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THE USE OF BLOOD AGAR FOR THE STUDY OF STREPTOCOCCI

By

JAMES HOWARD BROWN

NEW YORK
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PREFACE.

The purpose of this work is quite as much the description of methods as the recording of results, the latter being in a very high degree dependent upon the former.

The close association between methods and results is the most striking feature recognized in a review of the literature. Discordant results can be seen in most cases to have been due to different methods employed; *i.e.*, in those cases where the authors have described their methods sufficiently to permit their being understood. The review of literature is presented in tabular form because it seems to be most accessible and most useful in that form. The review of so many papers in textual form including all the items tabulated would have occupied much more space and would have been much less convenient for reference. Some but not all of the papers tabulated are discussed in the text. In the tables under such headings as *Media*, *Inoculation*, *Incubation*, *etc.*, are mentioned any details which the authors give regarding these; if these spaces are left blank it may be assumed that the author reviewed has published no information on these subjects. Under *Results and Conclusions* are presented only the results and conclusions of the authors reviewed. All quotations are in italics. By referring to the work of an author in each of the tables—The Use of Blood Agar, The Use of Blood Bouillon, etc., Fermentation Reactions, Pathogenicity and Virulence, Mutations and Variability, General Conclusions, etc.—a fairly complete review of the author's work is obtained. Reviews with respect to serology, epidemiology, pathological anatomy, and clinical medicine have not been included except incidentally. In each table papers are arranged chronologically so that a paper referred to in the text or bibliography is easily found in the tables by seeking first the year of publication and then the author's name. A historical review of each subject is obtained by reference to the reviews in the order in which they are listed.

In the Bibliography are included many papers which it has not been considered necessary to review in the tables. There are also in-

cluded a number of papers on other subjects than Streptococci because certain suggestions in regard to methods or technique have been obtained from them.

I take pleasure in expressing my gratitude to Dr. Theobald Smith under whose direction this work was started, and from whom during five years of association I obtained much of the training and many of the ideals which have resulted in this work. I am also grateful for the facilities of the Department of Comparative Pathology of Harvard Medical School and for assistance from Harvard University in the form of the Charles Elliott Ware Memorial Fellowship.

J. H. B.

May, 1917.

MONOGRAPH OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
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THE USE OF BLOOD AGAR FOR THE STUDY OF STREPTOCOCCI.*

BY JAMES HOWARD BROWN.

(*From the Department of Comparative Pathology, Harvard Medical School, Boston.*)

PLATES 1 TO 34.

(Received for publication, April 24, 1918.)

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* A thesis submitted to the Division of Medical Sciences of the Faculty of Arts and Sciences of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with assistance derived from the Charles Elliott Ware Memorial Fellowship.

In preparation for publication a number of minor changes and additions to the text have been made. The number of illustrations has been reduced and the remaining ones have been rearranged.

INTRODUCTION.

The usual form of reporting scientific work is to start with a review of the literature, to indicate one or more problems which have attracted the author's interest, and finally to report original work on the subject. Ideal though it may be thoroughly to familiarize oneself with all the work that has been reported and to employ it as a point of departure for new work, it is doubtful whether it is the method usually followed. It has not been in the present instance. The work here reported was practically complete before the thorough review and tabulation of the literature was made. In thus entering upon a thorough study of the appearances produced by various streptococci in blood agar it was realized that others might have made similar observations. Recent literature, however, indicated that the work which had been done had led to no generally accepted standardization of method or of definition of such terms as "*hemolytic*" and "*non-hemolytic*" as applied to the appearances produced by streptococcus colonies in or on blood agar. The importance of such factors as the amount and kind of blood used, the amount and composition of agar used, the method of inoculation, and the period of incubation was suspected. The work of Rosenow on "*Transmutations within the Streptococcus-Pneumococcus Group*" was an additional incentive. It seemed worth while for a single author to work at the subject of *The Appearances Produced by Streptococci in Blood Agar* from the ground up, employing a system of records which would allow nothing to escape observation and recording; and finally to compare the results with those previously reported with a view to being able to interpret the results of others and to explain the many disagreements among them. This is the plan here followed.

During "*A Study of Streptococci Isolated from Certain Presumably Milk-Borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914*" by Dr. Theobald Smith and myself there came to us for study material in the form of throat swabs, milk samples, pathological exudates, and a few strains of streptococci isolated by others. Most of the material came from cases of epidemic septic sore throat, secondary lesions, and suspected milk. Attention was fixed

on the streptococci found in this material, these being the organisms commonly considered to be the causative agents in such epidemics. In throats and in milk as ordinarily obtained, however, there are many streptococci normally present but harmless under ordinary conditions. The differentiation of the pathogenic from the non-pathogenic streptococci was therefore the question of primary importance. The method followed was to plate out the suitably diluted material directly in blood agar, a medium adopted because known to favor the growth of a large number of organisms and one in which they produce widely different appearances. This was found to be true of different strains of streptococci. A number of strains found pure in the material from secondary lesions furnished the clue to the type of streptococcus which was probably the etiological factor in the epidemics. In blood agar this type of streptococcus was easily distinguished by the production of clear colorless hemolyzed zones about the colonies. Such streptococci were found in varying numbers in the material from the sore throats and in the carefully drawn milk of only one or two cows which epidemiological data had placed under suspicion. A study of fermentation and agglutination reactions showed that there were two or three varieties of these hemolytic streptococci. Pathogenicity for rabbits was found to be correlated with hemolysis and with certain fermentation reactions. There were also present in the blood agar plates inoculated with material from throats and with milk sediment large numbers of colonies of other streptococci producing much less hemolysis and certain other characteristic features.

There arose immediately the question of naming these two most common types of streptococci. Such names as *Streptococcus mitior seu viridans*, *Streptococcus brevis*, *Streptococcus longus*, *Streptococcus pyogenes*, and *Streptococcus pathogenes* naturally suggested themselves. Davis had recently applied the name *Streptococcus epidemicus* to streptococci from a similar epidemic. There were reasons, however, which made us hesitate to apply any of the foregoing names to our strains. Not all of the more hemolytic strains produced long chains in fluid media while some of the less hemolytic ones did, hence *Streptococcus longus* and *Streptococcus brevis* did not seem to be apt terms. The capsules or greenish color of the colonies described by Davis for *Streptococcus epidemicus* were not observed by us. *Streptococcus pyogenes* was a term introduced by Rosenbach (1884) without reference to hemolysis. After considerable debate and as the result of inoculation experiments (Petruschky, 1896) it came to be regarded as synonymous with *Streptococcus erysipelatos* (Fehleisen, 1883), both included by Schottmüller (1903) under the name of *Streptococcus longus pathogenes seu erysipelatos* on the basis of hemolytic characters only, later limited by Andrewes and Horder (1906) to hemolytic streptococci having certain fermentative characters, but since used by various authors (Beattie and Yates, 1911; Rogers and Dahlberg, 1914) with regard to the fermentation reactions and without regard to hemolytic characters. We avoided the use of the name *Streptococcus mitior seu viridans* for the less hemolytic streptococci because many of them produced little or no green color on blood agar and all did produce more or

less hemolysis. In the presence of these difficulties we were impressed with the greater importance of describing the essential characteristics of the strains rather than of naming them. We described, "*two types of streptococci, with reference to hemolysis*," the less hemolytic being called the "*alpha type*" and the markedly hemolytic being called the "*beta type*." Occasionally streptococci were found corresponding to neither of these two types but were never present in sufficient numbers or with sufficient constancy to excite suspicion of their importance respecting epidemic sore throat and were, therefore, not discussed in the report of our studies as published in January, 1915.

I have now to report a more detailed study of the appearances produced by the growth of streptococci in blood agar. I have in cultivation between 125 and 150 strains of streptococci and pneumococci. More than half of these have been under observation for several years; others have been isolated from various sources from time to time. All of them have been under the author's care exclusively and there are complete genealogical records covering every transplant and every day of the existence of each strain since it was acquired. From these have been selected a number of representative strains for more intensive study, for photography, and report. These strains and their more conspicuous characteristics are listed in Table I. It will be observed that in this table the strains are grouped according to certain "*Types in Blood Agar*." Although the appearances of colonies of various streptococci on standard agar or glycerol agar have been described by various authors and though even since blood agar has come into general use a number of authors have proposed the use of such media as the Conradi-Drigalski litmus-lactose-crystal-violet agar, blood agar is now generally employed for the isolation and primary differentiation of streptococci. As already mentioned, it has the double advantage of being an excellent medium for the growth of streptococci and of being of differential value because of the different appearances produced by different strains. Below are the descriptions of the *alpha* and *beta* types as described by Smith and Brown (1915).

"*Type α.—As observed after forty-eight hours incubation the change produced by streptococci of this type may be described as a somewhat greenish discoloration and partial hemolysis of the blood corpuscles immediately surrounding the colony forming a rather indefinitely bounded zone one to two millimeters in diameter and surrounded by a second, narrow, clearer, not discolored, partly hemolyzed zone. The*

inner (discolored) zones were fairly constant in size and composition on all the plates. Under the microscope many of the corpuscles were seen to be present but obviously discolored, the discoloration differing a good deal in intensity for different strains of streptococci. The corpuscles remaining in the outer (clearer) zones were much fewer and never discolored. The outer zones were much more conspicuous on crowded plates than on plates containing only a few colonies. The deep colonies themselves were small (greater diameter, 0.5–1 millimeter) and biconvex or complex (lobulated or asteroid), the formation of biconvex or complex colonies being quite constant and characteristic for different strains."

There was no reason for calling this type *alpha* and the other *beta* except that the α type was the one first noted and commonly found in varying numbers on all the swabs whether the β type was also present or not. The β type, however, was the one found in secondary lesions and was regarded as the etiological factor in the epidemics.

"Type β.—Streptococci of this type produced hemolyzed zones on horse-blood-agar plates radically different from those of the alpha type. The former produced sharply defined, clear, transparent, completely hemolyzed, colorless zones two to four millimeters in diameter. Under the microscope no corpuscles were seen to remain within the zones. The colonies themselves were simple and biconvex, never complex (greater diameter, 0.5–1 millimeter)."

"There was nothing particularly distinctive about the surface colonies of either type on blood agar plates. All were round, glistening, grayish, translucent, flatly convex colonies surrounded by hemolyzed zones corresponding to their respective types."

In describing streptococci as of the *alpha*, *beta*, or *gamma* type, I refer to their appearances in blood agar only.

In the work about to be reported the medium used throughout, unless otherwise described, was standard veal infusion agar containing the aqueous extractives of 500 gm. of veal, 5 gm. of commercial salt, 15 gm. of agar-agar shreds, and 10 gm. of Witte peptone per liter. The final reaction was 0.8 to 1.2 per cent N acid to phenolphthalein. This agar was tubed in 12 cc. amounts. For the pouring of blood agar plates it was melted, placed in a water bath at 45°C. for about 15 minutes, two-thirds of a cubic centimeter of defibrinated blood added and evenly mixed. The tube of medium was inoculated at once with a loop or two of properly diluted 24 hour bouillon culture and poured into a Petri dish 9 cm. in diameter. The medium therefore contained 5 per cent of blood and formed a layer 2 mm.

thick in the Petri dish. After the medium had solidified, the moisture which otherwise would condense on the surface of the agar and on the lid of the Petri dish was allowed to evaporate by placing the inverted dish in the position shown in Plate 1, Fig. 1. Allowed to remain in this position for 15 to 30 minutes in a room free from disturbance the plates were rarely if ever contaminated. Broadhurst (1913) and Holman (1914) have emphasized the importance of employing the most favorable possible medium for the determination of fermentation reactions. It is not just to describe an organism as unable to ferment a test substance when the failure is due to the unsuitableness of the medium in which the substance is dissolved. *Bacillus coli* will not ferment dextrose in physiological salt solution. A medium should be used which will bring out the largest possible quota of fermentation reactions. Holman (1914) describes a suitable medium as one "*in which the organisms grow well independently of the carbohydrate added.*" The work of Gordon (1905), Andrewes and Horder (1906), Winslow (1912), Fuller and Armstrong (1913), and even of those using Hiss serum water is open to criticism from this standpoint. For similar reasons there should be emphasized the importance of using a blood agar that will reveal the maximum amount of differentiation. It is for this reason that the medium and proportions described above have been used. The importance of this will be illustrated after the fundamental types in blood agar have been described.

DESCRIPTION OF THE TYPES IN BLOOD AGAR.

Type α.—In Plate 15, Fig. 1 is the photograph of a horse blood agar plate of an α type streptococcus at the end of approximately 48 hours incubation. This plate may be described as containing "a good number of colonies well distributed." To the eye the deep colonies appeared as small biconvex greenish colonies (greater diameter, 0.8 mm.) surrounded by zones of 1.3 mm. diameter in which the blood corpuscles were distinctly greenish in color. Under the low power of the microscope (7 to 8 diameters) the greenish zone was less apparent, but in the outer portion of the zone there was possibly a slight hemolysis which in the photograph (Fig. 1a) appears quite distinct. It must be remembered, however, that red light is quite inactive to the photographic plate while a greenish light is less so, so that the actual amount of hemolysis was less than appears in the photograph. The surface colonies on this plate were round, flatly convex, glistening colonies, somewhat greenish by transmitted light, grayish by reflected light, and with little apparent alteration of the surrounding medium.

The word "apparent" is used because as a matter of fact there are invisible exchanges between the colonies and the surrounding medium which exert a decided influence on neighboring colonies of the same or different organisms. It has often been observed that in pure cultures the colonies in a plate containing only a few colonies will be much larger than in a plate containing many colonies even though in the latter plate the colonies may be well distributed and well separated from one another by several millimeters of apparently unaltered medium. The ability of a streptococcus colony so to alter the surrounding agar as to render it unsuitable for the growth of a so called *surface spreader* is well shown in Plate 18, Fig. 5. This blood agar plate at the end of 24 hours incubation contained only streptococcus colonies (mostly deep) with indefinite partly hemolyzed zones about them. When observed at the end of 48 hours incubation a hemolytic surface spreader had appeared as a contamination rapidly spreading over nearly half the plate (Plate 18, Fig. 5), but in its progress neatly circumventing every one of the deep streptococcus colonies leaving islands of sterile laked blood agar about 2 to 2.5 mm. in diameter above and around them.

The agar plate shown in Plate 15, Fig. 1 has been described as containing "a good number of colonies well distributed." It con-

tained about 100 colonies and this is a good optimum. The colonies are far enough apart to exert no apparent influence on one another and are able to attain their full development unhindered. Even if there were 200 colonies they might still be able to do so. If there were many more than this, however, two effects would be noticeable: (1) the colonies would be smaller, and (2) around each of them would appear a more or less distinct zone of partial hemolysis.

These effects suggest the following explanation: (1) The metabolic products formed by an organism are always detrimental to its growth. In a crowded plate there is less opportunity for the diffusion of these products outward because they are heing formed throughout the plate and rapidly reach a concentration which inhibits the growth of the colonies. (2) The hemolytic substance, whatever its nature, as it diffuses outward from the colonies must reach a certain concentration hefore it can produce hemolysis. If the colonies are few, diffusion into the surrounding medium takes place quite readily. If the plate is crowded with colonies the diffusion of the hemolytic substance from an individual colony is soon checked by the concentration of the substance throughout the agar, and a concentration in the immediate neighborhood of the colony is soon attained sufficient to bring about hemolysis with the formation of zones. The fact that notwithstanding the general diffusion of the hemolytic substance throughout the medium the zones are fairly sharply defined is probably due to the fact that the corpuscles act as an indicator with a sharp end-point; they lake abruptly and simultaneously when a certain concentration of hemolytic substance is reached forming a definite zone, though the concentration of the hemolytic substance within the zone may be only slightly above that outside the zone and the slope from one to the other very gradual.

At the end of 48 hours incubation, then, streptococcus B-7α produced small deep colonies surrounded by small zones of somewhat greenish discolored corpuscles with or without apparent partial hemolysis, depending upon the number of colonies in the plate. A plate similar to the one described had been in the incubator 48 hours. Other work prevented its being examined then. Rather than being allowed to remain in the incubator another night it was placed into the refrigerator. Next morning all the colonies were ***hemolytic***. The experiment was repeated, this time making careful observations before and after refrigeration. The hemolysis occurred during refrigeration. Duplicate plates were made, one treated as before and the other left in the incubator for 96 hours. The results were as follows:

Plate 1	(48 hours at 37°C.) Both plates alike; no hemolysis.	(48 hours in refrigerator) Distinct hemolyzed zones in less than 24 hours.
Plate 2		(48 hours at 37°C.) Colonies larger but no hemolysis.

It was later observed that at room temperature (18 to 20°C.) some hemolysis might occur while the plates were lying about on the desk waiting to be examined. Hemolysis has been seen to appear in 30 to 45 minutes at room temperature. The lesson is obvious. *A strain may appear to be non-hemolytic at one time and hemolytic at another unless care is taken to examine it under the same conditions each time.* Although Konrád (1909) and Sigwart (1909) noted the appearance of hemolysis about the colonies of green-producing streptococci at room temperature and in the refrigerator, the fact has not been appreciated by other authors. Authors who have considered hemolysis on blood agar unreliable as a differential character because variable may have encountered the above factors without recognizing them (*i.e.*, variations in appearance due to the number of colonies in a plate and the time and temperature of observation). The hemolysis occurring at lower temperatures is evidently not to be regarded as an artefact for it does not occur in an uninoculated plate similarly exposed after incubation, but does occur in definite relation to the colonies of streptococci. It is quite as definitely characteristic of certain strains of streptococci as is hemolysis under any other condition of temperature. I have therefore adopted as a routine procedure the incubation of blood agar plates for 48 hours (an observation being made at the end of 24 hours also) followed by refrigeration for 24 hours or longer and observations again made. The 48 hour period of incubation was adopted because while some strains reach their full differential development in 24 hours, others do not.

After refrigeration for 48 hours the blood agar plate of streptococcus B-7α first shown in Plate 15, Fig. 1 appeared as in Fig. 2 with small but definite hemolyzed zones around each deep colony and a less well defined hemolysis about the surface colonies. Examined with a good simple lens or under the low power of the microscope the deep colonies were seen to be surrounded by what may be

called "*double zones*" (Fig. 2a); *i.e.*, composed of a distinct inner collection of (in this case) greenish non-hemolyzed corpuscles next to the colony and an outer more or less hemolyzed ring (1.5 mm. in diameter in this case). By comparing Figs. 1a and 2a of the same colony before and after refrigeration it appears that the inner collection of greenish corpuscles has remained unchanged and unaffected by the hemolysis during refrigeration. Apparently the greenish discoloration of the corpuscles was accompanied by a fixation against hemolysis for (1) it preceded hemolysis, (2) the hemolytic substance must have passed out from the colony through the collection of corpuscles, and (3) it must have been present in greater concentration among these corpuscles next to the colony than farther out where hemolysis did occur. This subject will be developed farther in a later chapter. As it had been noted that the zone of greenish corpuscles was formed during incubation and that hemolysis took place during refrigeration, the blood agar plate was replaced into the incubator for another period of 48 hours. There appeared outside the hemolyzed zone another ring of greenish corpuscles not so apparent macroscopically in Fig. 3 but easily seen microscopically in Fig. 3a, a "*triple zone.*" Still another exposure to the temperature of the refrigerator resulted in the formation of a second ring of partial hemolysis, a "*quadruple zone*" easily seen macroscopically in Fig. 4 and microscopically in Fig. 4a. Apparently the alternate throwing off of rings of fixed corpuscles and of hemolysis in response to alternate incubation and refrigeration is limited only by the exhaustion of the medium and the accumulation of metabolic products which inhibit further growth and activity. Saito (1912) observed a similar ring formation but apparently did not detect its relation to temperature. He says:

"*Ich will nicht unterlassen, darauf hinzuweisen, dass ich bei dem Studium der hämolytischen Platten mancherlei sah, wofür ich in der Literatur noch keine Erklärung gefunden habe und was ich auch selbst nicht erklären kann. Namentlich bei Streptococcus acidi lactici konnte ich nicht selten beobachten, dass unter den gut gewachsenen Kulturen die Blutkörperchen besser erhalten waren als in den hämolytischen verwaschenen Höfen in der Umgebung. Bei meinem lanceolatus 4 und 5 habe ich einige Male etwas sehr Merkwürdiges beobachtet. Es war die Kultur von einem hellen Hof umgeben, in dem eine vollständige Zerstörung der roten Blutkörperchen stattgefunden hatte. Dann kam ein dunkler Ring, ein zweiter heller Ring,*

ein zweiter dunkler Ring, ein dritter heller Ring und dann der unveränderte Nährboden. Es ist sehr merkwürdig, dass ein von der Kultur in den Nährboden diffundierender Stoff nicht am stärksten an seinem Entstehungsort, sondern in einiger Entfernung wirkt, und dass sich Maxima und Minima der Wirkung abwechseln. Es beweist dies, dass bei dem Vorgang der Hämolyse und Hämoglobinzerstörung noch Dinge mitspielen, die sich noch der allgemeinen Kenntnis entziehen."

The concentric rings seen by Saito were in all probability the record of his taking the plates from the incubator to room temperature for observation and of their subsequent reincubation. I have seen as many as six concentric rings appear in some cases.

Little or nothing has been said about the surface colonies. The surface colonies of streptococci on blood agar are of much less differential value than the deep ones. Sigwart (1909) recognized this fact and it has more recently been emphasized by Krumwiede and Valentine (1915). A glance at Table III on The Use of Blood Agar will show that with very few exceptions, however, authors have confined their attention to surface growth, probably merely because it was found easier to keep blood agar plates on hand ready made and to smear or streak them with culture material than to inoculate them in fluid condition. At any rate, there has been a great neglect of the deep colony.

The surface colonies of streptococcus B-7α have already been described as they appeared after 48 hours incubation (Plate 15, Fig. 1). They were round, flatly convex, glistening colonies, somewhat greenish by transmitted light, grayish by reflected light, with little or no alteration of the surrounding medium. If there had been many more colonies on the plate their appearance would have been much as it was in this plate after refrigeration (Fig. 2); *i.e.*, there would have been a hazy ring of partial hemolysis about the surface colony. Taken from the incubator at various times for examination there may develop about the surface colonies zones similar to those in Fig. 4 and since the multiple zones of the surface colonies are much less distinct than those of the deep colonies the finer characters of the former may pass unobserved. A close examination reveals a radical difference in the appearance of the zones of the two kinds of colonies. The *quadruple zones* of the deep colonies are made up from the center outward of a collection of corpuscles, a ring of hemolysis, a ring of

corpuscles, and an outer ring of hemolysis. The zones of the surface colonies on the same plate appear *triple*, beginning with a ring of hemolysis next to the colony, then a ring of corpuscles, and an outer ring of hemolysis. This latter is the appearance described by Natvig (1905) as "*Type II*" (see Table III on The Use of Blood Agar). He says:

"*II. Um die Kolonien bildete sich ein ca. 1 mm. breiter, farbloser, matter Hof, und ausserhalb derselben ein ebenso breiter, grün gefärbter Hof. Ab und zu war auch aussen herum ein schmaler farbloser Saum zu sehen.*"

He states that only two of his strains produced this reaction and that it was not always equally apparent. Evidently he did not recognize the factors influencing it, though he offers the following explanation:

"*Hier schien ein Hämolyse, theilweise mit vollständiger Zersetzung des Blutfarbstoffes (innerer Ring), theils mit einer Umbildung des Hämoglobins zu grünen Farbstoffen, vor sich gegangen zu sein. Dagegen wurde eine Auflösung der Blutkörperchen nur gelegentlich und in geringem Grade bemerkt (der schmale farblose Saum). Die Umbildung des Hämoglobins in grüne Farbstoffe war offenbar nur ein Vorstadium einer vollständigen Decomposition des Blutfarbstoffes, denn wenn man die Entwicklung der Reaction verfolgte, beobachtete man zuerst nur ein schwache graugrüne Färbung der Kolonien selbst, später dann einen grünen Hof dicht an den Kolonien und noch später entfärbten sich sowohl die Kolonien als die dieselben nächst umgebende Partie, während der grüne Hof gleichsam nach aussen verlegt war.*"

It would appear that Natvig's study of surface colonies only led him astray. I have never witnessed the hemolysis of corpuscles after they have heen discolored green and am in doubt as to whether there may be slight encroachment of hemolysis on the outer edges of the collection of greenish corpuscles where the discoloration is very slight or may be due to only a few of the corpuscles being discolored. What Natvig observed was probably this: He took the blood agar plate from the incubator and noted the "*grünen Hof dicht an den Kolonien.*" The plate was reincubated and when again examined the colony was immediately surrounded by a ring of partial hemolysis and outside of this a second ring of greenish corpuscles. Under these circumstances it is but natural that he should have regarded the greenish discoloration as a "*Vorstadium*" of hemolysis, but as a matter of fact the "*grünen Hof*" first observed had not been hemolyzed; it was merely concealed by the overgrowing surface colony. The hemolysis occurred outside the first "*grünen Hof.*" If he had scraped off the colony from the agar he would have found the collection of green corpuscles beneath. The suhstance of the colony scraped off would have been seen to be grayish in color; it appeared grayish green at the time of the first observation because the light hy which it was seen passed through the green corpuscles lying beneath and beyond the edges of the colony; it appeared less green later because it had increased

in size and substance while the collection of greenish corpuscles beneath had not increased in size because limited by the ring of hemolysis and consequent lack of corpuscles surrounding it. I have many times observed the course of events here described. Mandelbaum (1907–08) describes it also, though he describes the discoloration as brownish rather than greenish, a difference due to differences in the media employed. He observed surface colonies and his description fits my own observations perfectly if it is assumed that instead of taking the cover off the Petri dish to observe the colonies from the top surface, he observed them through the medium from the bottom of the dish, not an uncommon procedure. He says:

"Die Kolonien des Mitior dagegen zeigen niemals die Färbung und strukturlose Form, die man bei ihrem Wachstum auf gewöhnlichem Agar zu sehen gewohnt ist. Die Farbe ist tiefbraun bis braunrot oder rostbraun, die Kolonien scheinen grob gekörnt. Untersucht man nun diese Kolonien mit dem Mikroskop, so entdeckt man, dass man von der eigentlichen Kolonie gar nicht sieht, sondern nur die Form oder, besser gesagt, den Abdruck, der durch die roten Blutkörperchen gebildet wird, die 'grobe Körnung' ist nichts anderes als eben die dicht zusammenliegenden roten Blutkörperchen. Die Rand der Kolonie ist durch eine dunklere Linie angedeutet. Ueber diese hinaus liegen noch auf eine kurze Strecke die unaufgelösten roten Blutkörperchen, die dieselbe Farbe zeigen wie die unmittelbar unter der Kolonie liegenden. Dann folgt der helle, farblose Hof, in dem die roten Blutkörperchen nur noch als 'Schatten' zu erkennen sind. Man kann die eigentliche graue Kolonie ruhig mit der Platinöse abtragen, ohne das mikroskopische Bild zu verändern."

Mandelbaum's deductions from the above observations are hardly justified but will be considered later (page 71).

Type β.—This type of appearance in blood agar may be described as a colony surrounded by a perfectly clear, colorless zone of hemolysis. The blood agar plate of such a streptococcus after 48 hours incubation is shown in Plate 6, Fig. 1. There is no trace of discoloration and when viewed microscopically no corpuscles are seen in the medium surrounding the colony (Fig. 1a). The colony itself is grayish by transmitted or reflected light. The distinguishing characteristics of this type appear much earlier than do those of the *alpha* type and are often quite well developed in 18 hours at incubator temperature. In 48 hours the zones and colonies are a little larger than at the end of 24 hours but no different qualitatively. The surface colonies are surrounded by similar zones.

The characteristics of this type have been recognized by most authors since Schottmüller (1903) described them as distinguishing *"Streptococcus longus pathogenes seu erysipelatos,"* and although *Streptococcus pyogenes* was a name originally employed by Rosenbach (1884) without reference to hemolytic char-

acter, the source of his strains makes it altogether probable that most of them were of this type. Neither did Rosenbach describe the fermentation reactions of his strains, but since the great majority of so called *hemolytic streptococci* from wound infections have been found to ferment dextrose, maltose, saccharose, lactose, and salicin but not raffinose, mannite, or inulin, I believe it is justifiable to restrict the name *Streptococcus pyogenes* to streptococci of the *beta* type in blood agar and having the fermentative characters just mentioned. This has been done by Andrewes and Horder (1906), if it may be assumed that by "*hemolytic*" they meant only this most conspicuous type of hemolysis. Many authors, however, have used the name *Streptococcus pyogenes* with reference to either the hemolytic or the fermentative character alone, both of which practices are full of possibility of error, since not all streptococci of the *beta* type produce the same fermentation reactions and many strains having the same fermentative characters produce radically different appearances in blood agar. *Streptococcus hemolyticus* and *Streptococcus vulgaris* are in so far as can be determined from the literature synonymous with *Streptococcus pyogenes*, though they may he more inclusive.

Having seen hemolysis take place more readily during refrigeration than during incubation in the case of streptococci of the *alpha* type one might expect the zones of the *beta* type shown in Plate 6, Fig. 1, to become much enlarged during refrigeration. As a matter of fact, however, after refrigeration for 48 hours they showed no change (compare Plate 6, Figs. 1 and 2), nor did they change during a second period of incubation and refrigeration though colonies transferred to bouillon were in all cases viable. The difference in behavior of the two types in respect to refrigeration is so great as to suggest different hemolytic substances or processes.

Type α'.—In describing the *beta* type it was stated that the surface colonies were surrounded by zones similar to those of the deep colonies. However, even in trying to distinguish streptococci of the *beta* type the employment of surface inoculation only can result in failure to recognize the *alpha prime* type which in so far as appearance is concerned is intermediate between the *alpha* and *beta* types. In the paper by Smith and Brown (1915) there are included among the *beta* strains two designated B-2 and B-6 respectively. They differed from all the other *beta* type strains in fermenting raffinose and were non-pathogenic when injected intravenously into rabbits. These were among the first strains isolated and studied by us and

our notes on them are less satisfactory than on strains isolated later. Replating these strains many months later I found that they did not produce the clear hemolyzed zones of the *beta* type but belonged to what I have since called the *alpha prime* type. One of these strains (B-6α') is shown in Plate 10, Fig. 1, after 48 hours incubation. It can scarcely be called anything but a *hemolytic streptococcus* for there are distinct hemolyzed zones and no apparent discoloration. By carefully comparing this figure with one of the *beta* type, however, (*e.g.*, Plate 6, Fig. 1) it is seen that the deep colonies of the *alpha prime* type appear somewhat hazy or poorly defined within the hemolyzed zones. Under the microscope (Plate 10, Fig. 1a) the reason for this haziness is apparent; a few corpuscles are seen to remain throughout the zone but are most numerous next to the colony. They were not noticeably discolored, possibly because not sufficiently numerous to impart their color to the mass. On the other hand, the zones of the surface colonies were practically like those of the surface colonies of the *beta* type because the thin collection of corpuscles near the colony was completely obscured by the overgrowing surface colony. Different strains of the *alpha prime* type differ with respect to the relative clearness and size of their zones, and strains of the *beta* type differ also with respect to the size of their zones, so that the surface colonies of one type may be quite indistinguishable from those of the other type. The deep colonies must be studied microscopically to differentiate them. Without doubt authors devoting their attention to the appearance of surface growth only have included many of these strains in the *Streptococcus longus pathogenes* group and finding them avirulent for rabbits or coming from clinically mild infections, as they often do, and perhaps finding that these strains differ also in their fermentation reactions from other *hemolytic streptococci* have concluded that hemolysis or fermentation reactions or both are of no significance with respect to virulence or pathogenicity. (For a review of the question of the relation of virulence to hemolysis, etc., see Table VIII on General Conclusions, etc.). In all probability many of the strains described (*e.g.*, Broadhurst, 1915; Rosenow, 1914; Thalmann, 1911; Sigwart, 1909; Fromme, 1908; Heynemann, 1908; Natvig, 1905) as producing a *hazy hemolysis* were of the *alpha prime* type, but most authors have been content to designate strains merely as *hemolytic* or *non-hemolytic*.

Superficially there is considerable resemblance between the *alpha prime* and *beta* appearances while quantitatively at least there is a great difference between the *alpha* and *alpha prime* appearances. Nevertheless I have indicated a relationship between the latter two by calling one *alpha* and the other *alpha prime*. This relationship is indicated not only by the presence of *fixed* corpuscles next to the colony but also by the response to refrigeration. After 48 hours incubation the plate shown in Plate 10, Fig. 1, was refrigerated for 48 hours. The zones were noticeably enlarged as shown in Figs. 2 and 2a. Placed into the incubator for another period of 48 hours the zones became still larger and rather diffuse but no multiple zones of concentric rings were formed. The relationship to the *alpha* type was clearly demonstrated by the appearance of the same strain in a rabbit blood agar plate instead of horse blood agar. In the rabbit blood agar plate treated in the same manner as the one just described the typical *alpha* appearance was produced (Plate 10, Figs. 3, 3a, 4, and 4a). After refrigeration there was seen a typical *double zone* with a broad collection of green corpuscles next to the colony. It must not be assumed, however, that the *alpha prime* appearance is never produced in rabbit blood agar. It has often been seen in rabbit blood agar and in human blood agar, but was not produced by this particular strain.

When after cultivation for more than a year it was discovered that streptococci B-2 and B-6 no longer could be classed among those producing the *beta* type of hemolysis, the idea was entertained that their hemolytic character had changed. Searching through the notes carefully, however, it was discovered that from the time of their isolation these strains had produced in horse blood agar zones of hemolysis described as slightly hazy or a little less clear than those of other hemolytic streptococci. In all probability the hemolytic character had not changed.

Type γ.—There remains at least one more type of appearance in blood agar to be described. By the *gamma* type is meant the growth of the streptococcus colonies within and on the blood agar plate without the production of any perceptible hemolysis or discoloration of the surrounding medium during incubation or refrigeration. A streptococcus of this type is shown in Plate 2. It requires no de-

scription. Streptococci causing this appearance in blood agar have been described by Mandelbaum (1907–08) as *Streptococcus saprophyticus*, by Zangemeister (1910) as *Streptococcus anhemolyticus vulgaris*, and were found by Rosenow (1914) among those from the joint fluid of cases of rheumatism.

Each of the above described blood agar types is fairly distinct from the others so that on seeing a streptococcus colony for the first time in a blood agar plate there is rarely any difficulty in deciding to which type it belongs. Incubation for 24 hours is usually sufficient to determine the type though it is best to confirm the diagnosis by incubating the plate for 48 hours and then refrigerating it. To insure purity the strain should always be replated until colonies of only one type develop. In a mixed colony the hemolytic (*beta*) type will completely obliterate the characters of the *alpha* or *gamma* type even though cocci of the latter types may be present in the colony in considerable numbers. This will be illustrated later (page 64). (See also Holman (1914) in Table VII on Mutations and Variability.)

VARIOUS REPRESENTATIVES OF EACH TYPE.

Belonging to each of the above types are organisms of widely different characters in other respects and the appearances in blood agar produced by different strains show quantitative differences within each type except the *gamma* type, this latter type being defined by purely negative characters. Streptococci of this type, however, do differ with respect to size of colonies, fermentation reactions, etc.

Type α.—In so far as the author has been able to observe, not only do all green-producing and brown-producing streptococci belong to this type but also the *Pneumococcus* and *Streptococcus* (*Pneumococcus*) *mucosus*.

In horse blood agar streptococcus A-BLα (Plate 12) differs from B-7α (Plate 15) described as the type form, in producing a broader central collection of greenish corpuscles surrounded after refrigeration (compare Plates 12 and 15, Figs. 2 and 2a) by a broader ring of partial hemolysis. The appearances produced by the two strains are, however, essentially similar. A greater difference is apparent by comparing Plate 12, Figs. 3 and 3a, with Plate 15, Figs. 4 and 4a, photographs taken after the second period of refrigeration. Both show quadruple zones about the deep colonies, but in the case of streptococcus A-BLα the second ring of corpuscles though distinct is quite narrow and is composed of relatively few corpuscles. Streptococcus D-4α (Plate 14) forms zones which after the second period of incubation and refrigeration (Figs. 3, 3a, and 3b) are much like those of A-BLα. The colonies themselves of D-4α, however, though less numerous, are noticeably smaller than those of A-BLα or B-7α. The deep colony in Fig. 3a viewed from the edge and appearing biconvex shows the quadruple zone less plainly than one (Fig. 3b) viewed from the broad side and appearing round. While streptococcus D-4α resembles A-BLα after the second period of refrigeration, streptococcus E-4α (Plate 17, Figs. 4 and 4a) resembles B-7α more closely, the second corpuscle rings of the latter two being

quite prominent. For some unknown reason, however, the peripheries of the quadruple zones of E-4α and A-BLα are surprisingly sharply defined (especially that of E-4α), while those of B-7α and D-4α blend more gradually with the surrounding medium. The plates of these four strains just described were poured from four tubes of the same lot of agar, contained the same amounts of the same lot of horse blood, and were inoculated and poured one after the other on the same day. They were incubated, examined, refrigerated, and photographed just as nearly simultaneously as possible. The four strains (Table I) were isolated from the throats of four different individuals during four different epidemics of sore throat, but in no case were they considered responsible for the patient's condition or the epidemic. Two of the strains were isolated in 1914 and had similar fermentation reactions (D-4α and E-4α) but were least alike in other respects. The other two were isolated 10 months previously. In blood agar their dissimilarities were much less conspicuous than their resemblances. All produced typical *alpha* type zones in horse blood agar.

The deep colonies of the four strains just described and in fact the deep colonies of most bacteria in agar are circular biconvex or lenticular bodies. This shape is so common as to be of little differential value and is probably largely determined by the natural cleavage of the agar itself under pressure from the growing colony. If a deep dextrose agar shake culture of a gas-forming organism is examined the gas bubbles in the agar are seen to be biconvex. Very small colonies may be spherical and the degree of convexity of colonies is not the same for all organisms or strains, but different deep colonies of the same strain appear round, oval, or biconvex merely because of their position with respect to the surface of the plate from which they are observed. An attempt to illustrate this by a projection drawing is shown in Plate 1, Fig. 3. Three colonies of the same size and shape are shown in the upper half of the figure as they might appear viewed laterally. Projected into the lower half of the figure are the same three colonies as viewed looking downward from the surface of the plate. The colonies appear biconvex, oval, and round respectively.

In plates made from throat swabs there were occasionally found

by Dr. Smith and myself a certain number of colonies which we called *complex* to distinguish them from the usual *biconvex* colonies just described. It was natural to suppose at first that these might be accidental variations of the biconvex form, due possibly to the fusion of colonies developing from two or more cocci close together in the medium. It was discovered, however, that the formation of either form of colony was "*quite constant and characteristic for different strains.*" Strains isolated from so called *complex* colonies over 2 years ago have been replated and reexamined. Plain agar and blood agar plates of two of these strains are shown in Plate 19. Figs. 1 and 2 are of streptococci F-2α and D-1α respectively, in horse blood agar after 48 hours incubation. In both figures the colonies are quite numerous and the zones of the deep colonies are plainly double and of the *alpha* type. Under the low power of the microscope the colonies were seen to be of the same form as those of the same strains in plain agar plates shown in Figs. 3 and 4. All of the deep colonies of streptococcus F-2α (Figs. 1 and 3) were small irregular conglomerate clumps; none was biconvex. Photomicrographs of three of these are shown in Figs. 3a, 3b, and 3c. The deep colonies of streptococcus D-1α (Figs. 2 and 4) were quite different from the preceding; some were biconvex (Fig. 4c), some were described as "ruptured biconvex colonies" (Fig. 4a), others were somewhat triangular with or without rupture (Fig. 4e), and still others had the form of rather elaborate radiate structures (Figs. 4b and 4d). The deep colonies of streptococcus F-2α were all asymmetrical; those of D-1α always symmetrical. Searching back through the notes it is found that these characteristics have been noted from the time these strains were isolated over 2 years ago. Both these strains formed in bouillon sediment difficult to disintegrate by shaking. Microscopically the bouillon cultures were seen to be composed of clumps of crooked chains.

The characteristics in bouillon agree with the descriptions of *Streptococcus conglomeratus* given by Kurth (1891) and by Thalmann (1910, 1911, and 1912). It has been impossible to identify the two varieties shown in Plate 28 with the two varieties (*A* and *B*) described by Thalmann.

Thalmann's description of *Streptococcus longissimus* led to the recognition of similar strains in my collection. A study of one of these (D–AD $8_2\alpha$) is shown in Plate 18.

The chief characteristics ascribed to this organism by Thalmann (1910) are: (1) the formation on "*feuchtem, leicht alkalischem Fleischwasserglyzerinagar*" of delicate round colonies of irregular size and with irregular borders (thread-like projections); (2) "*Auf Blutagar bildet der Streptococcus longissimus bei Zusatz von frischem Blut nach 20–24 Stunden bei 37° flache, hell bis bräunlich gefärbte, meist nicht ganz regelmässig geformte Kolonien, die einen grünlichen oder grünblauen Hof aufweisen. Die Hämolyse fehlt entweder vollkomen oder sie ist gering. Bei vorhandener Hämolyse sind in der Regel einzelne Blutkörperchen im hellen Hof sichtbar, oft ist die Hämolyse ringförmig, da die direkt auf den Kolonien aufliegenden Blutkörperchen ihren Farbstoff nicht abgegeben haben;*" (3) bouillon is said not to be clouded but an abundant thready fleecy sediment is formed which disintegrates fairly easily when shaken; (4) microscopically bouillon cultures are said to present very long (crossing several microscopic fields) smooth chains lying parallel in skeins, the elements being generally slightly ovoid and weakly Gram-positive. The characteristics of streptococcus D–AD $8_2\alpha$ agree with this description in every respect. Thalmann apparently employed only surface inoculation of his plates.

In Plate 18, Fig. 7, is shown a plain agar plate inoculated in fluid condition and then streaked. Only the surface colonies interest us. Some from the encircled area on the plate are shown in Figs. 7a and 7b under two powers of the microscope. The borders are irregular and filamentous (Fig. 7b). In horse blood agar after a period of 48 hours incubation the most striking feature was the presence around the deep colonies of colorless zones which macroscopically might have been mistaken for zones of the *beta* type (Fig. 1). They were somewhat cloudy, however, and microscopically (Fig. 1a) a small collection of greenish brown corpuscles or pigment was seen close to the colony. Under the microscope the outer hemolyzed portion of the zone could never be mistaken for the *beta* type; it was in fact much less distinct microscopically than macroscopically, and was quite diffuse, many unhemolyzed corpuscles remaining within it. After refrigeration the hemolysis about the deep colonies was much more distinct (Figs. 2 and 2a). If we confine our attention to surface colonies as did Thalmann, we find that his description is quite applicable—"*Die Hämolyse fehlt entweder vollkommen oder sie ist gering.*" Macroscopically, even after refrigeration, only a faint partly hemolyzed border was seen around the surface colonies and along the edges of the streaks. Microscopically it was hardly visible. By transmitted light the streaks were distinctly green, due to the greenish discolored

corpuscles which lay beneath the growth and extended nearly 1 mm. beyond its edges. Macroscopically the appearance of the deep colonies was that of the *alpha prime* type in horse blood agar. However, the greenish discoloration visible macroscopically under the surface growth and microscopically about the deep colonies causes this strain to be placed among those which produce the *alpha* type of zones in blood agar.

The strains above described producing the *alpha* type of appearance have all had certain characteristics in common (Table I). None of them produced capsules; none was soluble in bile or sodium taurocholate; and none fermented inulin. For comparison with the above there have been studied a number of strains of *Streptococcus* (*Pneumococcus*) *mucosus* and of pneumococcus. These strains were all encapsulated under suitable conditions, all were soluble in sodium taurocholate, and all fermented inulin. In blood agar all produced the *alpha* type of appearance.

Park and Williams (1905) and others have given reason for regarding the *Streptococcus mucosus* of Schottmüller as a pneumococcus rather than a streptococcus. It is in fact the Type III pneumococcus of Cole. The name *Streptococcus mucosus* was originally applied by Howard and Perkins (1901) to an organism isolated by them from a case of peritonitis resulting from a tubo-ovarian abscess. These authors did not employ blood agar or blood bouillon, did not observe the action of bile on their culture, and did not study the reaction toward inulin. From their description, however, it is altogether likely that their organism was either identical with the *Streptococcus mucosus* later described by Schottmüller (1903) or may have been a strain of *Streptococcus epidemicus* (Davis, 1912). These two organisms are remarkably similar morphologically and culturally but differ in respect to fermentation of inulin, solubility in hile, and type of appearance in blood agar, the former producing the *alpha* and the latter the *beta* appearance. The one might not inappropriately be called *Pneumococcus mucosus* and the other *Streptococcus mucosus* if the latter name had not already become so firmly associated with the pneumococcus-like organism. For many years (since 1903) European literature has applied the name *Streptococcus mucosus* to the organism described hy Schottmüller and known in this country as *Pneumococcus mucosus* or *Pneumococcus Type III*. Apparently *Streptococcus epidemicus* has not been encountered or recognized in Europe. Under these conditions it would seem a mistake to try to transfer the name *Streptococcus mucosus* to the latter organism. It would seem much hetter to continue to use the names *Pneumococcus mucosus* and *Streptococcus mucosus* synonymously, just as *Pneumococcus*, *Streptococcus pneumoniæ*, and *Diplococcus lanceolatus* are used.

Streptococcus mucosus Brig.-IIaα in horse blood agar differed from the streptococci above described most conspicuously in the size of the colonies. The deep colonies (Plate 20)[1] were biconvex discs over a millimeter in diameter and because of their size had a marked tendency to find the top or bottom surface of the agar, especially during the second period of incubation (Fig. 3). The surface colonies were even more distinctive. Instead of being rather small round greenish colonies as seen by transmitted light after the first period of incubation, they were quite large (many of them several millimeters in diameter), roundish, glistening, almost transparent "*tearful*" colonies which when first observed were quite convex but soon dried down to flat transparent films (Fig. 1). Beneath and close to the surface colonies were areas of pale greenish discoloration accompanied by lightening of the medium (partial hemolysis?). The typical deep colony, even before refrigeration was surrounded by a distinct partly hemolyzed zone within which near the colony was a good collection of pale greenish corpuscles (Fig. 1a). This zone might be called *double* except for a faint narrow second ring of corpuscles which is explained as follows: This culture was very sensitive to the lowering of temperature so that during the process of examination at room temperature the outer hemolyzed zone was seen to increase in size. This was after 48 hours incubation, but the plate had also been examined after 24 hours incubation at which time the zones consisted merely of light greenish, partly hemolyzed rings which, according to measurements then taken, extended just to where the faint second ring of corpuscles later appeared. The removal of the plate from the incubator at the end of 24 hours was therefore recorded by a slight hemolysis occurring during this observation followed by a little more greenish discoloration and fixation of corpuscles after replacement in the incubator. After refrigeration (Figs. 2 and 2a) the outer hemolyzed zone was still larger. Figs. 3 and 3a were made after a second period of incubation and refrigeration during which the colony became much larger so as to encroach upon the central collection of fixed corpuscles and to extend almost to the second ring

[1] The plate described and photographed was of agar made with beef infusion and Digestive Ferments Company peptone, but was apparently like a plate made with veal infusion agar and Witte peptone.

which at this time appeared darker, whether because of any alteration in it or because of some optical effect of its closer proximity to the colony I am unable to say. During the last period of incubation the hemolysis had continued to extend outward but a ring of fixed corpuscles did not appear.

Pneumococcus Cole I 109 (Plate 21) in horse blood agar[2] differed from streptococcus Brig.-IIaα in forming smaller colonies, especially the surface colonies being smaller, less mucoid, and less transparent. The hemolyzed zones were less influenced by low temperatures so that at no time did they reach the size of those of Brig.-IIaα. After the first period of refrigeration (Figs. 2 and 2a) both surface and deep colonies had distinctly *double* zones, the central collection of corpuscles being distinctly greenish. After reincubation for 24 hours there was little visible change (Figs. 3 and 3a) but the effect of this incubation was revealed by the subsequent refrigeration during which there appeared *quadruple* zones, very distinct about some of the deep colonies (Fig. 4^{II}) and less so about others (Fig. 4a). The quadruple zone was also apparent about the bottom surface colony (Fig. 4^{III}) but the zones of the top surface colonies were apparently double (Fig. 4^{IV}) if viewed microscopically. Macroscopically these latter would probably be described as being greenish by transmitted light and surrounded by a narrow ring of partial hemolysis.

Pneumococcus Cole II 45 in horse blood agar (Plate 24)[3] produced essentially the same appearance as pneumococcus Cole I 109. The most apparent difference was a more intense green color, the discolored corpuscles next to the colony quite obscuring the outline of the deep colony itself (Figs. 1a and 2a). At the end of 24 hours incubation the deep colonies were surrounded by *simple* zones of greenish discoloration, apparently partly hemolyzed, but at the end of 48 hours incubation the zones were distinctly *double* (Figs. 1 and 1a). The effect of refrigeration (Figs. 2 and 2a) was not to intensify the hemolysis but rather to render it more diffuse. The surface colonies showed only a faint indefinite ring of hemolysis. They were flat, roundish,

[2] Made with beef infusion and Digestive Ferments Company peptone.

[3] The plate photographed contained veal infusion—Witte peptone agar but was apparently like one containing beef infusion—Witte peptone agar. The result of using Digestive Ferments Company peptone will be described later.

and green by transmitted light, but by scraping off the substance of one of them it was seen to be gray, the green color by transmitted light evidently being due to the discolored corpuscles beneath the colony.

There is seen to be much resemblance between the growth in horse blood agar of the *Streptococcus mucosus* and the two pneumococci just described. Other strains that I have studied resemble them. They produce larger colonies than do the non-encapsulated *alpha* type streptococci. As distinguished from the latter their deep hemolytic colonies are more hemolytic, producing distinct double zones in 48 hours at 37°C. even when there are few colonies in the plate, while their surface colonies are if anything less hemolytic than those of the other streptococci. The latter fact probably accounts for the prevalent idea that pneumococci are non-hemolytic.

Type α'.—Streptococcus B-6α' has been described as producing the *alpha prime* type of appearance in horse blood agar and streptococcus D-AD $8_2\alpha$ has been referred to as approaching this type. In Plate 11 is shown another strain (streptococcus F-2α') which produced zones essentially like those of B-6α'. The hemolytic activity of the former, however, was somewhat the greater and was especially noticeable after the second period of incubation. At this time the zones of F-2α' were 5 to 6 mm. in diameter and perfectly clear except for the small central collection of corpuscles visible microscopically. The colonies themselves had also increased in size during the last incubation.

Type β.—In Plates 6, 7, and 9 are shown three strains of streptococci producing clear hemolyzed zones without corpuscles remaining next to the colony, the *beta* type. Of these the growth of A-STβ in horse blood agar has been described. In this medium streptococcus D-AD4β (Plate 7, Figs. 1 and 2) was indistinguishable from A-STβ. Both strains were from human sources, one from a throat and the other from peritoneal pus, but they differed in their fermentation reactions (Table I).[4] The deep colonies and hemolyzed zones of

[4] Only a few strains of streptococci similar to A-STβ have been described. It is a non-encapsulated, β hemolytic streptococcus, differing from *S. pyogenes* (*e.g.*,

a third streptococcus, B-2b (cow)β (Plate 9), were also like the preceding except that the surface colonies of the latter were slightly larger and possibly a little more moist in appearance. In the photographs, however, these three strains are hardly distinguishable from one another. Streptococcus B-2b (cow)β was isolated from the udder of a gargety cow in a dairy herd which from epidemiological data was suspected of being responsible for a sore throat epidemic at Canton, Mass., May, 1913 (Smith and Brown, 1915). It was studied in comparison with strains from the epidemics of Chicago (1912), Baltimore (1912), and Boston (1911) and found to be similar to them in all respects. This is the variety of streptococcus first carefully described by Davis and Rosenow (1912) and later called "*Streptococcus epidemicus*" by Davis (1912 and 1913). Some of the strains available for comparison came directly from Davis and he had examined the strain from the Boston epidemic sent to him from this laboratory. With regard to hemolysis, the descriptions of Davis and Rosenow correspond very well with my observations of surface colonies. The occasional greenish tint described by them will be considered later. In the case of the strain here described capsules had not until recently been noticed. It is stated by Davis (1912; see Table VIII on General Conclusions, etc.) to be frequently lacking. If, however, some of the moist surface growth from a blood agar plate (incubated barely over night) is suspended in a suitable india ink, covered with a cover-slip, and examined at once, the capsules are readily seen. They have been found to be present on all the strains of *Streptococcus epidemicus* in my possession even after several years of cultivation. The capsules of some strains are extremely perishable, being present in young surface colonies only, and rapidly disappearing

D-AD4β) in fermenting mannite but not lactose. Such streptococci were described by Smith and Brown (1915) as the cause of an epidemic at Norton, Mass. Holman (1916) has described a similar strain and in his classification of streptococci has grouped these together as *S. hemolyticus II* to distinguish them from other hemolytic streptococci. It would seem to us that since this streptococcus was found to be the cause of an epidemic, since the characteristics of our strains have remained constant for several years, and since these characters are such as to form one of the major groups in Table II, it may be justifiable to propose a name for this group—*Streptococcus alactosus* β (Smith and Brown).

after being suspended in india ink. The other characters tabulated by Davis (1913; Table VIII on General Conclusions, etc.) for the differentiation of *Streptococcus epidemicus* from *Streptococcus hemolyticus* are quantitative and hardly sufficient for diagnosis. The growth on an agar or blood agar slant or in a streak on a blood agar plate, taken in conjunction with a knowledge of the hemolytic type in blood agar plates, is the most constant and reliable means of differentiating these two hemolytic streptococci. In Plate 1, Fig. 2, is a drawing of three agar slants (*a*, *b*, and *c*). These slants were inoculated by placing one small loop of 24 hour bouillon culture of streptococcus into the condensation fluid of the slant and then streaking the inoculated fluid upward along the slant. The tubes were incubated over night in a standing position in the moist atmosphere of the incubator. At the end of this time, strains of *Streptococcus epidemicus* and of *Streptococcus mucosus* present the appearance shown on slant *a* of the figure. The growth on slant *a* is composed of flat, almost transparent, confluent, amebiform patches, small or large depending upon the number of organisms carried up onto the slant. The streak across the surface of a freshly poured blood agar plate exhibits the same characteristics even more conspicuously. *Streptococcus mucosus* is easily differentiated from *Streptococcus epidemicus* because the former produces the *alpha* and the latter the *beta* type of hemolysis in blood agar. The growth of *Streptococcus mucosus* on the slant may be somewhat more raised or mucoid than that of *Streptococcus epidemicus* but that does not alter the ameboid form of the growth. If the upper part of the slant is somewhat dry the growth there may take the form of small discrete colonies as shown on slant *b* of the drawing, but subinoculations from such colonies into bouillon and then onto suitably moist slants again reveal the characteristic amebiform patches. Similar slants or plates inoculated with other streptococci (including *Streptococcus pyogenes*) develop streaks of small, round, convex colonies, discrete unless crowded, as shown in slant *c* of the drawing. The pneumococcus often develops larger and flatter round colonies which if crowded may be confluent but quite distinct from the ameboid forms shown by *Streptococcus* (*Pneumococcus*) *mucosus* and *Streptococcus epidemicus*. Slants inoculated too heavily with any of the streptococci or pneumococci develop more

or less homogeneous film-like streaks without differential character. Ten or eleven strains of *Streptococcus epidemicus* in cultivation since 1911, 1912, and 1913 have up to the present time shown no change with regard to the above characteristic.

During tonsillitis epidemics A and B described by Smith and Brown (1915), milk from the cows of suspected dairies was examined first microscopically, then those samples which revealed large numbers of leukocytes were studied culturally. There was mastitis in both herds. From one cow of one herd there was isolated a hemolytic streptococcus like those isolated from patients in epidemic B. This streptococcus was B-2b (cow)β. From cows of each herd there were also isolated bovine hemolytic streptococci which differed in important respects (*e.g.*, fermentation reactions, pathogenicity for rabbits, and agglutination) from those of human origin. The bovine streptococci from both herds had the same fermentation reactions and showed some agglutinative relationship, but exhibited somewhat different appearances in blood agar which will now be described.

Streptococcus B-18 (cow)β is representative of those isolated from herd B suspected in connection with epidemic B. Small colorless hemolyzed zones about the deep colonies in horse blood agar plates were visible after incubation for 24 hours, while after 48 hours these zones were about 3 mm. in diameter (Plate 5, Fig. 1). There were no corpuscles remaining next to the colony (Fig. 1a) and no discoloration. These zones were therefore of the *beta* type and in fact differed in appearance from those of streptococcus A-STβ, D-AD4β, and B-2b (cow)β only in being slightly smaller and less sharply defined at their outer borders. These zones increased slightly in size during refrigeration (Figs. 2 and 2a).

Streptococcus A-18 (cow)β is representative of those isolated from herd A suspected in connection with epidemic A. Usually in horse blood agar this streptococcus had the following appearance: After incubation for 48 hours the deep colony (Plate 4, Fig. 3) is surrounded by a small (2 to 2.5 mm. in diameter), clear, colorless, sharply defined hemolyzed zone, free of corpuscles or discoloration and therefore of the *beta* type. Outside this zone which I will call the central zone is a broad (about 1 cm. in diameter), translucent, partly hemolyzed, well defined zone containing many apparently unaltered cor-

puscles. There is no discoloration. The area within the outer zone but outside the inner zone is noticeably darker than the outer portion of the outer zone. This dark area was less apparent to the eye than it is in the photograph and I think it is due to the diffusion of free hemoglobin outward from the inner completely hemolyzed zone. A similar shading of the medium is apparent around the zones of other *beta* hemolytic streptococci (*e.g.*, Plates 6, 7, and 9). The outer partly hemolyzed zones of streptococcus A-18 (cow)β were influenced by inconspicuous factors which are not fully understood. For instance, the plate shown in Figs. 1 and 2 was inoculated just 6 days before the one shown in Figs. 3 and 4; both were made of agar of similar composition having titratable acidities of 1.2 and 1.1 per cent N acid respectively; both contained similar amounts of the same lot of horse blood; yet the outer zones in Figs. 1 and 2 are hardly visible. They were more readily seen in the agar plate itself. In this instance the difference between the two agar plates was probably due to the difference of 6 days in the age of the blood used. The blood was kept without apparent alteration in the refrigerator in the interval, but had been drawn only 24 hours when used for the plate shown in Figs. 1 and 2. Little or no change was produced in the appearance of the zones of this streptococcus by refrigeration.

The broad outer zones produced by streptococcus A-18 (cow) β can hardly be considered due to acids produced since they were even more conspicuous in a medium of fermented veal infusion agar and washed horse blood corpuscles (Plate 4, Fig. 6). Streptococcus B-18 (cow) β, on the other hand, did not produce similar zones in this medium.

In the descriptions given above quotations and citations from other authors have been avoided. For these the reader is referred to Table III on The Use of Blood Agar and especially to the work of the following authors: Schottmüller (1903); Rosenow (1904); Kerner (1905); Natvig (1905); Ruediger (1906); Boxer (1906); Mandelbaum (1907–08); Konrád (1909); Sigwart (1909); Thalmann (1910); Saito (1912); Davis and Rosenow (1912); Davis (1912); Ruediger (1912); Rosenow (1914); Baerthlein (1914); Smith and Brown (1915); Krumwiede and Valentine (1915); Davis (1916).

INFLUENCE OF THE AMOUNT OF BLOOD AND OF AGAR USED.

As stated above, the use of 12 cc. of standard meat infusion agar plus 5 per cent of blood was found to give the maximum amount of differentiation. Schottmüller (1903) used 2 cc. of blood to 5 cc. of agar. There is nothing in his paper to indicate what size the Petri dish he used may have been. The size most commonly used is 8.5 to 9.0 cm. in diameter. In such a plate 7 cc. of blood agar would make a layer only 1.2 mm. thick and because of the usual unevenness of the bottoms of these plates this amount of medium does not make a satisfactory plate. Furthermore, a layer so thin dries out very quickly and is not a favorable medium. From the descriptions of Schottmüller it appears more likely that he used a smaller Petri dish (7 to 7.5 cm. in diameter) which is also on the market. 7 cc. of blood agar in this dish would make a layer 1.7 to 1.8 mm. thick. The layer of agar in Schottmüller's plates was therefore none too thick, and it is probably for this reason that he obtained good results in spite of the use of such a large proportion of blood. Unfortunately most authors have not given sufficient data to enable one to determine how thick the layer of agar in their plates may have been. Many of them have not even stated the proportion of blood to agar used (see Table III on The Use of Blood Agar). That the thickness of the layer of medium is important is obvious from the following considerations: If a colony is able to produce a clear hemolyzed zone 2 mm. in diameter, this zone will barely penetrate the layer of medium 2 mm. thick provided the colony is situated in the center of the layer of agar. The zone of a surface colony, however, is hemispherical and if it has a radius of less than the thickness of the layer of agar it will not appear clear because of the underlying non-hemolyzed corpuscles. Furthermore it is only the outer portion of the zone of a surface colony which is visible because the central portion is obscured by the overgrowth of the colony. If the zone of a deep colony has a diameter less than the thickness of the layer of agar, it will also appear clouded because it fails to penetrate the layer of medium. This source of error is largely overcome by examining the colonies and

zones microscopically because by focusing down onto the deep colony there is obtained an optical section at the level of the colony. Few authors have done this.

In Plate 6, Fig. 3, is shown a 48 hour horse blood agar plate culture of streptococcus A-STβ. This plate contained 5 per cent of horse blood in agar forming a layer 2 mm. thick. The clear colorless zones of the deep colonies are well above 2 mm. in diameter and are shown in the figure in marked contrast with the surrounding medium. In Fig. 6 is shown the same organism in the same agar containing only 2.5 per cent of the same horse blood, but in a layer of medium 4 mm. thick. Note the rather clouded appearance of the zones and the poorer contrast between zones and surrounding medium. In Fig. 7 is shown the same organism in the same agar containing 5 per cent of the same horse blood in a layer of medium 4 mm. thick. Note that only one of the deep colonies shown has a fairly clear zone because of its advantageous position in the center of the layer of blood agar; all other colonies located nearer one surface or the other have quite clouded zones. In Fig. 5 is shown an attempt to photograph a culture of the same dilution of the same organism in the same agar containing 25 per cent of the same horse blood forming a layer of medium 2.6 mm. thick. (Schottmüller used 28.5 per cent of blood.) This plate contained about the same number of colonies as did the plates shown in Figs. 3, 6, and 7 and each colony was surrounded by a laked zone of the same size as those in the other plates, but the zones were quite red with the released hemoglobin. The plate was therefore quite opaque to the photographic plate. Naturally these remarks regarding the most hemolytic organisms, the *beta* type streptococci, apply even more forcibly to those of the *alpha* type. Many of the details which have been described for the zones of the latter would have been completely concealed by the use of a thicker layer of medium or by a larger proportion of blood, and would have been lacking altogether in plates containing much smaller amounts of blood. A comparison of Figs. 4 and 5 with 6 and 7 of Plate 12 shows the appearance of a culture of streptococcus A-BLα in the same agar containing 5 and 10 per cent respectively of the same horse blood. Both plates were subjected to the same treatment, namely 48 hours incubation (Figs. 4 and 6) followed by 48 hours refrigeration (Figs. 5 and 7).

About 5 per cent of blood in a layer of agar about 2 mm. thick appears to be the optimum.

A review of the literature indicates that this conclusion is justified. Schottmüller (1903) using blood agar (2:5) makes no mention of the finer characters of the zones produced by streptococci. He states that none except *Streptococcus longus pathogenes* produced macroscopically visible hemolysis. I think he would undoubtedly have included some of the *alpha prime* zone producers under *Streptococcus longus pathogenes*. Rieke (1904) tried two extremes of blood agar mixtures, 2: 5 parts and 5 drops of blood to 5 cc. of agar. On the former his *Streptococcus mitior* strains produced no hemolysis, while on the latter they all produced hemolysis but only one of them produced any perceptible greenish tint. Natvig (1905) found blood agar 2: 5 of less differential value than 12 drops of blood to 10 cc. of agar. His observations were quite accurate for surface colonies. To continue to point out the results obtained by each author who has stated how much blood and agar he used would be a useless repetition of statements to be found in Table III on The Use of Blood Agar. Attention is called to the work of the following authors as reviewed in that table: Schottmüller (1903); Rosenow (1904); Rieke (1904); Natvig (1905); Boxer (1906); Silberstrom (1906); Mandelbaum (1907-08); Zangemeister (1909); Hoessli (1910); Thalmann (1910); LeBlanc (1912); Saito (1912); Rosenow (1914); Kligler (1915); Holman (1916).

INFLUENCE OF THE AGE AND THE KIND OF BLOOD USED.

Until now our attention has been confined almost wholly to the appearances produced by streptococci and pneumococci in blood agar made with defibrinated horse blood. Although a good number of workers have used horse blood for this purpose, the majority have not. In this laboratory it was available, freshly drawn once or twice each week. The blood used, however, was often 2 weeks old, and with the exception of the difference noted above in the case of streptococcus A-18 (cow)β no difference in the results obtained by using blood 24 hours old or after being kept in the refrigerator for as long as 2 weeks has been noticed. Most authors who have made any statement on the subject, have used rabbit or human blood. It was important, therefore, to make parallel series of experiments using each of these kinds of blood. In the plates are shown comparable figures of horse, rabbit, and human blood agar plates subjected to alternate periods of incubation and refrigeration. A description of each of the figures would be tedious. Remarks will be confined to a general statement and a description of any marked variations in appearance that may have resulted from the use of other than horse blood.

In general it may be said that, excluding the *alpha prime* type, there was never any danger of confusion of the other three types (*alpha, beta, and gamma*) by the use of horse, rabbit, or human blood agar provided the criteria above described were searched for. The differences in appearance due to the use of different kinds of blood were only quantitative. The exception in the case of the two *alpha prime* strains (B-6α' and F-2α') has been mentioned above (page 17). In rabbit blood agar both these strains produced the *alpha* appearance (compare Plate 10, Figs. 3 and 4 with Plate 11, Figs. 3 and 4). As already mentioned, this does not mean that the *alpha prime* appearance is never produced in rabbit blood agar, but it occurs less frequently than in horse blood agar. It has also been seen in cat blood agar. In human blood agar streptococci B-6α' and F-2α' produced zones which after 24 hours incubation had the typical

alpha prime appearance, and in fact after 48 hours incubation (Plate 10, Fig. 5 and Plate 11, Fig. 5) resembled the *alpha prime* type rather than the typical *alpha* type, though under the microscope a slight, almost doubtful greenish discoloration of the corpuscles next to the deep colony could be seen. There was hardly any change during refrigeration (Plate 10, Fig. 6 and Plate 11, Fig. 6) and the zones became larger (especially those of F-2α') during reincubation. The appearance of these two strains in human blood agar, therefore, resembled that in horse blood agar more closely than that in rabbit blood agar. The most notable difference between the human blood agar plates and the horse blood agar plates was an indefinite milky clouding of the hemolyzed zones in the human blood agar. This is apparent in the photographs. The point of greatest interest in connection with these two strains is that relying upon the usual macroscopic observation either of these strains might be called "*hemolytic*" in horse or human blood agar and "*non-hemolytic*" or "*green-producing*" in rabbit blood agar. This shows to how much confusion the use of different kinds of blood has led in the hands of different workers or of the same worker at different times, and how important it is for an author to state what kind of blood he has used.

Plate 15 shows some striking quantitative differences in the appearance of streptococcus B-7α on plates made of the same lot of agar thoughout but with horse, rabbit, and human blood. The blood was 24 hours old in each case. There were about the same number of colonies in each plate. All three plates were subjected to the same alternate periods of incubation and refrigeration. After 48 hours incubation (Figs. 1, 5, and 9), the three plates were almost identical in appearance, and after 48 hours refrigeration the only notable difference was in the amount of hemolysis, which was greatest in the horse blood plate (Fig. 2) and least in the human blood plate (Fig. 10), while in the latter plate the greenish discoloration was most pronounced. The human blood plate was practically uninfluenced by further incubation and refrigeration (Fig. 11), differing in this respect from both the others. The formation of distinct quadruple zones in the horse blood agar plate (Fig. 4) has been described (page 11). Reincubation of the rabbit blood agar plate resulted in a phenomenon not described above. The discoloration be-

came a brownish green and appeared to extend outward from the colony almost obliterating the ring of partial hemolysis which had appeared during refrigeration (Fig. 7). Microscopic examination showed that the brownish discoloration visible within the hemolyzed zone was due not to discoloration of the corpuscles but rather to a diffuse brownish stain or pigment coloring and clouding the medium. The photomicrograph (Fig. 7a) fails to reveal this because the different parts of the zone were alike opaque to the photographic plate whether they were brownish or greenish or contained corpuscles or not. During a second refrigeration (Figs. 8 and 8a) another ring of hemolysis appeared and to the eye the zones were quadruple though in the photograph they appear double.

Streptococcus A-BLα (Plate 12) behaved much like B-7α towards the different kinds of blood used in that there was much less hemolysis in the rabbit and human blood plates. Greenish discoloration and fixation of corpuscles were much more evident in the rabbit blood plate than in the other two plates, but the brownish pigment did not appear during the second incubation as it did in the case of B-7α. After a second period of refrigeration the zones were distinctly quadruple, and after a third period of incubation and refrigeration six rings could be seen though the outer two were quite faint.

Streptococcus D-4α (Plate 14) much like A-BLα in horse blood agar but differing from both A-BLα and B-7α in fermentation reactions was startlingly different from these two in its appearance in rabbit and human blood agar. The appearance in rabbit blood agar (Figs. 4 to 6) could almost be called the *alpha prime* type. Doubtless many authors seeing the growth of this strain macroscopically in rabbit blood agar (Fig. 4) after 48 hours incubation would call it "*hemolytic*" and would name it *Streptococcus longus pathogenes* rather than *Streptococcus viridans*. The surface colonies especially resembled those of ordinary hemolytic streptococci. Even after only 24 hours incubation the appearance was the same except that the zones were smaller. The small collection of slightly greenish corpuscles visible microscopically close to the deep colony and the increase in hemolysis during refrigeration and reincubation would serve to indicate that this strain did not belong to those producing the *beta* appearance, however.

Streptococcus E-4α (Plate 17) was essentially like B-7α in blood agar made of all three kinds of blood. Its fermentation reactions were different from those of B-7α which it resembled in blood agar and were the same as those of D-4α which it did not resemble in rabbit blood agar.

The plates described of the above four strains (A-BLα, B-7α, D-4α, and E-4α) with each kind of blood, were made at the same time, with the same samples of blood, each 24 hours old, the same agar, and were subjected to the same periods of incubation and refrigeration. They are strictly comparable.

At a different time the strain *Streptococcus longissimus* D-AD$8_{2\alpha}$ (Plate 18) was studied in blood agar made with horse, rabbit, and human blood. The growth in horse blood agar has been described (page 21). In this plate are given photomicrographs of both deep and surface colonies from each agar plate. The appearances differed conspicuously from those of the above four strains described. The large collection of greenish corpuscles about the colonies in the rabbit blood plates (Figs. 3 and 4) should be especially noted as contrasted with the few in the horse blood plate (Figs. 1 and 2); also the very clear and sharply defined ring of hemolysis about the deep colonies in the rabbit blood plate after refrigeration (Figs. 4 and 4a); the lack of hemolysis about the surface colonies as compared with the deep colonies; in both the horse blood and rabbit blood plates after refrigeration (Figs. 2 and 4) and in the human blood plate after refrigeration (Fig. 6) the marked hemolysis about the surface colonies as compared with that about the deep colonies in the same plate and as compared with the surface colonies on horse and rabbit blood agar.

The strain of *Streptococcus mucosus* (Brig.-IIaα, Plate 20) in rabbit blood agar produced smaller zones of hemolysis than in horse blood agar, but was otherwise the same in appearance.

On the other hand, Pneumococcus Cole I 109 (Plate 22) produced wider and clearer but less sharply bounded zones in rabbit blood agar (Figs. 1, 2, and 3) than in horse blood agar (Plate 21, Figs. 1, 2, 3, and 4).

In human blood agar the *Streptococcus mucosus* and Pneumococc Cole I 109 and Cole II 45 looked very much alike, all producing littl

hemolysis but broad collections of greenish corpuscles about the deep colonies (Plate 23, Figs. 5 and 6).

When the comparison of the appearances produced by different streptococci and the pneumococci in blood agar made with different kinds of blood was undertaken, it was hoped to find either that the appearances were the same or nearly the same for all kinds of blood or that the differences in appearance were quite uniform in direction; *i.e.*, that there was more or less hemolysis in one kind of blood agar and more or less greenish discoloration in another. With organisms of the *alpha* type such was not the case, however, at least with reference to horse and rabbit blood. Some strains produced markedly different appearances in blood agar made with different kinds of blood and others did not. The differences noticed in the case of some strains were quite opposite in direction from those noted in the case of other strains and the differences were not apparently correlated with other characters of the strain in so far as could be determined. In human blood agar, however, there was a general tendency for both green-producing streptococci and pneumococci to produce less hemolysis and more greenish discoloration of corpuscles than in horse or rabbit blood agar.

The variations in appearance of the *beta* type zones of hemolysis produced by streptococci A-STβ (Plate 6), D-AD4β (Plate 7), and B-2b (cow)β (Plate 9) in horse, rabbit, and human blood agar were quite insignificant. The zones in horse blood agar were most sharply defined, those in rabbit blood agar had rather indefinite borders, and those in human blood agar were slightly smaller and had a slightly milky clouded appearance not due to incomplete hemolysis.

Of the bovine strains of *beta* type streptococcus B-18 (cow)β (Plate 5) produced similar appearances in horse, rabbit, and human blood agar. Streptococcus A-18(cow)β (Plate 4) in rabbit and human blood agar produced smaller "*inner*" zones of hemolysis than in horse blood agar and very faint "*outer*" partly hemolyzed translucent zones.

It may be said, therefore, that in blood agar made with the three kinds of blood used there is much less variation in the appearance of

the *beta* type zones produced by streptococci than of the *alpha* type zones. There appears to be no danger of mistaking a *beta* type streptococcus for an *alpha* type streptococcus in blood agar, but certain of the *alpha* type streptococci when grown in certain kinds of blood agar may resemble the *beta* type so closely that only a careful microscopic study of the deep colonies can be relied upon to identify them.

Streptococcus D-1γ (Plate 2) grew alike in horse, rabbit, and human blood agar, producing no apparent hemolysis or discoloration in any of them.

Table III on The Use of Blood Agar should be consulted for the work of the following authors: Natvig (1905); Silberstrom (1906); Heynemann (1908); Zangemeister (1909); Saito (1912).

INFLUENCE OF THE COMPOSITION OF THE AGAR USED.

Reaction (*Acidity*).—A number of strains producing the *alpha* and *beta* types of appearance in horse blood agar were replated under comparable conditions in standard veal infusion agar, fermented[5] veal infusion agar, and standard (0.3 per cent) Liebig extract agar, of various reactions. Media made from Liebig extract is apparently sugar-free, while those made from meat infusion always contain a small amount of fermentable sugar. It was to eliminate the possible effect of fermentable sugar that the fermented veal infusion agar was also used. Any differences in the appearances produced by growth in these three different kinds of agar will be described later, and attention will be here confined to differences in reaction of each medium. It was found that the reaction (titratable acidity) of the veal infusion agar (both fermented and unfermented) might vary from 0.4 per cent to 1.5 per cent N acid to phenolphthalein without affecting the appearances of either *alpha* or *beta* type in blood agar, and the result was the same whether hydrochloric or lactic acid was added to the agar to increase its acidity. The two extremes mentioned were considered far enough apart to include any probable reaction of standard meat infusion agar as made in all laboratories. When various amounts of acid were added to standard Liebig extract medium an unexpected difficulty was encountered in that the blood underwent almost immediate laking when added to extract agar having a titratable acidity of about 0.7 per cent N acid or above. In order to eliminate entirely the possibility of spontaneous laking of blood during incubation of the extract agar it was found necessary to keep its reaction at or below about 0.4 per cent N acid. There were no variations in the *alpha* and *beta* appearances in this medium due to reactions between 0.15 per cent and 0.4 per cent N acid.

Since the only difference between the veal infusion agar and the extract agar was in the veal infusion or beef extract used, the differ-

[5] Made from fermented bouillon prepared from a veal infusion which had been fermented by *B. coli* for 16 to 18 hours, heated, reaction adjusted, peptone added, and made into bouillon.

ence in behavior towards blood must reside in these constituents. Other work had led the author to titrate the hemolytic activity of graduated amounts of various acids added to veal infusion bouillon and to Liebig extract bouillon. The results obtained after 2 hours at 37°C. by adding 5 per cent of an undiluted suspension of washed rabbit blood corpuscles (*i.e.*, a suspension equivalent to the original volume of the blood) to two series of tubes containing the different bouillons are shown in the following table.

Veal Infusion Bouillon.

Titratable Acidity (per cent normal)	11.3	9.4	7.45	6.2	5.1	4.15	3.35	2.4	1.4
Hemolysis.....	++++	+++	+	–	–	–	–	–	–

Liebig Extract Bouillon.

Titratable Acidity (percent normal)	10.75	8.7	6.45	4.65	4.5	3.55	2.6	1.65	0.75
Hemolysis.....	++++	++++	++++	++++	++++	+++	++	+	–

The results were the same whether hydrochloric, acetic, lactic, or butyric acid was used. A veal infusion bouillon made without the addition of peptone behaved like the veal infusion bouillon containing peptone.

Walbum (1914) has shown the importance of hydrogen ion concentration for hemolysis by various hemolytic agents and in the absence of other agents the hemolytic power of acids themselves is dependent upon the hydrogen ion concentration. It is therefore probable from the results recorded in the above table that the veal infusion bouillon with a titratable acidity of 7.45 per cent N had about the same hydrogen ion concentration as the Liebig extract bouillon with a titratable acidity of 1.65 per cent N. Clark (1915) has published titration curves of infusion and extract broths showing that the "*buffer*" or "*amphoteric*" property of infusion broth is much greater than that of the extract broth. The same is probably true of infusion and extract agars.

The "*reaction*" of the media is expressed in terms of per cent normal titratable acid because it is the method in general use among bacteriologists, but it should be noted that the mere statement of authors that agar or bouillon of a certain reaction was used means nothing unless it is also stated whether the medium was made from meat infusion or extract.

Kind of Meat and Peptone Used.—All the blood agar plates so far described, unless otherwise stated, were made with veal infusion and Witte

peptone agar. Until a year or so ago veal and Witte peptone were used for all standard media in this laboratory. Since then it has been more convenient to procure beef rather than veal. At about the same time our stock of Witte peptone was nearly exhausted and the continuance of the European war made it very difficult to obtain more. A similar product, however, was being manufactured by the Digestive Ferments Company in this country. Before adopting any changes in the constituents of the media, however, it was determined to compare carefully the appearances of various strains of streptococci in blood agar made with the following substances:

(*a*) Veal infusion and Witte peptone.
(*b*) Veal infusion and Digestive Ferments Company peptone.
(*c*) Beef infusion and Witte peptone.
(*d*) Beef infusion and Digestive Ferments Company peptone.
(*e*) Liebig extract and Witte peptone.
(*f*) Liebig extract and Digestive Ferments Company peptone.

The appearances of the various *alpha* zone-producing strains were influenced to different degrees by the above alterations in media. The most constant difference was that due to the use of meat infusion agar or Liebig extract agar. For most strains the colonies in extract agar were slightly smaller than in meat infusion agar. In the case of streptococci A-BLα, B-7α (compare Plate 16, Figs. 1 and 3), and E-4α (compare Plate 17, Figs. 5 and 7) there was less greenish discoloration and a smaller collection of fixed corpuscles next to the colony in extract agar than in infusion agar. This may result in a more conspicuous zone of hemolysis and was especially noticeable in the case of streptococcus B-7α (Plate 16, Figs. 3 and 4). *In no case was the difference in appearance in these various media great enough to cause confusion between the alpha and the beta types provided deep colonies were examined microscopically.*

With the pneumococci the differences were more conspicuous, and were different for each of the two strains studied. In Liebig extract and Witte peptone agar the colonies of pneumococcus Cole I 109 were small and brownish green, surrounded by small collections of brownish green corpuscles and small indefinite partly hemolyzed zones; the medium surrounding these zones was also somewhat lighter. This plate was not photographed but would have appeared just about like

Plate 23, Fig. 3. By comparison with the photograph of the veal infusion blood agar plate (Plate 23, Figs. 1 and 2) there is seen to be much less hemolysis and an obscuring of the colonies by the brownish green discoloration. With pneumococcus Cole II 45 the appearance in extract agar was almost the reverse of that described for Cole I 109. In Liebig extract Witte peptone agar (Plate 24, Figs. 5 and 6) it produced more hemolysis, appearing with practically clear colorless zones easily mistaken macroscopically for zones of the *beta* type. Microscopically, however, the edges of the deep colony were seen to be obscured by a thin collection of brownish green corpuscles (compare with Figs. 1 and 2).

There were no apparent differences in the appearances of the *alpha* type streptococci in extract media made with the two kinds of peptone.

In the case of the pneumococci the appearances in extract agar were noticeably influenced by the use of Digestive Ferments Company peptone instead of Witte peptone. Photographically the appearance of the Digestive Ferments Company peptone extract agar plate (Plate 23, Figs. 3 and 4) of pneumococcus Cole I 109 is the same as was the Witte peptone plate, but to the eye the discoloration of the former plate was quite green and that of the latter brownish green. The difference in color was more conspicuous in the plates of pneumococcus Cole II 45; in the Digestive Ferments Company peptone plate (Plate 24, Figs. 7 and 8) not only was the discoloration vividly green but the collection of corpuscles was so broad and thick as to conceal the deep colony itself, making it appear much larger than those in Witte peptone agar (compare Figs. 5a and 7a).

There were no differences in appearance of any of the *alpha* type zone producers (streptococci or pneumococci) due simply to the use of veal or beef infusion agar respectively except in the case of pneumococcus Cole I 109 which produced a broader collection of greenish corpuscles about the deep colony and less hemolysis in beef infusion agar than in veal infusion agar (compare Figs. 1 and 2 of Plate 21 with Figs. 1 and 2 of Plate 23). The medium shown in Plate 23 was made with Witte peptone too but other experiments indicated that it was the difference in the infusions rather than in the peptones that caused the differences between the two plates.

In either veal or beef infusion agar, however, there were differences produced by the use of Witte or Digestive Ferments Company peptone. Streptococcus Brig.-IIaα and pneumococcus Cole I 109 showed no variation with respect to the two kinds of peptone in veal or beef infusion agar. Streptococcus B-7α showed slight variation in this respect. In meat infusion agar made with Digestive Ferments Company peptone the greenish discoloration was very slightly more vivid. Streptococcus A-BLα showed more greenish discoloration when grown in agar containing Digestive Ferments Company peptone than in that containing Witte peptone. Pneumococcus Cole II 45 (compare Plate 24, Figs. 1 and 3) produced in Digestive Ferments Company peptone veal or beef agar a much heavier and more vivid green collection of corpuscles next to the deep colonies than in Witte peptone agar.

Of the streptococci producing the *beta* type of hemolysis A-STβ showed no variation with the use of veal or beef infusion or of either kind of peptone. In Liebig extract agar, however, hemolysis was much more extensive and occurred much more readily than in meat infusion agar as a comparison of Figs. 3 and 4 of Plate 6 will show. The photograph in Fig. 4 (extract agar) was taken after 24 hours incubation and yet the hemolysis is more extensive than that in Fig. 3 (veal infusion agar) taken after 48 hours incubation. In this length of time the zones in the extract agar plate shown in Fig. 4 were confluent over a large portion of the plate.

For streptococci C-64β and D-AD4β (both of which are strains of typical *Streptococcus pyogenes* in the most limited sense, *i.e.* with regard to hemolysis, fermentation reactions, pathogenicity, morphology, growth on agar slants, and as distinguished from *Streptococcus epidemicus*) the results in the various kinds of agar were the same as for streptococcus A-STβ just described.

From the above strains *Streptococcus epidemicus* (Davis) differs noticeably. Briefly the results were as follows: The appearances in Liebig extract agar whether made with Witte peptone or Digestive Ferments Company peptone were alike. There were clear colorless zones of hemolysis with no trace of discoloration about deep or surface colonies by transmitted or reflected light. In veal infusion agar (Plate 9, Figs. 3 and 4) there was clear hemolysis without discolora-

tion about the deep colonies, but the surface colonies in agar made with different kinds of peptone showed conspicuous differences. On Digestive Ferments Company peptone veal infusion agar (Fig. 4) the top surface colonies were a deep opaque green color by transmitted light and surrounded by narrow clouded rings of hemolysis. If on this medium surface colonies only were observed macroscopically the organism might readily be called *Streptococcus viridans*. When viewed microscopically (Fig. 4a), however, the greenish discoloration was seen to be due to a diffuse pigment obscuring the edges of the colony and extending outward into the zone of hemolysis, and not due to discolored fixed corpuscles as seen in zones of the *alpha* type. On the Witte peptone veal infusion agar the surface colonies were translucent with well defined edges and without discoloration except for yellowish centers which appeared less opaque to the eye than they do in the photograph (Fig. 3). The yellow nucleus appears as a dark patch in the center of the colony shown in Fig. 3a, but the differences between Figs. 3a and 4a are very conspicuous. No changes occurred during refrigeration. The appearances in beef infusion agar were the same as in veal infusion agar.

The differences in appearance of the top surface colonies of the *Streptococcus pyogenes* and *Streptococcus epidemicus* on meat infusion Digestive Ferments Company peptone blood agar are shown macroscopically in Figs. 5, 6, and 7 of Plate 8. Streptococci D-AD4β and C-64β are strains of *Streptococcus pyogenes* while streptococcus X-38β is a strain of *Streptococcus epidemicus* (from the Baltimore epidemic, 1912). The three plates were of the same medium, inoculated, incubated, and photographed simultaneously.[6]

A *bovine streptococcus* of the *beta* type of hemolysis (A-18 (cow)β) showed no variation in appearance due to the use of veal or beef or of Witte or Digestive Ferments Company peptone. In Liebig extract blood agar only the small clear central hemolyzed zones appeared during 24 hours incubation, but during the next 24 hours at incubation temperature the broad outer zones not only appeared but were so nearly completely hemolyzed as almost to obliterate the inner zones.

[6] Note that the large thin patches of growth are bottom surface colonies, while the dark round colonies are on the top surface.

Streptococcus saprophyticus (D-1γ) produced neither hemolysis nor discoloration in any of the agars used except that around some of the colonies in Liebig extract blood agar there was a very faint, hardly visible, trace of indefinite lightening of the medium which will be mentioned again later.

Although our present object is satisfied by having shown that important differences in appearance may result from the use of different kinds of commercial peptone, in view of the experiments about to be described, it was important to determine whether or not the presence of fermentable sugar was responsible for the different results obtained by the use of Witte or Digestive Ferments Company peptone. Solutions of neither peptone gave any reaction for sugar with Fehling's or Benedict's reagents. No recognizable trace of acid or gas was formed by the growth of *Bacillus coli* and various actively fermenting streptococci in either peptone solution.

Influence of Dextrose in Agar.—The point of departure for this series of experiments was the paper by Ruediger (1906) on The Cause of Green Coloration of Bacterial Colonies in Blood Agar. Ruediger studied a number of strains of hemolytic *Streptococcus pyogenes* and of green-producing pneumococci in blood agar with and without the addition of glucose. He says:

> *" * * a large number of typical S. pyogenes cultures produce no hemolysis in plates made of glucose blood agar, although they produce very extensive hemolysis made of plain agar and blood. If these glucose blood agar plates are allowed to remain in the incubator for 36 to 48 hours, the streptococcus colonies become distinctly green and are surrounded by a green halo."*

We have made comparative studies of several strains of *beta* hemolytic streptococci including *Streptococcus pyogenes*, *Streptococcus epidemicus*, and *Streptococcus alactosus* (Smith and Brown) in both veal infusion blood agar and Liebig extract blood agar with and without the addition of 1 per cent dextrose. The appearance of streptococci A-STβ, B-2b (cow)β, and D-AD4β, in veal infusion blood agar has already been described. *Streptococcus pyogenes* C-64β was in every respect like D-AD4β and *Streptococcus epidemicus* X-32β and X-38β were just like B-2b (cow)β. All these strains produced the same appearance in veal infusion blood agar and in Liebig extract blood agar except that in the latter hemolysis occurred a little

more readily and a little more extensively than in the former. Clear colorless zones were produced by all these strains in less than 24 hours in both kinds of agar. In the case of streptococcus A-STβ the effect of the addition of 1 per cent of dextrose to the infusion and extract blood agar was the same (Plate 26, Figs. 7 and 8). As observed after 24 hours incubation there were distinct colorless hemolyzed zones about the deep colonies, completely hemolyzed and free from corpuscles where penetrating the agar or as viewed microscopically in optical section. Instead of being 2.5 or 3.0 mm. in diameter, however, these zones were only 1.3 mm. in diameter. The surface colonies where well scattered apart were without hemolysis, but where crowded together were surrounded by small partly hemolyzed rings 1.25 mm. in diameter. By transmitted light the surface colonies could be imagined to have a slightly greenish tint. After 48 hours incubation the zones of the deep colonies were slightly larger and microscopically there was visible a slight collection of brownish pigment (not corpuscles) next to the colony. Beneath the area of crowded surface colonies there was generalized partial hemolysis and a brownish discoloration obliterating the individual zones noted after 24 hours incubation. The plates had a diffuse brownish red tone more intense in the extract agar plate, and the greenish tint was less apparent than after 24 hours incubation. As distinguished from these plates streptococci C-64β and X-32β (also others not photographed) produced appearances in veal infusion dextrose blood agar similar to that just described (Plate 26, Figs. 1 and 4), but in Liebig extract dextrose blood agar (Figs. 2 and 5) produced no hemolysis except a slight indefinite diffuse lightening of the medium in the neighborhood of the colonies. In the photographs of these plates most of the colonies are not visible but are present in about the same numbers as in the plate to which dextrose was not added. To the eye by transmitted light after 24 hours incubation there was no discoloration in the extract dextrose blood agar plate of streptococcus C-64β (*pyogenes*), but in a similar plate of streptococcus X-32β (*epidemicus*) the patches of bottom surface growth and the top surface colonies where close together had a slight greenish tint. After 48 hours incubation the plates of both these streptococci (and others like them) were of a brownish red color and the areas of greenish

discoloration were less apparent. In neither kind of dextrose blood agar were there distinctly green colonies surrounded by green halos as described by Ruediger.

Ruediger, however, used fresh defibrinated rabbit blood rather than horse blood which I used in the experiments just described. He also used a sugar-free agar containing Witte peptone, salt, and agar, but no meat infusion or extract, with and without the addition of 1 per cent of dextrose. He says that using this medium *Streptococcus pyogenes* "*forms green colonies in plates of glucose blood agar*," as did also the pneumococci. In the same agar without dextrose, *Streptococcus pyogenes* produced hemolysis while the pneumococci did not "*produce more than a faint trace of green*," due, he thinks, to a trace of sugar in the blood. Ruediger concludes that:

"*The green coloration of bacterial colonies in blood agar plates is dependent upon the production of acid and the action of this acid (probably lactic acid) on the red corpuscles.*"

The above experiments have been repeated with Ruediger's agar and rabbit blood, without dextrose, with 0.1 per cent of dextrose, and with 1 per cent of dextrose. Streptococcus D-AD4β (*pyogenes*) (Plate 8) produced clear colorless zones in the sugar-free agar (Fig. 2), like those in veal infusion agar (Fig. 1) except that those in Ruediger's agar were a little smaller, due probably to poorer growth since the colonies themselves were also smaller. In the 0.1 per cent dextrose agar (Fig. 3) the zones were without discoloration but incompletely hemolyzed; microscopically (Fig. 3a) there was seen a small amount of reddish brown (not green) pigment close to the deep colony. In 1 per cent dextrose agar (Fig. 4) hemolysis was still less apparent; after 24 hours incubation there was present merely a small (1 mm. in diameter) partly hemolyzed zone about the deep colony but after 48 hours incubation there was present outside of this a second broad indefinite zone of less hemolysis, and between the two a red ring of hemoglobin. Microscopically (Fig. 4a) there could be seen close to the colony a collection of reddish brown pigment. There was no greenish discoloration.

Streptococcus A-STβ (*S. alactosus* β) produced clear colorless zones in the sugar-free agar (Plate 6, Fig. 8, clouded by scratches on

the Petri dish). In the 0.1 per cent dextrose agar (Fig. 9) the deep colonies had small partly hemolyzed zones surrounded, after 48 hours incubation, by broad indefinite slightly hemolyzed areas. Microscopically next to the deep colony (Fig. 9a) there was seen a collection of brownish pigment. The surface colonies were a deep chocolate brown color by either reflected or transmitted light. In 1 per cent dextrose agar (Fig. 10) the entire plate was a deep chocolate brown color and the colonies were obscured by the discoloration.

Streptococcus B-2b (cow)β (*epidemicus*) also produced clear colorless zones in sugar-free agar (Plate 9, Fig. 5). In 0.1 per cent dextrose agar there was produced a smaller hemolyzed zone with a small amount of brownish pigment visible microscopically close to the deep colony (not photographed). In 1 per cent dextrose agar (Figs. 6 and 6a) there was still less hemolysis but broad areas of indefinite faint hemolysis about the smaller zones. Surface colonies were opaque and brownish.

The above three varieties of hemolytic streptococci, therefore, produced abundant brownish pigment and a diminished amount of hemolysis in dextrose blood agar but little or no greenish discoloration. The faint broad outer areas of slight hemolysis were probably due to acids formed as I will presently show.

The appearance of streptococcus B–6α' (Plate 10, Fig. 7) in veal infusion horse blood agar was scarcely influenced by the addition of 1 per cent of dextrose. The colonies were slightly larger and brownish green discoloration about the colonies slightly increased, but hemolysis was not reduced.

Having stated that in plain blood agar the pneumococci produce green coloration, Ruediger offers the following explanation for the failure of *Streptococcus pyogenes* to do so:

"(*1*) * * *these organisms probably do not readily ferment the muscle sugar which is found in plain agar, and (2) they produce such rapid hemolysis that the green would not be detected, because one can see no green coloration after the red corpuscles have been completely hemolyzed.*"

If this were true an actively fermenting organism which does not produce hemolysis should produce greenish discoloration in dextrose agar. Such an organism is streptococcus D-1γ (*saprophyticus*) which

has been described as producing neither hemolysis nor discoloration in meat infusion blood agar. The addition of 1 per cent of dextrose to veal infusion blood agar resulted after 48 hours incubation in a general brownish discoloration of the entire plate. The colonies were brownish by transmitted light and when viewed microscopically the edges of the deep colonies were seen to be bordered by a brownish pigment. There was no hemolysis nor greenish discoloration (Plate 3, Figs. 1 and 1a). I have already mentioned the fact that in Liebig extract blood agar this streptococcus produced a very slight indefinite lightening about some of the colonies. In extract blood agar containing 1 per cent of dextrose (Fig. 2) the light areas about the colonies were distinctly visible and were in fact indefinitely bounded zones of partial hemolysis more apparent macroscopically than microscopically (Fig. 2a). The edges of the deep colony were obscured by a small collection of brownish pigment. There was no trace of greenish discoloration. With this streptococcus the results in Ruediger's rabbit blood agar with and without dextrose were qualitatively the same as in Liebig extract agar. In the sugar-free agar there was seen (Fig. 3) a very slight indefinite hemolysis; in the same agar plus 0.1 per cent dextrose (Fig. 4) there was more hemolysis; while if 1 per cent of dextrose was added (Fig. 5) there was more brownish discoloration about the colony. In Fig. 5a the appearance is not unlike that of *alpha* zone-producing streptococci, but to the eye the dark ring about the colony was seen to be composed not of green corpuscles but of a deep red or brownish red pigment in the agar and not confined to the corpuscles. There was no greenish discoloration.

From the above experiments it is evident that the green coloration of bacterial colonies in blood agar plates is not dependent wholly upon the production of acid and the action of this acid on the red corpuscles. It may be that the presence of a certain amount of fermentable sugar favors the production of green coloration by those organisms which are capable of producing it, but it does not follow that the acids formed produce the green coloration. I tried to test this directly by adding various amounts of various acids (lactic, acetic, butyric, hydrochloric) to various kinds of bouillon and agar containing various kinds of blood. Laking or brownish discoloration

was invariably produced but never any greenish discoloration. Anthony (1909) adopts Ruediger's explanation and says: "*In our laboratory all the hemolyzing strains and also the green ones, when tested and retested in glucose agar blood plates, gave green colonies.*" I can not account for the results of Ruediger and of Anthony unless their hemolyzing strains were not of the true *beta* type.

Inasmuch as hemolysis is reduced or inhibited by the presence of fermentable sugar in the blood agar, it is evident that the *beta* type of hemolysis is not produced by acids formed, a fact recognized by Sachs (1909) and by Braun (1912). It is altogether probable that the partial hemolysis produced by streptococcus D-1γ in Liebig extract agar and in Ruediger's agar (especially in the presence of dextrose) was due to the acids formed by the colonies. It has been shown above (page 41) that extract bouillon is a very poor buffer solution as compared to meat infusion bouillon. Similar experiments have shown that a peptone and salt solution (such as is the basis of Ruediger's agar) is even a poorer buffer solution than extract bouillon. In extract agar or Ruediger's agar, therefore, a slight amount of acid formation would raise the hydrogen ion concentration in the neighborhood of the colonies sufficiently to cause some hemolysis. In meat infusion agar, on the other hand, the hydrogen ion concentration is kept down by the buffer effect of the medium and so acid hemolysis does not readily occur. Probably the outer, indefinite, broad, slightly hemolyzed zones formed by the other streptococci (*e.g.*, A-STβ, D-AD4β, B-2b (cow)β, etc.) in extract agar and Ruediger's agar containing dextrose are to be similarly explained. The diminution in the size of the central zones produced by the *beta* streptococci in meat infusion dextrose blood agar appears to be explained by the partial inhibition of hemolysin production in sugar-containing media. In Ruediger's blood agar plus 0.1 per cent of dextrose can be seen both the diminished inner clearer zone produced by hemolysin and the broad outer indefinite clouded zone of acid hemolysis. In Ruediger's blood agar or extract blood agar plus 1 per cent of dextrose only acid hemolysis is present. The reason for the partial or total inhibition of hemolysis in media containing sufficient fermentable sugar is yet to be discussed.

Ruediger (1906) simply noted it. Sachs (1909) states that dextrose bouillon diminished hemolysis only by checking the growth of the culture earlier because of a development of acid. Sachs' explanation can hardly be true of growth in blood agar since in dextrose blood agar the colonies become larger than in sugar-free blood agar, and yet in the former there is little or no hemolysis while in the latter large clear zones are produced. Kuhn (1912) concludes that the hemolysis in dextrose blood agar or in dextrose blood bouillon is not reduced by the acids formed but that in the presence of fermentable sugar the organisms adopt a saprophytic or "*herbivorous metabolism*," while in the absence of sugar they adopt a parasitic or "*carnivorous metabolism*" and hence attack the blood cells. Lyall (1914) found hemolysis in fluid media to be inhibited by the presence of dextrose, even in the presence of calcium carbonate, and by titration of amounts of acids produced in media without calcium carbonate found that the inhibition did not appear to be definitely associated with the amount of acid produced. There are various facts which indicate that streptolysin is a product of the protein metabolism rather than the carbohydrate metabolism of streptococci, as for example the facts that it passes through only the coarsest filters and with difficulty (M'Leod, 1911), it is inactivated by moderate degrees of heat (Besredka, 1901; Ruediger, 1903, 1906, and 1907; M'Leod, 1911; Braun, 1912; von Hellens, 1913), it is non-dialyzable (Besredka, 1901; Ruediger, 1903, 1906, and 1907), and is destroyed by peptic digestion (Ruediger, 1903, 1906, and 1907).

Lyall (1914) also found the inhibition of hemolysis by streptococci in fluid media apparent within 30 minutes after dextrose was added, and I have found washed streptococci suspended in fermented veal infusion bouillon much more hemolytic (during 2 hours incubation) than when suspended in unfermented veal bouillon or in fermented bouillon plus 1 per cent of dextrose. The fermented bouillon was certainly the least favorable for the growth of the streptococci during the period of incubation and the time was hardly sufficient for the development of a high degree of acidity. Apparently, therefore, in the presence of fermentable sugar the protein metabolism of the streptococci is reduced to a minimum and for this reason the formation of streptolysin is reduced or inhibited altogether. The other possibilities which suggest themselves are that (1) the acids formed may prevent the formation of streptolysin by acting upon the growing streptococci in some way; (2) the acids may inactivate the streptolysin as it is formed; and (3) the acids may render the blood corpuscles insusceptible to hemolysis by the streptolysin. Inasmuch as the purpose of this paper is descriptive rather than explanatory, it appears unnecessary to consider this subject further at this time.

Ruediger's view, that the green coloration of bacterial colonies in blood agar is due to acids produced, was based not only on his observation that hemolytic streptococci produced green colonies in dextrose blood agar but also upon the fact that:

" * * *pneumococci which produce distinctly green colonies in ordinary plain blood agar, or glucose blood agar plates do not produce more than a faint trace of green in plates composed of sugar-free agar and 0.3 to 0.4 cc. of fresh defibrinated rabbit blood.*"

He continues:

" * * *but if 1 per cent of glucose, lactose, or inulin is added to the sugar-free agar, the cultures again produce deep green colonies in blood agar plates made with it.*"

I have studied both pneumococci and green-producing streptococci in Ruediger's sugar-free agar with and without dextrose. In Plate 25, Figs. 7 and 8, are shown photographs of pneumococcus Cole II 45 after 48 hours incubation in these two media. In sugar-free agar (Fig. 7) the deep colonies were surrounded by broad indefinitely bounded partly hemolyzed zones. The surface colonies were surrounded by scarcely any hemolysis. By transmitted light both surface and deep colonies were distinctly greenish brown. Microscopically there was seen next to the deep colony (Fig. 7a) a narrow but heavy collection of dark green corpuscles. In the same blood agar plus dextrose (Fig. 8) there was less hemolysis but more greenish brown discoloration. The result is, therefore, the same as Ruediger's except that there is more than a "*faint trace*"of green in rabbit blood sugar-free agar plates.

A green-producing streptococcus (B-7α) was observed in (1) Ruediger's rabbit blood sugar-free agar, (2) the same medium plus 0.1 per cent dextrose, and (3) plus 1.0 per cent dextrose. These plates are shown in Figs. 5, 6, and 7 of Plate 16 after 48 hours incubation. Brownish green discoloration was most intense in the 1.0 per cent dextrose agar plate and least intense in the sugar-free agar plate, but even in the latter the discoloration was quite distinct. Hemolysis was most conspicuous in the 0.1 per cent dextrose agar plate. (The dark ring at some distance from the colony in Fig. 5a is not green but is of red hemoglobin.)

Since it was suspected that the greenish discoloration in the sugar-free agar might be due to the presence of a small amount of sugar in the rabbit blood, washed corpuscles were used instead of defibrinated blood. Horse corpuscles instead of rabbit corpuscles were also tried, as it had been discovered that there was apt to be less greenish discoloration of horse blood than of rabbit or human blood. Ruediger's sugar-free agar plus washed corpuscles is such a poor medium that the streptococcus colonies in it are very small and therefore not comparable with those in media containing sugar or serum. Liebig extract agar or agar made with fermented veal infusion (*Bacillus coli* 16 to 18 hours) supports growth much better and is also sugar-free. Several strains of green-producing streptococci were observed growing in extract agar and in fermented infusion agar plus washed horse corpuscles with and without the addition of dextrose. In these media without dextrose all the strains produced small partly hemolyzed zones without apparent discoloration though small collections of corpuscles could still be found next to the deep colonies. On the other hand, if dextrose was added to the medium there were found distinct collections of brownish green corpuscles about the deep colonies. The fermented infusion agar proved to be the better medium because it supported growth better in the absence of dextrose or serum and at the same time favored the production of greenish discoloration no more than did Liebig extract agar. The three media used were:

(1) Fermented veal infusion agar + washed horse corpuscles.
(2) Fermented veal infusion agar + washed horse corpuscles + 0.05 per cent dextrose.
(3) Fermented veal infusion agar + defibrinated horse blood.

There was no great amount of discoloration in any of the plates and macroscopically they appeared very much alike. In all of them after 48 hours incubation the deep colonies were surrounded by small partly hemolyzed zones which did not penetrate the agar and so were somewhat obscured by the overlying and underlying medium. Refrigeration resulted in an increase in the size of the hemolyzed zones revealing more clearly the colonies and the corpuscles about them. Microscopically the following differences were revealed: In the fer-

mented veal infusion agar plus washed corpuscles the deep colony was surrounded by very few corpuscles the color of which was doubtful because of their scarcity. In the same medium plus 0.05 per cent dextrose there was around the deep colony a moderate collection of slightly greenish corpuscles. In fermented infusion agar plus defibrinated blood there was around the deep colony a small collection of very slightly greenish corpuscles. In the medium containing dextrose the colonies were considerably larger than those in the other two media and so it is doubtful whether the greenish discoloration and larger collection of corpuscles in the former were due merely to better growth or to the more direct action of the dextrose. A very small percentage of dextrose was used because there could hardly have been more in standard unfermented infusion agar plus defibrinated blood and because a larger amount of dextrose resulted in a brownish rather than a greenish discoloration of the colonies and medium.

The experiments of Ruediger indicating that the formation of acid is the cause of the greenish discoloration by bacterial colonies in blood agar are quite inconclusive. The formation of acid alone does not result in greenish discoloration in blood agar. The failure of green-producing organisms to produce greenish discoloration in sugar-free blood agar may be due to poor growth rather than to failure to produce acid. The question of the cause of the green coloration is evidently much more complex than was supposed by Ruediger.

INFLUENCE OF ANAEROBIOSIS.

Parallel series of blood agar plates of several strains were studied under aerobic and anaerobic conditions. The strains studied were a streptococcus producing the *beta* type of hemolysis (B-2b (cow)β), a streptococcus producing the *alpha* type of appearance (A-BLα), the strain of *Streptococcus mucosus* (Brig.-IIaα), and the two strains of pneumococci (Cole I 109 and Cole II 45). The agar used was beef infusion agar made with Digestive Ferments Company peptone. Both horse and rabbit blood were used. Anaerobic conditions were secured by placing the plates in desiccators from which the oxygen was absorbed by a good amount of pyrogalic acid and sodium hydrate. The tightness of the desiccators was indicated by the existence of a partial vacuum when they were opened after 48 hours incubation. The lack of oxygen within the desiccators was indicated by the peculiar burgundy red color of the reduced blood agar, turning rapidly to the bright red color of oxyhemoglobin soon after the desiccators were opened.

Streptococcus B-2b (cow)β (*epidemicus*) grew equally well anaerobically and aerobically, and produced clear colorless hemolyzed zones of the same size and appearance under both conditions. It will be remembered, however, that aerobically in blood agar made with Digestive Ferments Company peptone the surface colonies of this strain and others of the same variety were slightly greenish by transmitted light. This was especially noticeable in rabbit blood agar. When grown anaerobically there was no trace of this greenish tint, the surface colonies being whitish and transparent by transmitted light.

Streptococcus A-BLα (*viridans*) grew rather poorly anaerobically (Plate 13, Figs. 1 to 6). The colonies in both horse and rabbit blood agar were very small after 48 hours incubation and showed no discoloration and very faint traces of hemolysis (even after refrigeration) slightly more apparent in the rabbit blood agar (Figs. 1 and 4). After reincubation aerobically the very small hemolyzed zones were slightly more conspicuous (Figs. 2 and 5), but when again refrigerated there appeared conspicuous almost clear hemolyzed zones about

the small, partly hemolyzed zones in the horse blood plate (Fig. 3) but not in the rabbit blood plate (Fig. 6). In the photomicrograph (Fig. 3a) the zone of the deep colony from the horse blood plate appears double, but there was no apparent discoloration of the collection of corpuscles next to the colony.

Streptococcus Brig.-IIaα (*mucosus*) (Plate 20) grew better anaerobically (Figs. 4, 5, and 6) than did the one just described but still did not grow so well as it did aerobically. After 48 hours incubation anaerobically the colonies in the horse blood agar plate (Fig. 4) were surrounded by small definite colorless zones which in optical section appeared clear (Fig. 4a), but when viewed macroscopically appeared clouded because they were not large enough to penetrate the layer of agar. There was no trace of discoloration or collection of corpuscles next to the colonies. The corresponding rabbit blood agar plate (not photographed) was exactly like the horse blood plate in appearance. During refrigeration the zones in the rabbit blood simply became larger but those in the horse blood plate (Fig. 5) not only became larger but in doing so left a faint thin ring of corpuscles at the place formerly marking the edges of the first zone. We have in this case what may be called a *"reversed alpha zone"* (Fig. 5a); *i.e.*, instead of there being a collection of corpuscles next to the colony there is a ring of corpuscles separated from the colony by a small zone of hemolysis. In this case the corpuscles were not perceptibly discolored, but this may have been due to their being so few in number. After another period of incubation aerobically and refrigeration another faint ring of corpuscles and another ring of hemolysis appeared (Figs. 6 and 6a) making a quintuple zone. There was a doubtful greenish discoloration of the second ring of corpuscles. The rabbit blood plate similarly incubated and refrigerated showed but one ring of corpuscles of which discoloration was doubtful.

Pneumococcus Cole I 109 (Plates 21 and 22) formed smaller colonies anaerobically than aerobically. Unlike the streptococci described it did not produce similar appearances in horse and rabbit blood agar during 48 hours incubation anaerobically (Plate 21, Fig. 5 and Plate 22, Fig. 4). In the horse blood agar there was only a faint trace of hemolysis about a few of the colonies (Plate 21, Fig. 5[1]), while in rabbit blood agar there were broad almost completely hemo-

lyzed zones (Plate 22, Figs. 4 and 4a). There was no discoloration and no collection of corpuscles next to the colony in either plate. As usual, by refrigeration hemolysis was increased (Plate 21, Fig. 6 and Plate 22, Fig. 5). Reincubation aerobically of the rabbit blood plate resulted in the formation of a dark intensely greenish pigment (not corpuscles) about the deep colonies (Plate 22, Figs. 6 and 6a) which remained unchanged during a second period of refrigeration (not photographed). After similar periods of aerobic reincubation and re-refrigeration the zones in the horse blood plate resembled those of streptococcus Brig.-IIaα just described except that there was less uniformity among the zones of the pneumococcus (Plate 21, Figs. 7 and 8).

Pneumococcus Cole II 45 (Plate 25) grew as well anaerobically as aerobically and produced essentially similar appearances in horse and rabbit blood agar. During 48 hours incubation anaerobically typical clear colorless hemolyzed zones of the *beta* type (Figs. 1 and 4) were formed. It will be remembered that in the same medium under aerobic conditions typical *alpha* type zones were produced exactly like those shown in Fig. 3 of Plate 24. Refrigeration caused no perceptible change in the plate which had been incubated anaerobically except a very slight increase in size of the zones in rabbit blood agar (Plate 25, Figs. 2 and 5). Reincubation aerobically for 24 hours caused the zones to become still larger (Figs. 3 and 6), and in the rabbit blood agar there appeared a collection of corpuscle-free greenish pigment about the deep colonies (Fig. 6^{a-1}). In horse blood agar there was a very conspicuous difference between pneumococcus Cole I 109 and pneumococcus Cole II 45 after 48 hours incubation anaerobically. The former produced hardly any perceptible hemolysis, while the latter produced clear *beta* type zones (compare Plate 21, Fig. 5 with Plate 25, Fig. 1). This experiment was repeated many times with exactly the same medium for both strains, incubating them simultaneously in the same desiccator. The result was always the same. Thinking that possibly this difference might be diagnostic for all pneumococci of types I and II (Cole), I obtained two other strains from Dr. Cole. The strains were labeled I 125 and II 37. Aerobically these grew like the ones studied but anaerobically produced no visible growth. A strange phenomenon agreeing quite well

with the above distinction did appear, however. Horse blood agar plates streaked with a loop of 24 hour bouillon culture of pneumococcus Cole II 37 showed no apparent growth anaerobically even under the low power of the microscope, but in the line of the streak there was a quite conspicuous line of partial hemolysis without discoloration (Plate 25, Fig. 9). This was also repeated several times with the same result. Pneumococcus Cole I 125 similarly treated produced no perceptible alteration of the medium. An attempt will be made later to interpret this result, but it is sufficient now to point out that anaerobically both strains of pneumococcus Cole II produced hemolysis in horse blood agar while neither strain of Cole I did so.

The general results of the anaerobic incubation of blood agar plates of the organisms described may be summarized as follows: (1) the production of greenish discoloration and the fixation of corpuscles, or, in other words, the formation of *alpha* type zones was inhibited in all cases; (2) in many cases the cultures grew less well anaerobically than aerobically, and in such cases there was correspondingly less hemolysis, but (3) in general hemolysis was not decreased by anaerobic cultivation except in the case of pneumococci type I (Cole) which were practically non-hemolytic in anaerobic horse blood agar plates.

EFFECT OF THE GROWTH OF ONE STREPTOCOCCUS ON THAT OF OTHERS.

Marmorek (1902) announced that the filtrates of bouillon cultures of streptococci of human origin would no longer serve as media for the growth of other streptococci of human origin. This has become known as *Marmorek's test* and has been used by various authors as a means of differentiating pneumococci from streptococci. Marmorek regarded this reaction as highly specific and as indicating "*l'unité des streptocoques pathogène pour l'homme.*". He regarded as a "*variant*" a strain of streptococci isolated from a scarlet fever patient, which grew slightly in streptococcus filtrates. A "*Streptococcus der Druse*" (*Streptococcus equi*) grew well in the filtrates from strains of human origin and was therefore regarded as belonging to a distinct species. In reviewing the literature it is surprising to find that Marmorek's test has not met with more skepticism.

In applying the test to various strains of streptococci there have been encountered some strains which behaved as Marmorek described and others which did not. It was found also that more accurate and more graphic results could be obtained by modifying Marmorek's technique as follows: Instead of bouillon filtrates, standard agar or 5 per cent serum agar plates are used, preferably the latter. 12 cc. of the melted agar at 45°C. are inoculated with several loops of a 24 hour culture of a given strain of streptococcus; the plate is poured, and incubated for 48 hours. At the end of this time there are no macroscopically visible colonies but the agar appears slightly clouded, due to the presence of myriads of minute colonies which can be seen microscopically throughout the medium. Growth appears to be complete in this length of time since no change is noticed if the plate is incubated longer. Inasmuch as the arrest of growth of an organism under such conditions may be due either to the accumulation of metabolic products or to the exhaustion of certain essential nutritive substances from the medium, it is well not to speak of the medium as being saturated or exhausted but to speak of it as being *metabolized* by the organism with which it is inoculated. If a metabolized standard agar, serum agar, or blood agar plate is streaked with the same strain as that by which it has been metabolized no growth will occur in the line of the streak. Parallel streaks of other strains may

also be made on the same plate and the amount of growth observed after 24 or 48 hours incubation. In Plate 28 is shown photographically the result of such an experiment with four strains of streptococci and one pneumococcus. The streaks of these different strains are numbered as follows on each serum agar plate:

(1) Pneumococcus Cole II 45
(2) Streptococcus A-BLα
(3) Streptococcus B-2b (cow) β
(4) Streptococcus D-AD4β
(5) Streptococcus A-18 (cow) β

The plates shown were of standard agar plus 5 per cent horse serum. The plates shown in Figs. 1, 2, 3, 4, and 5 were metabolized as described above for 48 hours by strains 1, 2, 3, 4, and 5 respectively, the metabolizing strain being indicated by a circle around the number in each figure (*e.g.* (1) in Fig. 1). In Fig. 6 is shown a control plate of the same medium which was incubated sterile (not metabolized) for 48 hours. After 48 hours incubation each agar plate was streaked with 24 hour bouillon cultures of each of the five strains mentioned. After another period of 48 hours incubation the results were as shown in the photographs. The relative amount of growth in each streak is indicated by the following signs placed under the numbers on each plate:

− = no visible growth, macroscopically or microscopically.
+ = slight growth, visible microscopically but hardly visible macroscopically.
++ = fair growth, visible macroscopically.
+++ = good growth.

Naturally only macroscopically visible growth is shown in the photographs. On the control plate (Fig. 6) all strains grew about equally well. In no case was there any growth of the strain by which a plate had been metabolized. If Marmorek's reasoning were applied to the result shown in Fig. 1, it would be concluded that strain 2 was a pneumococcus since it failed to grow on a medium metabolized by a pneumococcus. In Fig. 2, however, it is seen that the pneumococcus (strain 1) did grow on the medium metabolized by strain 2 which therefore would be regarded as a streptococcus. However, if

it is a streptococcus, a medium metabolized by strain 2 should not support the growth of other human streptococci, but as a matter of fact strains 3 and 4 (both streptococci of human type) did grow fairly well on this medium. On the other hand, it is seen in Fig. 3 that strain 2 did not grow on a medium metabolized by strain 3 (a human strain isolated from a cow's udder by Smith and Brown, 1915), but did grow slightly on a medium metabolized by strain 4 (Fig. 4) which is a typical *Streptococcus pyogenes hominis*, while on this latter medium the pneumococcus (strain 1) also grew as well as did streptococcus strain 2. Finally, although strain 5 is a bovine streptococcus and grew on media metabolized by any of the four strains of human origin, none of the latter showed a trace of growth on medium metabolized by the bovine strain (Fig. 5). Marmorek's strain of *Streptococcus equi* grew well in the filtrates of streptococci of human origin and is therefore regarded by him as of a different species. I suspect that none of the streptococci of human origin would have grown in a filtrate from his strain of *Streptococcus equi*, but Marmorek does not mention having tried this. The above and other experiments indicate that Marmorek's test is not specific. A pneumococcus may grow readily in standard bouillon, but if the bouillon is metabolized by *Bacillus coli*, the pneumococcus may fail to grow in it. It will often fail to grow in such a bouillon even after the reaction has been restored and a fermentable sugar added to it. Yet no one would think of regarding this as a specific reaction indicating even a remote relationship between the pneumococcus and *Bacillus coli*. Apparently the ability of a streptococcus or any other organism to grow in a medium metabolized by another organism is dependent wholly upon the ability of the former to tolerate the metabolic products of the latter or to utilize certain nutritive substances not utilized by the latter. This is well illustrated by the general characteristics of streptococcus A-18 (cow)β (streak 5 in Figs. 1 to 6 of Plate 28). This strain was more vegetative than the other four strains; it produced more acid in sugar media, indicating a higher acid tolerance; it grew more abundantly in all standard media; it remained viable for a longer time in stock culture tubes; thus it probably metabolized the medium more thoroughly than did the other strains, rendering it unfit for their growth, and on the other hand was able to utilize media

which had been metabolized by the others, carrying the metabolism still farther. It must not be assumed, however, that the ability of strains to utilize metabolized media is wholly a quantitative relationship. It is quite conceivable that a relatively poorly growing organism may produce certain substances which even in small quantities may inhibit the growth of organisms of greater but different metabolic activity.

The above described plate method of metabolizing media was the most satisfactory. Metabolizing the agar in tubes was also tried. After 48 hours incubation the tubes of agar full of minute colonies were melted (thereby sterilized at the same time), poured into plates, and streaked with various strains. The results were quite irregular and unsatisfactory. Such results may have been due to the fact that in the tubes metabolization took place largely under anaerobic conditions, while after pouring the plates the streaks grew under aerobic conditions, or the irregularity of the results may have been due to the effect of heat required to melt the agar. The following quotation from Rahn (1917) illustrates this possibility and also the possibility of error in the use of bouillon culture filtrates as used by Marmorek.

"Eijkmann, as the first, found that B. coli reached its maximum growth in gelatin at 37° in a few days, and that this gelatin, after hardening at 20°, would not support growth after streaking with a young culture of the same organism; but after this gelatin had been heated at 60° for half an hour, B. coli grew on it as well as on fresh gelatin. Broth in which B. coli had grown became fit again for growth of the same bacillus after filtration through porcelain. The inhibition of growth is, in this case, due to a compound which resembles a toxin in many respects."[7]

In Plate 29, Figs. 2 and 3, are shown blood agar plates metabolized by streptococcus B-7α and pneumococcus Cole II 45 respectively, and then streaked with streptococci A-18 (cow)β, B-2b (cow)β, D-AD4β, and A-STβ. Growth occurred in all streaks but least in the streak of B-2b (cow)β which is a strain of *Streptococcus epidemicus*. The control plate is shown in Fig. 1.

The appearance of mixed growths on blood agar has received little attention. Holman (1914) found that *"hemolytic streptococci, growing*

[7] Rahn in Microbiology, Marshall, 1917.

*on blood agar in mixed culture with a green-producing form, show an apparent preponderance, * * .*" I have been able to confirm this observation and to extend it to mixed cultures of hemolytic streptococci and pneumococci or non-hemolytic (*gamma* type) streptococci. In mixed growths on blood agar the appearance of the hemolytic streptococci will completely overshadow that of the green-producing or non-hemolytic strains so that the presence of the latter would not be suspected. In Plate 29, Fig. 4, is shown a blood agar plate streaked as follows:

Streak II = streptococcus D-1γ, pure.
Streak III = streptococci D-1γ and D-AD4β, mixed.
Streak IV = streptococcus D-AD4β, pure.

Streak III resembles streak IV in appearance, so that the presence of the non-hemolytic streptococcus in the streak is completely obscured. In Fig. 5 of Plate 29 is shown a blood agar plate streaked as follows:

Streak 1 = streptococcus A-BLα, pure.
Streak 2 = streptococci A-BLα and D-AD4β, mixed.
Streak 3 = streptococcus D-AD4β, pure.
Streak 4 = streptococcus D-AD4β and pneumococcus Cole II 45, mixed.
Streak 5 = pneumococcus Cole II 45, pure.

Streaks 2 and 4 are exactly like streak 3 in appearance, the presence of the green-producing streptococcus and the pneumococcus being completely concealed. Subcultures in blood agar of growth scraped from streaks 2 and 4 showed that the growth in streak 2 consisted of about equal numbers of the green-producing and hemolytic streptococci, while in streak 4 there were actually about ten times as many viable pneumococci as hemolytic streptococci.

THEORIES AS TO THE CAUSES OF THE VARIOUS APPEARANCES IN BLOOD AGAR.

Type β.—In looking at the clear, colorless, sharply defined, hemolyzed zones about the colonies of *beta* type hemolytic streptococci in blood agar (for example, Plate 6, Fig. 1) one's first impression is that the blood in these zones has been totally destroyed or consumed. This appearance gave rise to such descriptive expressions as:

" * * *élégante auréole d'hémoglobine dissoute*" (Marmorek, 1902).
" * * *völlige Resorption des Hämoglobins*" (Schottmüller, 1903; Kerner, 1903).
" * * *vollständige Decomposition des Blutfarbstoffes*" (Natvig, 1905).
"*Diese Eigenschaft, die roten Blutkörperchen zur Auflösung zu bringen und deren Blutfarbstoff zu zerstören*" (Mandelbaum, 1907–08).
" * * *deutliche Resorptionshöfe*" (Laabs, 1910).
"*Zerstörung von Blutfarbstoff*" (Loening, 1910).
"*Resorptionshöfe des Pyogenes*" (Thalmann, 1912).
"*Hämopepsie*" and "*Hämoglobinopepsie*" (Baerthlein, 1914).

It is to be noted, however, that in blood bouillon hemolytic streptococci never produce more than a laking of the blood. In the lower part of the undisturbed tube there is a change in the color of the hemoglobin from a bright cherry red to a "*burgundy red*" color. These phenomena attracted the attention of Zangemeister (1909) who found by spectroscopic analysis that the burgundy red discoloration was due to the reduction of hemoglobin. Zangemeister cites the following experiments:

(1) On laked blood agar plates hemolytic streptococci did not produce colorless zones, provided "*wirklich alle roten Blutkörper aufgelöst waren.*"

(2) Pulverized saponin scattered on the surface of sterile blood agar plates produced clear colorless zones. (Zangemeister cites Neufeld and Händel to the effect that saponin produces only release of hemoglobin from blood corpuscles.)

From these experiments Zangemeister concludes that "*Die Aufhellung entsteht: dadurch, dass Blurfarbstoff aus dem betroffenen Bezirk austritt und sich durch Diffusion im umgebenden Agar verteilt.*" The diffusion of the hemoglobin was shown by pouring over the inocu-

lated plate of blood agar a layer of plain agar which became red above the colonies of growing streptococci. Before the reading of these experiments similar conclusions had been reached as a result of the following experiments (Plate 30).

(1) In Fig. 2 is shown a horse blood agar plate which was heavily inoculated with a typical hemolytic (*beta*) *Streptococcus pyogenes*. As shown in Fig. 2 after 24 hours incubation the blood of the plate was completely laked and the medium filled with myriads of minute colonies visible microscopically. There was apparently no less hemoglobin, however, than in the sterile laked horse blood agar control plate shown in Fig. 3. In Fig. 1 is shown a sterile plain agar plate merely for photographic comparison with the other two plates. All three plates were handled in a parallel manner and photographed simultaneously as shown. If the plate shown in Fig. 2 had been incubated longer than 24 hours, it would have become brownish, but it must be remembered that streptococci of this type produce clear, colorless, hemolyzed zones in 18 hours. When crowded, as in Fig. 2, however, only laking of blood is produced and there is no apparent disappearance of hemoglobin.

(2) In Figs. 4, 5, and 7 are shown three *beta* type hemolytic streptococci (B-2b (cow)β, D-AD4β, and A-STβ respectively) growing in laked blood agar. There is no trace of zones or of discoloration about the colonies. The apparent slight indefinite lightening of the medium about the surface colonies in the photographs is an artefact due to refraction or reflection of light from the convex surface colonies. The method of preparing these plates was to add to defibrinated horse blood two volumes of sterile distilled water, to centrifuge the laked blood at a speed of 3,000 revolutions per minute for 30 minutes and then to add to each tube of melted agar 2 cc. of the laked blood from the top layer in the centrifuge tube. It will be appreciated that 2 cc. of the laked blood contained the hemoglobin from $\frac{2}{3}$ cc. of blood. The lowering of the salt content of the medium by the addition of the hypotonic laked blood was compensated for by using tubes of agar which originally had contained 12 cc. of agar but which had contracted to the extent of about 1.5 cc. by being allowed to "dry" in the room for some days. In the course of these experiments an unexpected difficulty was encountered which probably has led to

wrong conclusions on the part of others who have worked with laked blood agar. Loening (1910) and Holman (1916) assert that hemolytic streptococci produce hemolyzed zones on laked blood agar. If one or two volumes of distilled water are added to defibrinated blood (horse, sheep, rabbit, or human) there appears to be instantly a complete laking, the fluid becoming practically transparent. If, however, this laked blood is added to clear agar, it can be seen that the resulting mixture is quite clouded, much more so than was either the laked blood or the medium before mixing the two. The clouding of agar by laked blood led to the following observations:

A few drops of laked blood added to standard bouillon or physiological salt solution shows the same clouding. The addition of a small crystal of chemically pure sodium chloride to 1 cc. or less of laked blood in a test-tube causes marked clouding.

If the laked blood is examined microscopically under a cover-slip no corpuscles can be seen, but after the clouding by any of the above methods the corpuscles are seen to have reappeared, not as shadows but as well formed corpuscles having a deeper color than the surrounding fluid almost like that of the corpuscles of unlaked blood.

If a drop of blood is placed under a cover-slip and a drop of distilled water at the edge of the cover-slip, hemoglobin is seen diffusing outward from the corpuscles exposed to the distilled water and the corpuscles themselves appear to become slightly smaller, due to the fact that they have become spherical through the taking in of water. During this process the outlines of the corpuscles become faint and finally disappear from view. If, next, a small crystal of salt is placed at the edge of the cover-slip the corpuscles will be seen to reappear and to assume a disc-like form.

If laked blood is centrifuged at 3,000 revolutions per minute for 30 minutes, there is thrown down a light voluminous poorly refractive fluffy sediment of so called corpuscle shadows which in this case are really intact spherical corpuscle membranes filled with water or rather fluid of the same osmotic tension, presumably, as the surrounding fluid.

If a crystal of salt is added to laked blood and it is then centrifuged there is thrown down a compact, dark colored sediment occupying about one-third the volume of the sediment mentioned in the previous paragraph. The sediment from the salted blood is composed of disc-like corpuscles.

The problem throughout appears to be one of osmosis. When the fluid outside the corpuscles is made hypotonic there is an outward diffusion of hemoglobin and an inward diffusion of water resulting in a spherical corpuscle which occupies more volume. At the same time the exchange of solutes for solvent and the stretching of the corpuscle membrane lowers the refractive index of the cor-

puscle until it approaches that of the surrounding fluid, the corpuscle becoming invisible and the fluid transparent. When salt is added to the laked blood the reverse process takes place; water passes out from the corpuscle allowing it to contract to its normal discoid form. The contraction of the cell membrane and the concentration of its contents result in its having more color and a higher refractive index so that it is again visible.

If a blood agar plate is made from laked blood which has not been thoroughly centrifuged to remove all the corpuscle membranes, these undergo the restoration described above so that the agar is somewhat clouded. Colonies of hemolytic streptococci growing in such a medium produce hemolyzed zones (Plate 30, Fig. 6). Inasmuch as apparently completely laked blood had been used for this plate, it would be but natural to suppose that the zones formed were due to destruction of hemoglobin. I think the evidence indicates, however, that the zones were due not to a destruction of hemoglobin but to a dissolution of the remaining corpuscle membranes.

Baerthlein (1914) tabulates 18 strains of streptococci among those organisms which in blood agar produced "*Hämopepsie*," defined as a complete destruction of the entire blood, hemoglobin and stromata, causing the medium to become hemoglobin-free and transparent. This conclusion was arrived at by staining sections of the colonies and zones in blood agar and finding that in the central portions of the zones no corpuscle remains could be found. Baerthlein's assertion that the hemoglobin is also destroyed is less conclusive, for the virtual absence of hemoglobin in the zones may be accounted for by diffusion outward.

It must be noted that as long as the hemoglobin remains within the corpuscles embedded in the agar the latter remains hemoglobin-free, and that if in the neighborhood of the colony the hemoglobin is released from the corpuscles it will tend to diffuse outward into the surrounding hemoglobin-free agar; *i.e.*, among the corpuscles outside the zone. That this diffusion alone can account for the apparently colorless zones is shown by the following experiment in which no use is made of saponin or other hemolyzing reagent.

(3) A sterile standard blood agar plate containing 5 per cent of defibrinated horse blood was poured. After it had solidified, a number of small holes (about 5 mm. in diameter) were made in the agar by means of suction by the mouth applied to a piece of sterile glass tubing. These holes in the agar were then filled with laked blood

agar containing a little more than 5 per cent of laked blood so that when first photographed the holes filled with laked blood agar appeared darker than the surrounding blood agar (Plate 30, Fig. 8). After incubation over night the hemoglobin from the laked blood agar plugs had diffused outward and could to some extent be seen as a diffuse darkening in the surrounding agar. The plugs of laked blood agar appeared as clear colorless artificial zones (Fig. 9). From this experiment it is evident that laking of the blood about a bacterial colony and diffusion of the hemoglobin outward are sufficient to produce clear colorless hemolyzed zones. That these are the factors resulting in the formation of the *beta* type zones by streptococci is indicated by Experiments 1 and 2 described above and by the following observations:

(*a*) The zones produced by a *beta* type hemolytic streptococcus in 5 per cent blood agar can be seen to be surrounded by more or less definite dark rings of outwardly diffusing hemoglobin.[8]

(*b*) If a blood agar plate contains too many colonies of *beta* hemolytic streptococci so that there is little room for diffusion outside the zones, these appear reddish though transparent.

Although destruction of the corpuscle membranes and outward diffusion of the released hemoglobin are sufficient to account for the production of *beta* type zones and are essentially the only factors involved in the production of these zones during 24 or even 48 hours incubation, yet it does not follow that *beta* type hemolytic streptococci are incapable of causing a visible alteration of hemoglobin. In fact, if a thickly seeded blood agar plate is incubated for longer than 48 hours there sooner or later occurs a gradual brownish discoloration and fading of the laked blood agar until after a time (several days) it appears only a little darker than a blood-free agar plate. Similar changes are apparent in blood bouillon. During 24 or 48 hours in-

[8] If diffusion alone is responsible for the disappearance of the hemoglobin from the zone, there must remain within the zone just as great a concentration of hemoglobin as in the dark ring surrounding the zone, but whereas within the clear zone the remaining hemoglobin is relatively inconspicuous because so much lighter than the surrounding blood agar, the same amount of hemoglobin in the surrounding blood agar causes it to be much darker than the unaltered medium farther out. These dark rings can be seen in practically all the photographs of *beta* hemolytic streptococci.

cubation there is apparent only a laking of the blood accompanied by a reduction of the hemoglobin to the burgundy red color of reduced hemoglobin. In time, however, the medium becomes slightly brownish yellow and gradually fades out until almost as light as blood-free bouillon.

Most authors who have noted the different appearances produced by hemolytic organisms in blood agar and in blood bouillon have regarded the hemolytic processes in the two media as qualitatively different in some respect. It has been suggested that the temperature at which it is necessary to add the blood to the agar has an injurious effect on the blood corpuscles (Kraus, 1903); that the corpuscles are injured by the solid media (Bitter, 1886); that "*In der Bouillonkultur hat der Vibrio freihe Hand in der Auswahl seiner Nährmittel; in der Agarkultur ist er auf seine nächste Umgebung angewiesen. In der Bouillonkultur wird er leicht lösliche Eiweissstoffe zunächst angreifen, und diese werden daher längere Zeit vor der Zerstörung schützen. In der Agarkultur muss er alles erreichbare Nährmaterial aufzuzehren suchen, da er auch mit seinen löslichen Fermenten nur auf mehr oder weniger kurze Entfernungen wirken kann*" (Meinicke, 1904); or that "*durch den kolloidalen Charakter der Blutagarplatte eine Beschleunigung der Hämolyse herbeigeführt werden*" (Pribram as quoted by Mandelbaum).

Nothing in the experiments and observations described above indicates a difference in the hemolytic process as it occurs in blood agar or in blood buillon. The differences in appearance are due solely to the solid or liquid character of the medium, with the possibility of colony formation and localized hemolysis (zone formation) in the former.

It has been definitely shown by Besredka (1901), Ruediger (1903), Kerner (1905), M'Leod (1911), Braun (1912), and von Hellens (1913) that markedly hemolytic streptococci in suitable culture media produce a filterable hemolytic substance known as "*streptolysin.*" It is natural to assume that this is the substance produced by similar streptococci causing hemolysis in blood agar. Since the further investigation of streptolysin involves the use of fluid media rather than solid media it is not thought necessary to discuss it at length here. (See Table IV on The Use of Blood Bouillon and Other Fluid Media.)

Type α.—Natvig (1905), employing surface inoculation of blood agar plates, noted that some streptococci produced colonies which were surrounded by zones of hemolysis bordered on the outside by rings of greenish corpuscles. Natvig has already been quoted (page 13) and an interpretation of the appearance which he describes has been given. His view that the greenish discoloration of corpuscles is a "*Vorstadium einer vollständigen Decomposition des Blutfarbstoffes*" is not supported by the appearance of the zones of deep colonies which are made up of

greenish corpuscles next to the colony and partial hemolysis farther out. In the formation of these "double" *alpha* type zones the hemolytic substance must pass outward from the colony through the greenish corpuscles, and if these corpuscles were subject to hemolysis one would expect them to be hemolyzed first. Mandelbaum (1907–08) recognized the significance of this appearance and formulated rather an elaborate theory to account for it. He says:

"Rote Blutkörperchen, die unmittelbar mit den Kokkenleibern des Mitior in Berührung kommen, können von dessen Hämolysinen nicht mehr angriffen werden."

*"Der Streptococcus mitior liefert also zunächst ein Toxin, dass an den Kokkenleib gebunden zu sein scheint und dass nicht in das umgebende Nährmedium zu diffundieren vermag. Dasselbe wirkt schädigend auf den Blutfarbstoff der roten Blutkörperchen ein. Derselbe wird in eine Verbindung von braungelber bis rostbrauner Farbe übergeführt (Hämoglobintoxin). Dieses Toxin wird erst bei der Berührung mit roten Blutkörperchen von der lebenden Bakterienzelle gebildet. * * * Rote Blutkörperchen, deren Farbstoff durch das Hämoglobintoxin der Mitior geschädigt wurde, können durch das Hämotoxin (Hämolysin) des Mitior nicht mehr aufgelöst werden. Denn sonst müssten doch die roten Blutkörperchen, die unmittelbar unter der Kolonie des Mitior liegen, zuerst aufgelöst werden, da die Hämolysine erst durch diese wie durch ein Filter in die Umgebung diffundieren. In der Tat aber bleiben sie ungelöst, während die anderen roten Blutkörperchen, die nicht durch das Hämoglobintoxin gewissermassen 'immunisiert' wurden, durch die Hämolysine zur Auflösung gebracht werden, was auf der Agarplatte makroskopisch in der 'Hofbildung' erkannt wird."*

He accounts for the lack of hemolysis by *Streptococcus mitior* in blood bouillon by the same theory.

"In der Blutbouillon nun kommen wohl alle Blutkörperchen mit dem Bakterienleib in Berührung, der Mitior bildet nun, genau wie auf der Blutagarplatte, ein Hämoglobintoxin, durch das die roten Blutkörperchen unempfindlich gegen das Hämolysin dieses Bacteriums werden."

Aside from the objectionable use of the terms *"Toxin"* and *"immunisiert"* there is also open to criticism Mandelbaum's statement that this so called toxin is bound to the bodies of the cocci and does not diffuse into the surrounding medium. He studied the colonies and their zones under the microscope and it seems strange that he did not appreciate the fact that many of the discolored *"immunisiert"* corpuscles are not in contact with the bacterial bodies but are at a considerable distance from the colony. Had he studied deep colonies this could not have escaped his notice. The formation of alternate rings of discolored corpuscles and of hemolysis as observed by Saito (1912) and described above (pages 8 to 14) as being caused by variations in temperature, is directly opposed to Mandelbaum's hypothesis of *"ein Toxin, dass an den Kokkenleib gebunden zu sein scheint."*

With the failure of this hypothesis there naturally disappears Mandelbaum's explanation of the behavior of *Streptococcus mitior*

in blood bouillon. The following hypotheses would appear to meet the known facts more accurately.

(1) It may be assumed that there are elaborated by the *Streptococcus mitior* colony two substances one of which (*a*) produces hemolysis and the other of which (*b*) produces changes in the corpuscles visible as a greenish or brownish green discoloration of the intact corpuscles rendering them resistant to hemolysis by the first substance. It must be assumed that in the development of the colony the second substance (*b*) is elaborated first or at any rate reaches a concentration sufficient to produce fixation of the corpuscles next to the colony before the hemolytic substance (*a*) is present in sufficient concentration to produce hemolysis. The hemolytic substance acts well at refrigerator temperature, but at temperatures much below that of the incubator the fixing substance (*b*) either is not produced or is inactive. Alternate exposure to the temperatures of the incubator and of the refrigerator results in the production of alternate rings of fixed corpuscles and of hemolysis.

The above hypothesis would explain the appearances in blood agar very well and is partially supported by the fact that streptococci have been found to produce an extracellular *streptolysin*, but this substance has been demonstrated only in cultures of markedly hemolytic streptococci (probably only those of the *beta* hemolytic type). On the other hand, it is doubtful whether there has been demonstrated any substance corresponding to the hypothetical fixing or green-producing substance. Ruediger (1906) considered green production due to acid formation but there have already been given (page 46 and following) reasons for rejecting this view. They are briefly as follows:

(1) There are actively fermenting streptococci (*gamma* type) which even in dextrose blood agar produce no greenish discoloration.

(2) In the standard blood agar which I have used there is very little fermentable sugar and yet there is abundant greenish discoloration by *alpha* type streptococci.

(3) Although in dextrose blood agar, streptococci of the *beta* type may produce a small amount of greenish color in the substance of surface colonies (*S. epidemicus*) or of brownish pigment about the deep colonies (*S. pyogenes* and others), they never produce the collection of fixed and discolored corpuscles next to the deep colonies characteristic of the *alpha* type zones.

Before describing a modification of the above hypothesis there will be described some experiments which show that blood corpuscles which have been fixed under the influence of the growth of *alpha* type streptococci are no longer subject to hemolysis by streptococci of the *beta* type.

A 12 cc. tube of horse blood agar in fluid condition at 45°C. was heavily inoculated with several loops of streptococcus B-7α. The agar was poured into a Petri dish and incubated 48 hours. At the end of this time the plate contained no macroscopically visible colonies and showed no hemolysis but was of a brownish green tint and contained many small colonies visible microscopically. At this time the plate was streaked with four strains of streptococci of the *beta* hemolytic type and the plate reincubated for 48 hours. The plate was photographed and is shown in Fig. 2 of Plate 29. There was no trace of hemolysis associated with any of the streaks and consequently it was necessary to photograph the plate by reflected light to show the growth in the streaks. A plate was similarly heavily inoculated with pneumococcus Cole II 45 and later streaked with the same four hemolytic streptococci. The results were exactly the same as with the plate just described (Plate 29, Fig. 3).

A standard blood agar plate was streaked across with a loop of 24 hour bouillon culture of streptococcus B-7α and immediately afterward this streak was crossed by similar streaks of hemolytic streptococci A-STβ and D-AD4β. After 24 hours incubation the plate appeared as in Plate 27, Fig. 1. Streak I is of streptococcus B-7α and is greenish by transmitted light but without hemolysis. Streaks IIa and IIIa are of streptococci A-STβ and D-AD4β respectively and show wide zones of hemolysis without discoloration. The hemolysis is not interrupted at the crossing of streak I by streaks IIa and IIIa. At the crossing, however, edges of the hemolyzed streaks are indented where the corpuscles under streak I had become fixed before the hemolysis had advanced that far. At this time streak I was again crossed by streaks of the two hemolytic streptococci and the plate again incubated. After reincubation for 24 hours the result was as shown in Fig. 2. It is seen that in crossing streak I the hemolysis of streaks IIb and IIIb is interrupted, the corpuscles underlying the growth of streptococcus B-7α having been fixed by it and rendered

resistant to hemolysis by streptococci A-STβ and D-AD4β. Exactly similar results were obtained by making streak I of pneumococcus Cole II 45 and streaks II and III as before (Figs. 3 and 4).

The above experiments tend to discredit another conclusion of Mandelbaum's, arrived at as follows: Mandelbaum (1907–08) found that the brownish red corpuscles sedimented from a 48 hour bouillon culture of *Streptococcus mitior* and suspended in fresh bouillon were not hemolyzed by *Streptococcus mitior* but were hemolyzed by *Streptococcus pathogenes*. He concluded that the hemolysin formed by *Streptococcus mitior* was different from that formed by *Streptococcus pathogenes*. Mandelbaum's observation was correct; the brownish discolored corpuscles from blood bouillon cultures of *Streptococcus mitior* are readily hemolyzed by *Streptococcus pyogenes* in bouillon. If, however, these same brownish discolored corpuscles are mixed with agar, poured into a Petri dish, the plate streaked with *Streptococcus pyogenes*, and incubated, no hemolysis is produced. There are several possible explanations. There is no reason to believe that hemolysis by *Streptococcus pyogenes* takes place any less readily in blood agar than in blood bouillon. Unfortunately it is impossible to remove the brownish corpuscles from the blood bouillon culture without including also many streptococci, so that when the corpuscles are mixed with agar the medium is at the same time very heavily inoculated with *Streptococcus mitior* which continues to grow and to influence the blood corpuscles during incubation of the plate, so that by the time the streak of *Streptococcus pyogenes* has also grown sufficiently to show any hemolytic action the brown corpuscles can no longer be assumed to be in the same condition as when added to the agar. No way to overcome this experimental difficulty has been found. It is suspected that blood corpuscles in bouillon are less profoundly altered by *Streptococcus mitior* than they are in agar, possibly because of lower oxygen tension in the bouillon.

There are, however, other reasons than that urged by Mandelbaum for suspecting that the hemolytic activity of streptococci of the *alpha* type is different qualitatively from that of streptococci of the *beta* type. Apparently all authors who have given attention to the subject agree that the hemolyzed zones of *Streptococcus pyogenes* on a blood agar plate are practically fully developed within 24 hours, while hemolysis by *Streptococcus mitior* or by pneumococci develops after a much longer period of incubation and in many instances is not apparent within

48 hours. Furthermore, it has been noted by Sigwart (1909) and is apparent from the work described above that if plates containing colonies of *alpha* type streptococci or pneumococci are placed in the refrigerator, hemolysis appears or becomes much more extensive, the zones becoming larger, while the zones of colonies of *Streptococcus pyogenes* and other *beta* type streptococci change little or not at all. We must assume that if the same hemolysin is produced by both types of streptococci it must be present in larger amount about the colonies of the more hemolytic *beta* type, and if, therefore, it is suspected that refrigeration exerts its influence by lowering the resistance of the blood corpuscles to hemolysis, those about the more strongly hemolytic colonies should show the influence of refrigeration most. Such, however, is not the case.

Further discussion of this problem must be introduced by reference to a recent theory as to the cause of the discoloration of blood corpuscles by pneumococci and streptococci. By spectroscopic analysis it has been found that the brownish discoloration of blood corpuscles by certain streptococci in blood bouillon consists of a change within the corpuscles of oxyhemoglobin to methemoglobin (Rieke, 1904; Boxer, 1906). It is stated by Cole (1914) that the brownish or brownish green discoloration of corpuscles in blood agar is also the result of methemoglobinization. As a result of studies with pneumococci in fluid media Cole concludes that:

*" * * the formation of methemoglobin by pneumococci occurs as a result of reduction and oxidative processes occurring in the neighborhood of the bacteria * * , and without producing substances capable of isolation."*

Blake (1916) repeated Cole's experiments using *Streptococcus viridans* instead of pneumococci and arrived at the same conclusions as did Cole. The results of these authors are also in agreement with those of Rieke (1904) who found that methemoglobinization was enhanced by free access of oxygen and that killed bouillon cultures (60°C. for 1 hour) or sterile filtrates did not produce methemoglobinization. He concludes that:

"Diese Umwandlung des Oxyhämoglobins in neutrales Methämoglobin ist eine Folge der physiologischen Funktionen der Streptokokken, sie ist geknüpft an den Lebensprozess derselben."

In agreement with these conclusions are our observation that in the refrigerator, where the life processes may be assumed to be reduced to a minimum, the fixation and discoloration of corpuscles about the colonies of streptococci and pneumococci in blood agar are brought to a standstill while hemolysis is not inhibited, and that on being again incubated the fixation and discoloration of corpuscles is resumed. Fixation and discoloration of corpuscles did not occur under anaerobic conditions. Possibly the methemoglobinization of corpuscles about the colonies of *alpha* type streptococci and pneumococci is due to

processes of reduction and oxidation in the neighborhood of the colonies rather than to some methemoglobinizing substance elaborated by the colonies. If such is the case, however, it is surprising to note at what distances from the colonies methemoglobinization occurs, as is shown by the formation of rings in response to refrigeration and reincubation.

(2) Let us now return to a discussion of hemolysis by *alpha* type streptococci and pneumococci. Reasons are mentioned above (page 74) for suspecting that the hemolysis produced by such organisms is due to a different factor than that which is instrumental in producing the zones of markedly hemolytic streptococci.

Cole (1914) has described a "*hemolytic endotoxin*" which is released from pneumococci by their autolysis or disintegration by other causes. I have been able to repeat Cole's experiments to the extent of obtaining a hemolytic autolysate. It seems possible that the marked hemolysis occurring in the refrigerator about colonies of pneumococci after 48 hours previous incubation may be due to a hemolytic substance released by autolysis of the pneumococci. The resemblance of the zones produced by pneumococci to those produced by *alpha* type streptococci strongly suggests that they are due to similar processes. However, these streptococci are much less easily disintegrated or autolyzed than pneumococci, and I have been unable to obtain from streptococci anything resembling a "*hemolytic endotoxin.*" Holman (1916) regards methemoglobin production as due to reduction. He says:

"*The bacteria use the oxygen in the medium in which the red cells are embedded and thereby change the osmotic tension, this is followed by the abstraction of oxygen from the oxyhemoglobin, until we have the various stages of the reduction indicated. * * The red blood cells containing this reduced hemoglobin are, I believe, more liable to spontaneous hemolysis.*"

He would, therefore, regard the hemolysis produced by green-producing streptococci as "*spontaneous,*" due to reduction, and not due to the action of a hemolysin.

More work must be done before any conclusions as to the validity of one or the other of the above hypotheses can be reached.

The work of the following authors as reviewed in Table III on The Use of Blood Agar and Table IV on The Use of Blood Bouillon is of special interest: Rieke (1904); Natvig (1905); Ruediger (1906); Boxer (1906); Mandelbaum (1907–08); Zangemeister (1909); Sachs (1909); Anthony (1909); Zangemeister (1910); Loening (1910); Thalmann (1912); Cole (1914); Blake (1916); Holman (1916).

PERMANENCE OF CULTURAL CHARACTERISTICS.

The literature of this subject is reviewed in Table VII on Mutations and Variability. In this table have been included not only all references which have been encountered with reference to mutability or variability of streptococci but also the references to constancy of cultural characteristics. Even if mere loss of virulence be excluded, a majority of the authors who have expressed themselves in any way with reference to variability or constancy have reported instances of variation or have expressed themselves as regarding such variation as a fact. Except for mere loss of virulence, the variations reported consist principally of inconstancy of appearances in bouillon, in blood agar, and of fermentative power.

Some of our strains have been in cultivation for about 6 years, notably those from the Boston epidemic (1911). Most of the strains here reported were isolated in the early summers of 1913 and 1914.

Appearance in Bouillon.—The appearance in bouillon, usually associated with length of chain must be regarded as of minor importance. It is true that most of the more virulent *beta* hemolytic streptococci grow in fairly long chains, cloud the bouillon slightly or only moderately, and produce a correspondingly abundant sediment, but length of chain or failure to cloud bouillon is not necessarily associated with virulence. Avirulent strains may produce longer chains with abundant sediment and no clouding of bouillon or may produce short chains and no sediment, clouding the bouillon well. Some strains which when first isolated do not cloud the bouillon well, do so after prolonged cultivation, but others produce no clouding after 3 years of cultivation. Among the strains which do not cloud bouillon are those which produce both the *alpha* and *beta* types of hemolysis.

Fermentation.—Broadhurst (1913) and Holman (1914) have shown that supposed variations in fermentation reactions may be due to the use of inferior media. The latter has rightly advised the use of a medium in which the organisms will grow well in the absence of fermentable substance. Fermented bouillon (plus 1 per cent of test substance) was used by Smith and Brown (1915). In view of Hol-

man's (1914) recommendation of the use of serum bouillon many of the "negative" tests of the strains reported by us have been repeated, fermented bouillon plus unheated sterile horse serum being used. After an interval of many months the fermentation reactions of the strains described in this paper have also been retested, plain fermented bouillon being used in most cases. In neither set of experiments has there been any variation from the results of earlier tests. The validity of our results in fermented bouillon (without serum) may be attributed to the fact that a result was never considered negative unless there was macroscopically a distinct growth in the medium. Fermented bouillon as prepared in this laboratory has been found to be a much better medium than Liebig extract bouillon. There are various ways of preparing fermented bouillon, however. The fermentation is usually carried out with *Bacillus coli* because it is readily available in all laboratories and is an active fermenter of many sugars.

Gordon (1905) used "*ordinary beef broth freed from sugar by cultivating B. coli therein for 3 days at 37°C. and then sterilized, filtered, rendered slightly alkaline and tinted with litmus.*" Andrewes and Horder (1906) probably used the same medium or beef extract bouillon. Walker (1911) used Gordon's medium for at least part of his tests ("*The test media were prepared as directed by Gordon, at first with ordinary bouillon freed from sugar, but later, and for the bulk of the results * * from 'lemco'*"). Although Broadhurst (1913) made a comparative study of results obtained with fermented and meat extract bouillon and found the former to be superior, she used meat infusion bouillon that had been fermented for 7 days by *Bacillus coli*. A number of authors state that they used sugar-free or fermented bouillon without stating the manner of its preparation. The fermented bouillon used in this laboratory was prepared according to the method of Smith and Walker (1897) by fermenting the meat infusion with *Bacillus coli* for 16 or 18 hours at 37°C., boiling, neutralizing, adding 1 per cent of peptone and 0.5 per cent of salt, and finishing as standard bouillon. Each lot of fermented bouillon was tested for the presence of fermentable sugar before being used (*Bacillus coli* in fermentation tube). Heinemann (1915) made fermented bouillon from meat infusion fermented 24 hours by *Bacillus coli*. The fermented bouillon used by Gordon and that used by Broadhurst are subject to criticism in two respects: (1) fermentation by *Bacillus coli* for more than 18 hours is not only unnecessary to remove all the fermentable sugar but is positively injurious to the medium, since after the exhaustion of the sugar *Bacillus coli* attacks the protein constituents forming ammonia and other products of protein decomposition which inhibit the growth of other organisms; (2) since Witte peptone (also

Digestive Ferments Company peptone) contains no fermentable sugar, nothing is to be gained by its presence in the medium at the time of fermentation, and in fact a better medium is produced if the peptone is not acted upon by *Bacillus coli* which grows perfectly well in the meat infusion only.

Fermented bouillon as prepared in this laboratory has been found satisfactory for bringing out the full complement of fermentation reactions of all the streptococci studied except *Streptococcus mucosus* which failed to ferment lactose, raffinose, and salicin in fermented bouillon alone but did ferment them in fermented bouillon plus horse serum. On the other hand, several strains of pneumococci, including those described in this paper, require the addition of serum to fermented bouillon to bring out their full complement of fermentation reactions. It is suspected that many of the strains of pneumococci reported by various authors not to ferment inulin would have done so in more suitable media. I have not encountered any pneumococci which did not ferment inulin in serum bouillon. The amount of serum added need not be large; 10 per cent is ample and usually 1 drop of serum to 1 cc. of fermented bouillon is sufficient.

Appearance in Blood Agar.—Variations in appearance on blood agar have been reported by many authors since Schottmüller's (1903) introduction of this medium for the differentiation of streptococci. It was about 2 years ago that the first photographs of the blood agar plates here shown were made. In the report published by Smith and Brown (1915) it is stated that in repeated tests extending over a period of 14 months we had observed some loss of hemolytic activity for certain strains. This loss of hemolytic activity never took the form of the production of less clear zones of hemolysis but always the formation of slightly smaller zones. Never was there observed the greenish discoloration of corpuscles by strains which had not produced it from the first; in other words, there was never a transition from *beta* to *alpha* types. "*Strain B-15 from a cow forms an apparent exception, but this strain has in the course of our studies split up into a series of forms differing in their laking capacity, some being non-hemolytic at present*" (Smith and Brown, 1915). The strain B-15 just referred to was one of the first isolated by us and in view of what is now known about the influence of commonly ignored factors on the gross appearance of streptococcus colonies in blood agar it seems

likely that this strain may either have been impure to start with or its variable appearance may have been due to unrecognized influences. When the strains of streptococci described in this paper were first photographed most of them had been in cultivation for 1 or 2 years, but in so far as could be determined from careful notes their appearances in blood agar had not changed since they were isolated. Since then they have been replated many times, but no noteworthy changes have occurred. In the plates there can be compared photographs taken more than a year apart.

Streptococcus.

D-1 γ	Compare Fig. 1, Plate 2 with Fig. 3, Plate 2.
A-18 (cow) β	Compare Fig. 3, Plate 4 with Fig. 5, Plate 4.
B-18 (cow) β	Compare Fig. 1, Plate 5 with Fig. 3, Plate 5.
A-ST β	Compare Fig. 1, Plate 6 with Fig 3, Plate 6.
D-AD4 β	Compare Fig. 1, Plate 7 with Fig. 1, Plate 8.
B-2b (cow) β	Compare Fig. 1, Plate 9 with Fig. 3, Plate 9.
A-BL α	Compare Figs. 1 and 2, Plate 12 with Figs. 4 and 5, Plate 12.
B-7 α	Compare Figs. 1 and 2, Plate 15 with Figs. 1 and 2, Plate 16.
E-4 α	Compare Figs. 1 and 2, Plate 17 with Figs. 5 and 6, Plate 17.

In the case of the last four strains listed above slight differences appear between the photographs taken on the two dates. In the photograph of streptococcus B-2b (cow)β taken in March, 1916 (Plate 9, Fig. 3), the surface colonies appear darker than in the photograph taken a year earlier (Plate 9, Fig. 1), though the deep colonies appear the same. The same difference to a lesser degree is noticeable in the case of streptococcus A-STβ (Plate 6, Figs. 1 and 3). The notes do not indicate that this difference was apparent to the eye and it appears to be largely an artefact due to the use of different kinds of photographic plates each year.

In the case of streptococci A-BLα, B-7α, and E-4α it will be seen that in 1916 the characteristic double zones had appeared during 48 hours incubation, while in 1915 they were fully developed only after refrigeration. It has already been shown that the early development of double zones may be brought about by the presence of large numbers of colonies in a plate, but this does not appear to be the cause in the instances under consideration. This difference can apparently be due to very slight and unknown differences in the medium. It does

not indicate a change in the properties of the streptococci for it has been seen to come and go during intervals of only a few days in different plates inoculated from the same culture tube. It presents another reason for regarding the appearances in blood agar as incomplete until the plates have been refrigerated. A comparison of the 1916 plates with the 1915 plates after refrigeration shows remarkable uniformity in appearance. A comparison of rabbit and human blood agar plates made a year apart showed the same permanence of characteristic appearances. In none of these strains was there the slightest tendency to change from an *alpha* to a *beta* type or *vice versa*.

The permanence of apparently minor characteristics of all the strains studied has been surprising. It is difficult to believe that there are to be found in the human throat a dozen or more fixed varieties of streptococci, and yet there have been isolated from a single swab eight or nine strains which have retained individual characteristics for many months. This will seem to be contrary to the results of Rosenow (1912) and others who have reported variations in the cultural characteristics of streptococci, but it is not necessarily so. Many of the variations reported may easily have been due to unrecognized differences in media or technique employed over various intervals of time, but it should be pointed out that the present effort for several years has been to keep strains of streptococci under as uniform and as favorable conditions as possible so as to determine whether or not under these conditions cultural characteristics could be relied upon for the differentiation of varieties of streptococci. These strains have not been subjected to conditions likely to cause variations. The nearest approach to such conditions has been (1) passage of some strains through rabbits as described by Smith and Brown (1915), (2) keeping of some strains on plain agar slants rather than blood agar slants for several months, and (3) the drying of droplets of bouillon cultures of several representative strains on sterile coverglasses for 3 months. No changes were brought about by any of these procedures. Rosenow, on the other hand, subjecting strains to repeated animal passage and to various adverse conditions has reported many fundamental changes in the characters of strains so treated. I have made no attempt to repeat his work. It is believed, however, that a clear definition of various types of appear-

ance in blood agar under carefully defined conditions will do much to discourage the mistaking of differences in appearance due to variable conditions for true variations or mutations of the organisms themselves.

In this connection it may be worth while to mention the manner in which our stock cultures are kept. To the surface of standard meat infusion agar slants two or three drops of defibrinated horse blood are added and the tubes incubated 48 hours to test their sterility. The condensation fluid of these slants is inoculated and streaked upward along the slant. Longevity of the culture is much greater if the inoculated tubes are incubated only long enough for a fair amount of visible growth to appear, usually just over night. They are then placed in the refrigerator at 40 to 45°F. and kept there until discarded. The cotton plugs of these tubes have been dipped quickly into sterile paraffin and at the time the tubes are inoculated are set afire and pushed down into the tubes. The paraffin in the plugs is not sufficient to make them air-tight but probably does help to prevent rapid drying of the medium. No caps or seals of wax or paraffin are necessary. Molds never cause trouble. On this medium all strains except *Streptococcus mucosus* and the pneumococci are ordinarily renewed once in 4 or 5 months. Of fifty strains of various types which were recently allowed to go 7 months without renewal all but two remained viable. The pneumococci and *Streptococcus mucosus* are usually renewed each month but are viable after 2 months. All inoculations of animals or test media are made from fresh bouillon cultures which are always inoculated directly from the stock tubes. Experiments are never conducted with cultures inoculated from one test medium to another in an indefinite sort of way, but always from fresh bouillon cultures taken directly from stock cultures. The stock cultures are kept in the refrigerator and are used for no other purpose than this. The keeping of stock cultures has not been entrusted to assistants.

For a review of the literature the reader is referred to Table VII on Mutations and Variability.

OCCURRENCE OF VARIOUS TYPES OF STREPTOCOCCI.

The markedly hemolytic (*beta* type) streptococci are commonly present in various suppurations (otitis media, osteomyelitis, adenitis), erysipelas, and in sore throats, rarely in clinically normal throats. Green-producing (*alpha* type) and indifferent (*gamma* type) streptococci are commonly found in normal throats, but though often present in mixed culture they have not been found by me under conditions that would indicate them as the cause of acute suppurations. The blood agar plates inoculated with material from acute sore throats or from normal throats are, therefore, quite homogeneous and characteristic in appearance. On the other hand, plates inoculated with material from chronic sore throats resemble those from convalescent throats; they are very heterogeneous in appearance often containing colonies of the *alpha*, *alpha prime*, *beta*, and *gamma* types with a great variety of quantitative differences among themselves, so that as stated above (page 81) as many as eight or nine distinguishable varieties of streptococci may be obtained from a single swab. Three such plates are shown in Plate 32. The finding of the *alpha prime* type is characteristic of convalescent or chronic sore throats. In several cases of acute sore throat material was obtained at intervals of a day or two. The *beta* type colonies were present in large numbers at first, gradually yielding to the appearance of the *alpha prime* type and the great variety of *alpha* type colonies characteristic of convalescent throats, until finally only the typical *alpha* and *gamma* types remained in the normal throat. The succession of types followed in this way strongly suggests the change of one type into another. Yet when these seemingly changing strains have been isolated they have remained for months or years in cultivation with their characters fixed just as they were when isolated. It can not be said whether or not they were actually changing in the throats from which they were taken. It seems strange that there should be this succession of types from markedly hemolytic to moderately hemolytic, less hemolytic, and green-producing. Still it must be remembered that the latter are always present in greater or less numbers. The

fact that the great variety of types to be found in plates from convalescent throats can be arranged in an almost perfect series with reference to their appearance does not mean that they are derived one from another. The various strains of streptococci illustrated in the plates can be arranged in an almost perfect series as is shown in Plate 31, but each of these strains came from a different individual and from five different epidemics in five different localities; there can scarcely be supposed any genetic relationship among them. But if they all appeared on the same plate (and just as good a series has been seen on a single plate) the temptation to regard them as derived one from another would be strong. One is reminded, however, of what occurs in a bed of cultivated plants which is later abandoned. During the abnormal condition of artificial cultivation there is an almost pure culture of the plant cultivated. Abandoned the next season the loose upturned soil supports the growth of a great variety of weeds. Finally, however, after another season or two the native grass crowds in from the edges of the bed choking out most of the weeds. In the case of the streptococci a close series of forms can be noted by fixing the attention on a single character; *i.e.*, the amount of hemolysis. If in the case of the flower bed the attention is similarly fixed on a single character, *e.g.* the height of the plants or the color of the flowers, there can also be arranged a close series among the weeds. To indicate an actual genetic series, however, either the various forms must be found to differ with respect to the single character only or the same series must be confirmed by other variable characters. With these streptococci such a series is not apparent. The fermentation reactions and the morphology of the various strains do not suggest the same series as do the hemolytic types.

Reviewing the literature one is impressed with the similarity of blood agar plates inoculated with vaginal or uterine secretions to those inoculated with material from throats. Most authors working with vaginal streptococci agree that the streptococci found in cases of severe puerperal infection are of the markedly hemolytic type. They also agree that ante partum in normal cases there are usually to be found only non-hemolytic or green-producing streptococci. Post partum in clinically normal cases there are usually found only the non-hemolytic or green-producing types, though in a minority of cases hemolytic streptococci may also be found. Heynemann (1908) and Zangemeister (1910) regarded the

finding of hemolytic streptococci in clinically normal cases as of grave significance or at least as potentially grave. Fromme (1908) and Heynemann (1908) mention having found intermediary (moderately hemolytic) types in convalescent cases of puerperal infection.

The general subject of the relation of hemolysis to the source of strains is reviewed in Table VIII on General Conclusions, etc. The work of the following authors as reviewed in Table VIII on General Conclusions, etc., and in Table III on The Use of Blood Agar should be especially noted: Heynemann (1908); Fromme (1908); Lüdke and Polano (1909); Konrád (1909); Sigwart (1909); Zangemeister (1910); Rosenow (1910); Fabre and Bourret (1910); Gminder (1912); Puppel (1912); Ruediger (1912); Sitzenfrey and Vatnick (1913); Davis (1914); Gilmer and Moody (1914); Hartzell and Henrici (1915); Rosenow (1915); Broadhurst (1915); Rosenow and Dunlap (1916); Rosenow and Oftedal (1916); Davis (1916); Smillie (1917).

CLASSIFICATION OF STREPTOCOCCI.

The difficulty encountered in trying to identify the strains of streptococci isolated by Dr. Smith and myself has already been mentioned. The reason for such difficulty is that various authors in attempting to name or classify streptococci have fixed their attention upon different characters as criteria. Such names as *S. pyogenes*, *S. erysipelatos*, *S. epidemicus*, *S. lacticus*, *S. equinus*, *S. equi*, *S. anginosus*, *S. fecalis*, *S. salivarius*, *S. rheumaticus*, *S. saprophyticus*, and *S. mastitidis* were suggested by consideration of the source of the organisms. Such names as *S. longus*, *S. longissimus*, *S. brevis*, *S. conglomeratus*, and *S. lanceolatus* were suggested by morphological peculiarities. Such names as *S. pathogenes*, *S. mitior*, and *S. mitis* have reference to pathogenicity or virulence. Such names as *S. hemolyticus*, *S. viridans*, *S. anhemolyticus*, *S. mucosus*, and *S. acidi lactici* refer to certain cultural characteristics.

Comparatively few authors have considered all the above criteria in grouping or classifying the strains studied by them. Generally speaking, the German authors have given most of their attention to the morphology of streptococci and their appearance on blood agar, with little attention to fermentation reactions; the English authors have given more attention to fermentation reactions, but very little to appearance on blood agar; American authors have been divided in giving their attention to one or the other set of characters and a number have tried to correlate the two. Source and pathogenicity for laboratory animals have engaged the attention of authors of all nationalities.

In view of the emphasis being placed on one or another character it is to be expected that the various names and classifications of streptococci should overlap. Such questions as the following arise. Is *S. pyogenes* a *S. hemolyticus* and what are its fermentation reactions? Is *S. mitior* (Schottmüller) synonymous with *S. mitis* (Andrewes and Horder)? Is *S. longus seu erysipelatos* (Schottmüller) synonymous with *S. longus* (von Lingelsheim) or with *S. erysipelatos* (Fehleisen)? Do *S. longissimus* and *S. conglomeratus* belong to the hemolytic,

non-hemolytic, or viridans groups? Do authors regard hemolytic green-producing streptococci as *S. hemolyticus* or as *S. viridans?* A review of the literature reveals the need for answers to these and many similar questions. The present nomenclature of streptococci is almost chaotic.

The designation of streptococci as *S. longus* and *S. brevis* as proposed by von Lingelsheim (1891) appears to be of little value not only because it is a matter of common laboratory experience that length of chain is readily influenced by conditions of cultivation, but also because *S. longus*, defined by von Lingelsheim (1899) as including those streptococci which have a tendency to grow in chains of six or more elements, includes practically all streptococci. In fact, under *S. longus* (*pyogenes*) von Lingelsheim (1912) includes in addition to the ordinary *S pyogenes* (1) *Obligate anaerobic streptococci;* (2) *S. conglomeratus;* (3) *S. longissimus;* (4) *S. mitis seu viridans.* He excludes *S. lacticus*, *S. mucosus*, and the pneumococci. His inclusion of "*S. mitis seu viridans*" (presumably *S. mitior seu viridans*) under *S. longus* (*pyogenes*) is in direct disagreement with the classification of Schottmüller (1903) who distinguished (1) *S. longus pathogenes seu erysipelatos*, (2) *S. mitior seu viridans*, and (3) *S. mucosus.* Von Lingelsheim fixed his attention upon morphology, Schottmüller his upon source and appearance on blood agar. Adopting the test substances proposed by Gordon (1905), Andrewes and Horder (1906) distinguished (1) *S. equinus*, (2) *S. mitis*, (3) *S. pyogenes*, (4) *S. salivarius*, (5) *S. anginosus*, (6) *S. fecalis*, and (7) pneumococci. This classification was based largely upon the correlation of fermentative characters and source. *S. pyogenes* and *S. anginosus* are merely mentioned as being "*hemolytic*" while *S. fecalis* is indicated as "*non-hemolytic*" on blood agar. Mandelbaum (1907-08) described *S. saprophyticus* as producing neither hemolysis nor discoloration on blood agar. He also recognized *S. mitior* as described by Schottmüller. *S. longissimus* and *S. conglomeratus* were described by Thalmann (1910) who says that they produced on blood agar either no hemolysis or greenish partly hemolyzed zones. He describes *S. brevis* as being non-hemolytic and usually producing more or less green color. Thalmann (1911) described strains from four cases of angina which produced on blood agar neither hemolysis nor discoloration and which he regarded as varieties of *S. pyogenes* though he had previously (1910) described the latter as producing clear hemolyzed zones. Zangemeister (1910) recognized "*hämolytische*" streptococci and *S. viridans* as described by Schottmüller, and designated those streptococci which produced neither hemolysis nor discoloration on blood agar as *S. anhemolyticus vulgaris* which is therefore apparently synonymous with *S. saprophyticus* (Mandelbaum). Rolly (1912) distinguished (1) *S. hemolyticus* (apparently synonymous with *S. longus pathogenes seu erysipelatos* (Schottmüller), (2) *S. anhemolyticus*, (3) *S. viridans*, and (4) *S. mucosus.* Davis (1913) characterized (1) *S. hemolyticus*, (2) *S. epidemicus*, (3) *S. mucosus*, (4) *S. pneumoniæ*, and (5) *S. viridans* with ref-

erence to appearance on blood agar, presence or absence of capsules, solubility in bile, fermentation of inulin, and pathogenicity for rabbits. His use of the terms *S. hemolyticus* and *S. viridans* is evidently much more restricted than is their use by Rolly and Schottmüller respectively.

Lyall (1914) proposed to differentiate streptococci as (1) *Hemolytic*, (2) *Methemoglobin Producers*, and (3) *Indifferent*, according to their action on a suspension of sheep corpuscles. By taking into consideration also the fermentation of inulin, salicin, and raffinose, he distinguished (1) *Pneumococci and Pneumococcus mucosus*, (2) *S. pyogenes*, (3) *S. viridans and Variants*, and (4) *S. fecalis and other streptococci*. Lyall's scheme may be criticized from two standpoints: (*a*) the differentiation of *Methemoglobin Producers* from *Indifferent* strains by his method is an artificial one since some strains may be *Methemoglobin Producers* with respect to the blood of one species of animal but *Indifferent* with respect to the blood of another species; (*b*) too many strains fall into groups 3 and 4; *i.e.*, *S. viridans and Variants* and *S. fecalis and other streptococci;* furthermore strains of *S. fecalis* are green producers and therefore belong to the *S. viridans* group as originally defined by Schottmüller. The grouping proposed by Kligler (1915) is incomplete and not sufficiently definite.

The classification of Holman (1916) has much to commend it. It is practical, definite, and recognizes the names which have already been applied to various streptococci. Holman classifies streptococci with reference to their being hemolytic or non-hemolytic on blood agar and with respect to their behavior towards lactose, salicin, and mannite, though he also recognizes inulin-fermenting varieties of most of the non-hemolytic streptococci.

The scheme proposed by Holman should be extended and rearranged, however. The *alpha*, *beta*, and *gamma* appearances in blood agar should be recognized instead of dividing all streptococci into hemolytic and non-hemolytic groups. Although lactose, mannite, and salicin are of primary differential value, raffinose and saccharose should not be ignored. Notwithstanding the statements of Broadhurst and Holman that the fermentation of raffinose and of inulin run almost parallel to each other, I do not find it so, nor does it appear so in Broadhurst's tables. Of 666 strains tabulated (Table II), 102 act differently towards raffinose and inulin, and of these strains 59 are reported by Broadhurst (1915). With three substances there are eight fermentative complexes. By duplicating these eight complexes under hemolytic and non-hemolytic groups Holman was able to differentiate all the type forms of Andrewes and Horder and some others. With four substances there are sixteen complexes, with five there are thirty-two, and with six there are

sixty-four. If streptococci are divided into a hemolytic and a non-hemolytic group the number of complexes is doubled, and if three types of appearance in blood agar are recognized the number of complexes is tripled. If, as seems advisable, the *alpha*, *beta*, and *gamma* types are recognized in blood agar and if six test substances for fermentation are employed, there would be 192 possible complexes, many of which have not been encountered among the streptococci. This would seem to be an impossible number of complexes to keep in mind or to work with. Even Andrewes and Horder tabulated only 94 complexes. However, as a working basis in the absence of a natural classification I have employed a tabulation of 192 complexes which obviates the reduplication and ambiguity of some of Andrewes and Horder's so called "*variants*" and which preserves the simplicity of Holman's scheme without ignoring the differential value of saccharose, raffinose, and inulin as test substances.

In Table II streptococci are placed according to "*types*" in blood agar; "*groups*" with respect to fermentation of mannite, lactose, and salicin; and "*subgroups*" with respect to fermentation of saccharose, inulin, and raffinose. The types are indicated by α, β, and γ. The groups are numbered 1 to 8, and the subgroups 0.1 to 0.8 inclusive. By this means the hemolytic and fermentative properties of a streptococcus can be referred to by a letter, a number, and a decimal. Examples: $\alpha 1.2$ indicates a green-producing streptococcus which ferments lactose, salicin, saccharose, and raffinose but does not ferment mannite or inulin. $\beta 1.2$ indicates a streptococcus having the same fermentative powers as the above but a different type of appearance in blood agar. $\alpha 1.1$ indicates a streptococcus differing from $\alpha 1.2$ only with respect to raffinose. It is surprising how soon and how easily the different numbers are carried in mind. This is made possible by the orderly alternation of positive and negative signs so that, for instance, it is soon learned that groups 1 to 4 are mannite non-fermenters while groups 5 to 8 are mannite fermenters; the groups indicated by odd numbers are salicin fermenters while the non-fermenters are represented by even numbers, etc. The signs in the subgroups are just the reverse of those in the groups. It may be asked what has determined the particular order in which the test substances are tabulated. The order is evidently mannite, lactose,

salicin, saccharose, inulin, and raffinose.[9] This order was adopted not because it represents any natural relationship of the test substances or any metabolic gradient, but because it was found to bring the well known groups to the top of the table and the typical members or subgroups to the left where they are easily remembered. It appears rather remarkable that so many of the well known strains which have been given specific names by various authors should by this means fall into subgroup 0.1 of groups 1 to 5. What may be called the "*typical member*" of each group is therefore indicated by the numbers 1.1, 2.1, 3.1, or 4.1, etc. This means simply that the "*typical members*" ferment saccharose but do not ferment inulin or raffinose, while the "*atypical members*" act differently towards one or more of these three substances. In describing *Streptococcus salivarius* (α2.1) as not fermenting raffinose it is realized that Andrewes and Horder describe the "*type form*" as $\pm$ with regard to raffinose. However, the more restricted use of the name is justified by the fact that only 24 per cent of the *Streptococcus salivarius* strains tabulated by these authors did ferment raffinose while 76 per cent did not ferment it. The non-raffinose-fermenting strains should be regarded as being "*typical members*" of this group.

In Table II is indicated the number of strains belonging to each subgroup which have been reported by Smith and Brown (1915), Hartzell and Henrici (1915), Kligler (1915), Broadhurst (1915), Smillie (1917), and Davis (1916). The strains of these authors have been selected because they have employed all the six test substances listed in this table and have indicated whether their strains produced greenish discoloration, hemolysis, or neither on blood agar, corresponding roughly to the *alpha*, *beta*, and *gamma* types.[10] It should be noted that of the 666 strains listed in this table 381 or nearly 60 per cent of them are "*typical members*" of the various groups. Among the *beta* type streptococci there are very few "*atypical members*," 94 per cent being "*typical.*" Streptococci of the *gamma* type are the

[9] Dextrose and maltose are assumed to be fermented in all cases unless specifically stated otherwise. I have never encountered a streptococcus which failed to ferment these two sugars in a favorable medium.

[10] From Broadhurst's paper I have not included strains of doubtful types such as are labeled "*haze*," "*haze-hemolysis*," "*hemolysis?*," and "*no color-haze.*"

least homogeneous with regard to the fermentation reactions, the least numerous, and the least studied. The name of the "*typical member*" of each group should not be employed for the "*atypical members*" of the group since many of these names as *Streptococcus salivarius*, *Streptococcus equinus*, etc., indicate the common habitat or source of these strains, and there is at present no reason to believe that such strains as α5.4 or α5.5 come from the same sources as α5.1 or that they are any more nearly related to α5.1 than to α1.4 or α1.5. The use of the term "*variant*" as used by Andrewes and Horder is also objectionable since there is no evidence that their so called variants are variants of their "*type forms*." There is no apparent objection, however, to calling group α1 the *Streptococcus mitis group* or α5 the *Streptococcus fecalis group*, etc., so long as it is understood that the inclusion of the various subgroups or atypical members within the groups is purely an arrangement for convenience based upon a certain similarity of fermentative characters which may or may not indicate a natural relationship. The tabulation employed has been very useful. It is perfectly definite. It provides space for all the streptococci so far described with sufficient detail to be classified according to hemolytic and fermentative characters and for many not yet described. The group may be determined and indicated as Holman has done, without indicating the subgroup, if desired. Recognition is given to the names of streptococci already described by various authors.

There should also be mentioned certain names which have been applied by various authors to streptococci the description of which with reference to fermentation or appearance in blood agar is not sufficiently complete to enable them to be placed in the table just described. *Streptococcus rheumaticus* (Poynton and Paine, 1902) represents probably a number of different varieties found in cases of rheumatism, belonging probably to the *alpha*, *alpha prime*, and *gamma* types (Rosenow, 1914). *Streptococcus bovinus* (Winslow, 1908) belongs to group 1.2, and from the fact that it was found in large numbers in bovine feces, I suspect that it belonged to the *alpha* type, but its appearance on blood agar was not described by the author. Most of Broadhurst's (1915) strains were of the *alpha* type. *Streptococcus gracilis* (Escherich) can not be grouped because of insufficient data. *Streptococcus versatilis* (Broadhurst, 1915) apparently belongs to group 5.2 and to the *alpha* or the *gamma* type, mostly to the former. *Streptococcus mucosus* (Howard and Perkins, 1901) and the pneumococci belong to the α1.4 or the α5.4 type and groups, and are further distinguished by the presence of

capsules, solubility in bile, etc. *Streptococcus longissimus* (Thalmann, 1910) in all probability belongs to the *alpha* type but its fermentation reactions are not given. Strain D-AD$8_{2}\alpha$ (described above, page 21) which is undoubtedly a *Streptococcus longissimus* is also a *Streptococcus salivarius* (α2.1). Strains D-1α and F-2α of *Streptococcus conglomeratus* (Kurth, 1891) belong to groups α1.1 (*Streptococcus mitis*) and α2.2 respectively. Thalmann (1912) came to the conclusion that *Streptococcus conglomeratus* was not a distinct species or variety but merely a degeneration form of various other streptococci brought about by growth under unfavorable conditions. This view may be supported by the fact that I have found strains of *Streptococcus conglomeratus* belonging to the *beta* type as well as those of the *alpha* type. Heinemann (1906) came to the conclusion that *Streptococcus lacticus* (Kruse) is not a distinct species but merely a name for various non-pathogenic, vigorous lactic-acid-producing streptococci commonly found in milk as contaminations. This view would seem to be supported by the work of Saito (1912) whose descriptions would indicate that his strains of *Streptococcus acidi lactici* belonged to the *alpha* type. They all coagulated milk and fermented lactose and dextrose, but differed with respect to mannite. These facts are in harmony with these strains belonging to groups α2 (*Streptococcus salivarius*) and α5 (*Streptococcus fecalis*). In the last edition of Kolle and Wassermann's Handbuch der pathogenen Mikroorganismen (1912) von Lingelsheim gives a tabular summary of characteristics of *Streptococcus longus*, *Streptococcus lacticus*, the pneumococcus, and *Streptococcus mucosus*. There is nothing in this table to differentiate various non-hemolytic or green-producing streptococci from *Streptococcus lacticus*.

The descriptions of the following streptococci are not sufficiently complete to classify them with respect to appearance in blood agar and fermentation of sugars. *Streptococcus pyogenes bovis* is stated by a number of authors (Glage, in Kolle and Wassermann, 1912) to be indistinguishable from *Streptococcus pyogenes hominis* but distinct from *Streptococcus agalactiæ contagiosæ bovis*. The streptococci causing mastitis in cows have been called *Streptococcus mastitidis*, *Streptococcus agalactiæ*, *Streptococcus mammitis bovis*, etc. From the literature it cannot be determined whether they refer to the same or different organisms. Mastitis in sheep and goats is produced by *Diplococcus lanceolatus ovium* (Gaertner) and *Streptocotcus ovis* (Wiemann). The former is described as being encapsulated but non-hemolytic. The latter has no capsule but is hemolytic.

The streptococci mentioned in the preceding two paragraphs need to be again studied by modern methods.

Addendum.—Since this article was written there has appeared a paper by Francis G. Blake on *The Classification of Streptococci.*[11] He proposes a scheme of classification "*for clinical purposes.*" On the basis of appearance in blood agar

[11] Blake, F. G., *J. Med. Research*, 1917, xxxvi, 99–133.

plates the streptococci are divided into two divisions (1) *Streptococcus hemolysans* and (2) *Streptococcus viridans*. Blake says:

"Further subdivision of Streptococcus hemolysans by means of fermentation reactions is of no practical value. Streptococcus viridans, however, is divided into three subgroups by its fermentation reactions in lactose and mannite, a procedure which is of value in that it gives some indication of the source of the strain in question. These subgroups are (a) Streptococcus buccalis characterized by the fermentation of lactose and failure to ferment mannite, (b) Streptococcus fecalis characterized by the fermentation of both lactose and mannite, and (c) Streptococcus equinus characterized by the failure to ferment either lactose or mannite. Limitation of the carbohydrate-fermentation reactions to the use of lactose and mannite reduces the number of unclassified or variant strains to a negligible minimum."

While this procedure may serve most clinical purposes the introduction of new specific names is a question of biological nomenclature and is not justified entirely by clinical purposes. If the names proposed by Blake are to indicate so few differential characters, would it not serve clinical purposes just as well to speak of a lactose + mannite − *Streptococcus viridans* (or more briefly αL+M−) as to introduce new names of less specific significance than those already in use? Progress would seem to lie in the direction of better differentiation of the species or varieties already recognized. Only in this way can it be discovered whether or not such varieties are of clinical or other practical or theoretical importance.

SUMMARY.

In the foregoing pages there have been illustrated and described in greater detail than heretofore the *alpha* and *beta* types of appearance produced by streptococci in blood agar. There have also been described the *alpha prime* and *gamma* types not heretofore described as such.

There has been emphasized the importance of studying the deep colony microscopically, the finer differential characters of the surface colonies being obscured by the overgrowth of the colonies.

Between 5 and 10 per cent of blood in sufficient agar to make a layer of medium 2 mm. thick reveals the maximum amount of differentiation by colonies of various streptococci.

The *beta* and *gamma* types streptococci produce essentially similar appearances in blood agar made with defibrinated horse, rabbit, or human blood. Some strains of *alpha* type streptococci and pneumococci produce macroscopically widely different appearances in blood agar made with different kinds of blood, differences so great that a strain may appear hemolytic in one medium and non-hemolytic in another; but microscopically the differences are seen to be quantitative rather than qualitative so that there need be no confusion among the various types.

Streptococci of the *alpha* type when numerous in a blood agar plate produce hemolysis more readily than when there are fewer in the plate.

Defibrinated blood is suitable for use in blood agar so long as there is no marked laking, the corpuscles are readily shaken up, and the blood is in apparently good condition.

Standard meat infusion agar should be used rather than meat extract agar.

The titratable acidity of the meat infusion agar should be in the neighborhood of 1 per cent N acid to phenolphthalein but may vary as much as 0.5 per cent below or above this reaction without exerting a marked influence on the appearances produced by growth of the streptococci.

Either veal or beef may be used in making the agar. Differences in appearance produced by the use of Witte or Digestive Ferments Company peptone are relatively slight.

The presence of small amounts of dextrose in the medium (as little as 0.05 per cent) produces a marked effect. Actively fermenting *gamma* type streptococci may produce an appreciable amount of acid hemolysis, especially noticeable in meat extract agar, probably because of the lower "buffer effect" of this medium as compared with meat infusion agar. Greenish or brownish discoloration similar to the *alpha* appearance is not produced by *beta* or *gamma* type streptococci in dextrose blood agar, though if sufficient fermentation takes place there may be a general brownish discoloration of the entire plate beginning in the neighborhood of the colonies. The most noticeable effect of the presence of dextrose in the blood agar is the partial or total inhibition of hemolysis by actively hemolytic *beta* type streptococci. In sugar-free media the *alpha* type streptococci still produce their characteristic double zones, though growth may be so scant as to make their differentiation difficult. The discoloration and fixation of corpuscles by *alpha* type streptococci is encouraged by the presence of a small amount of dextrose in the medium.

Under anaerobic conditions *alpha* type streptococci and pneumococci produce *beta* type zones.

If a *beta* type streptococcus is present in a colony or streak mixed with an *alpha* or *gamma* type organism the presence of either of the latter may be completely masked by the former.

Corpuscles in blood agar which have become discolored greenish or brownish in the neighborhood of colonies or streaks of *alpha* type streptococci or pneumococci are no longer subject to hemolysis by *beta* type streptococci.

Colonies of *beta* type streptococci in laked blood agar do not produce zones of hemolysis or decolorization; they produce no apparent alteration of the surrounding medium. A blood agar plate heavily inoculated with *beta* type streptococci is laked but not decolorized during 24 or 48 hours incubation. More prolonged incubation results in gradual brownish discoloration and decolorization. A similar result is obtained by the growth of *beta* type streptococci in blood bouillon. As a result of the above and other observations it is con-

cluded that the clear colorless zones produced by *beta* type streptococci within 24 hours are the result of laking of the blood corpuscles and diffusion outward of the released hemoglobin.

The *alpha* type zones are probably best explained by the theory that (1) in the neighborhood of the colony there are processes of reduction and oxidation which result in methemoglobinization of the blood corpuscles, apparent as a brownish or greenish discoloration; and (2) there is released a hemolytic substance which diffusing outward from the colony attacks the corpuscles outside the region of methemoglobinization. Methemoglobinization occurs only at incubator temperature; hemolysis occurs readily at room or refrigerator temperature. A combination of these factors results in the formation of alternate concentric rings of methemoglobinized corpuscles and of hemolysis about the *alpha* type colonies when blood agar plates are exposed to alternate periods of incubation and refrigeration.

There is reason to suspect that the hemolytic substances produced by *alpha* and *beta* types streptococci may not be the same.

When kept under suitable conditions the minute cultural characteristics of different strains of streptococci persist during long periods (several years) of cultivation on artificial media. This fact does not prejudge the possible effect of adverse conditions on streptococci.

The succession of various types of streptococci noted in convalescent throats and reported by others as occurring on other mucous membranes is regarded as a true succession of different strains accomplished by changing conditions rather than as a variation in the characters of the strains present.

In the absence of a natural classification of streptococci there is presented a convenient tabulation in which the names already applied have been retained and definitely defined with respect to type of appearance in blood agar and fermentation reactions. Although the specific names retained are in some instances given a more limited application than that intended by the authors who have described them, they are in no case given a different meaning.

In any system of classification or characterization that may be adopted it is urged that the loosely used terms "*hemolytic*" and "*nonhemolytic*" be replaced by recognition of the *alpha*, *beta*, and *gamma* types.

In view of the influence of many commonly ignored factors on the appearances in or on blood agar it is urged that in describing such appearances authors give full details regarding the composition and amount of medium used, the period of incubation, and subsequent treatment, method of inoculation, etc.

APPENDIX.

A Simple System of Notes for Preserving in Accessible Form the Genealogy and Other Details in the Study of Bacterial Cultures.

In view of the fluctuating variations and mutations of streptococci reported by a number of observers it was realized at the beginning of the work by Dr. Smith and myself that it was important to preserve a complete genealogical history of every strain studied by us from the time it came into our possession. Such a record we had and it has been continued and elaborated. It is quite common for bacteriologists to attach a "generation" number to each transplant of their strains so that they are able at any time to determine how many transplants or so called generations a particular strain has passed through. However, the kinds of media on which the strain has been cultivated; the age, reaction, and composition of each lot of medium; the length of time spent on it; the time and temperature of incubation and of storage; the kind, age, and condition of blood or ascitic fluid (if any) added to the medium, and if necessary the condition of the animal from which the blood or ascitic fluid was drawn, are all details which may be quite as important as the number of generations through which a strain has passed. To say that a strain has undergone "*spontaneous*" mutation or variation is merely to say that the causes or conditions of such changes have escaped recognition. It is important, therefore, to employ a system of notes which will record as many details as possible. It is equally important that such a system shall be simple and elastic, requiring practically no time or attention beyond the recording of observations, essential with any system of notes. Finally, when recorded, the notes must be accessible, for the most accurate observations are of little value if lost in a mass of notes.

A knowledge of the way in which records are kept is of importance in the proper valuation of work involving the question of variation. An apparent mutation may quite as readily be the result of an error in record as of an error in observation or of working with a mixed

culture. No apology is therefore due for describing a system of notes which is by no means entirely original but has been made possible by and is a development of methods found in use in this laboratory.

Media.—The laboratory assistant who makes the media keeps a journal in which is described in detail the making of each lot of medium; the materials entering into it, especially the source and condition of the meat, the temperature and time relations, and the reaction as determined by titration at various stages and that of the finally tubed and sterilized product. A serial number entered in the journal and recorded on each tube or flask of the medium makes it possible for one using the medium to refer to the journal at any time.

Animals.—The assistant having charge of the animals gives to each animal entering the laboratory a serial number which is entered on a blank with the animal's identification, history, and occasional observations as to its weight and general condition. When the animal is assigned to a worker for use, the blank passes to his care and on it he records his own notes regarding the use of the animal, clinical notes, and finally the autopsy. The blank is finally filed with those of all other animals used in the laboratory.

Cultural Notes.—Each strain of bacteria is given a certain designation, number, or name. Many bacteriologists designate strains by a serial number or by the date of their isolation. Rather than a serial number which soon becomes quite large and is not suggestive I employ one or more letters followed by a small number. The letters may represent the epidemic (as is the case with many of the strains reported here and by Smith and Brown in 1915) or may suggest the name of the patient or of the person, locality, laboratory, or material from which the strain was obtained. The number following the letter indicates the number of the strain obtained from the source indicated by the letter.

Examples.—B-6 represents the 6th strain isolated from epidemic B.
Ad4 represents the 4th strain isolated from a patient by the name of Adams.
D-Ad4 indicates the 4th strain isolated from Mr. Adams during epidemic D.

Any system of designation convenient to the bacteriologist may be used. It is important that it shall be *brief* for convenience, *suggestive*

so that the origin and facts connected with it are constantly suggested, and *elastic* so that it will answer all purposes. The remainder of the system of notes here described is illustrated by the sample cards in Plate 34.

"W4" is the designation of a swab received and the card accompanying the swab is filed at the beginning of the notes which are to be recorded on cards, each card representing a separate tube or plate culture. On the upper left-hand corner of each card is the designation (W4) followed by a fraction. The denominator of the fraction is the number of the individual card. Note that the denominators run serially, 1, 2, 3, 4, etc. The numerators, on the other hand, follow no apparent order; they are the numbers of the tube or plate from which the culture in hand was inoculated. For example, tube 3 (number in denominator) of bouillon (yellow card) was on April 4, 1915 (date of inoculation in upper right-hand corner of card), inoculated from a colony on agar plate 1 (blue card). This is indicated by transferring the number 1 from the denominator of the blue card to the numerator of the yellow card. A glance at the fractions on yellow cards Nos. 2, 4, and 5 (denominators) shows that each of these also has 1 for a numerator and therefore that they also were inoculated from colonies on agar plate 1. It will be noticed that some small letters (a, b, c, and d) have been added to the designation (W4) on cards Nos. 2, 3, 4, and 5 respectively. These small letters indicate the colonies on plate 1 from which each tube of bouillon was inoculated and the colonies will be found described in the notes on blue card No. 1. While W4 was the designation of the swab, W4a, W4b, W4c, and W4d are, therefore, the designations of strains isolated from the swab. Cards Nos. 6 and 7 (denominators) represent plates which, as indicated by their numerators, were inoculated from bouillon cultures Nos. 2 and 3 respectively; furthermore a comparison of dates in the upper right-hand corners shows that these bouillon cultures were about 24 hours old when plates 6 and 7 were inoculated from them. The condition of the bouillon cultures at this time is described in the notes on cards Nos. 2 and 3 under date of 5-IV (April 5). Observations of cultures are noted under the proper date (and hour, if significant) on the corresponding cards, the numbers being found on the culture tube and

on the upper left-hand corner of the card, and the cards being filed in the order of the serial denominator numbers and consequently according to the dates of inoculation found on the upper right-hand corner of the cards.

Suppose there is taken from the refrigerator a culture tube bearing the number W4b$\frac{17}{18}$ represented by the card of the same number (the last card on Plate 34) and that it is desired to know the history of this strain. The fraction $\frac{17}{18}$ refers to the card with 17 in the denominator (*i.e.*, $\frac{9}{17}$) which in turn refers to card 9 (*i.e.*, $\frac{7}{9}$) and this to $\frac{3}{7}$ and and thence to $\frac{1}{3}$ and $\frac{0}{1}$, the "0" indicating the "original" specimen. The above series of cards is therefore:

$\frac{0}{1}$

$\frac{1}{3}$

$\frac{3}{7}$

$\frac{7}{9}$

$\frac{9}{17}$

$\frac{17}{18}$ Taking these cards in hand it is found by counting them that the strain is in its sixth generation or transplant. Furthermore, there is on these six cards the complete genealogy of the strain and the notes of each generation with exact dates and a key to full details regarding each tube of medium. It is found that the strain was isolated into bouillon from a blood agar plate, replated on blood agar, grown in bouillon once more, passed through a rabbit, and recovered from the rabbit onto the agar slant which has been taken from the refrigerator. One can delve as much farther into the details as is desired without being confused by the mass of notes representing collateral experimental excursions, but if one cares to examine the records of any of the latter this can readily be done.

So much may be said for the *use* of the completed record, but what of the *making* of it? The simplicity of recording the cultures and taking the notes can be appreciated only by trying it. There is on the desk a small box of blank cards on which have been stamped, by means of a cheap rubber stamp with movable type, various headings which are convenient for the work in hand. The use of these stamped headings is in itself a great saver of time and adds to the legibility. The use of cards of different colors for different media,

etc., facilitates the picking out of the right card from either the box of unused cards or from the card index. The colors are soon fixed in memory so that the selection of the right color requires no additional thought or labor. Each tube of medium that comes to hand is labeled, *e.g.* Bouil. 1027, the number referring to the record of the assistant who has prepared the medium and which may be consulted if desired. It is desired to inoculate this tube of bouillon from a culture bearing the following label Agar 800 W4b $\frac{9}{10}$. Having been inoculated it is labeled Bouil. 1027 W4b $\frac{10}{\ }$. A bouillon (yellow) card is selected, and the number of the medium recorded under the word ***Bouillon*** with any other remarks regarding the medium that may be of particular interest. The date is written in the upper right-hand corner of the card and the culture number, W4b $\frac{10}{\ }$, in the left corner. But one thing is lacking, the denominator. Turning to the card index it is found that the last card filed under this strain had 18 for a denominator. The next one will therefore be 19 which is placed on the tube label Bouil. 1027 W4b $\frac{10}{19}$ and on its card. The tube is then incubated and the card filed. Any notes subsequently made on this culture are written under their respective dates on the card. Both sides of the card are used if necessary and occasionally a second card is inserted. The cards belonging to each strain or from each swab (as in the present illustration) are set apart in the card index drawer by slipping a little tab over the edge of the first card.

The labor involved is no greater than that of keeping any system of notes; the tube must be labeled; the number, date, and medium entered; and subsequent notes recorded. The labor of making these entries on cards is no greater than that of entering them on papers or in a book. The cards in the index need be referred to only to find the denominator of the last card, certainly no more trouble than to find the right page in a book. In fact the same system of numbering may be followed in a book or on loose sheets of paper, but the colored cards have the advantage of making the notes readily accessible; the colored edges of the cards are always in view and indi-

vidual cards are easily taken out, studied (as in the tracing of the genealogy), and replaced.

To the system as above described may be added another refinement which does require a little extra labor but which need be used only occasionally. There is a so called "comb" (Plate 34) on the rulings of which are printed certain suggestive titles or key-words to facts in the notes about which one may wish to inquire. The red and black rulings of the comb are continued along the top edges of the cards. (The edges of 1,000 new cards may be ruled in a few minutes by ruling them all at once in the box in which they are bought.) The rulings on the edges of the cards are hardly visible from the face of the individual card but are quite conspicuous forming continuous lines when the cards are viewed from their upper edges as they are packed together in the index drawer. By means of a card punch, using the edge of the comb placed over the edge of the card as a guide, notches can be cut into the edge of the card corresponding to the key-words on the comb. The comb can be moved along in the index and by means of the appropriate notch the record of any particular feature (*e.g.*, a capsule stain, or a fermentation of lactose) can be immediately selected from among hundreds of cards. The cards may be punched at any time after taking the notes and this feature may be used as much or as little as desired, but I often find it convenient to mark in this way some special record or experiment that I may wish to refer to later. Together with the colors of the cards this feature renders the notes readily accessible.

1,000 cards occupy a space of $8\frac{1}{2}$ inches in the index drawer. During the past year I have used about 3,000 cards costing $1.50 per thousand. The files of old cultures are conveniently stored in the pasteboard boxes in which the cards were bought. With very little expenditure of time, effort, space, or money I have the complete and readily accessible genealogical records of nearly one hundred fifty strains of streptococci.

A Simple and Inexpensive Method of Making Photographic Records of Growth on Agar and Blood Agar Plates.

Although detailed genealogical records were kept for all the strains reported by Smith and Brown (1915) and for all strains isolated by

me since then, and although from the first careful descriptions with measurements of colonies and zones of hemolysis or discoloration on blood agar plates were made, it was realized that in a series of experiments which were to extend over a period of several years even the memory assisted by notes might make mistakes and that certain details which escaped observation might be preserved photographically. About 2 years ago, therefore, while the strains then being studied were fresh in memory a series of representative strains for intensive study was selected, and of them together with many strains obtained since photographic records consisting now of well over 500 photographs have been made. The advantages are:

1. The photographs are permanent records of appearances in blood agar, not subject to the suspicion of faulty notes or memory.

2. Details, unnoticed or the importance of which was not realized at the time the culture was observed, are preserved and discovered in the photographs many months later when, except for the photographic record, they might be regarded as newly acquired characteristics. It is surprising how the emphasis in one's observations will shift from certain characteristics to others after intervals of several months.

3. Photographs of the same plate or colony on successive days of incubation or other treatment can be viewed and compared side by side. In no other way can the gradual development of a culture and of its minute characteristics be appreciated so well.

4. The appearance of the growth of the same or different strains on the same or different media can be compared at the same or different times, even though one or more of the strains may have been lost long ago.

Obvious though these and other advantages are, to be practical the method of making the photographs could not be too laborious or expensive. The object was to develop methods which could be employed in almost any well equipped bacteriological laboratory and therefore to avoid the use of expensive apparatus on the market for such purposes. The apparatus used for making all the photographs shown in the plates accompanying this thesis is shown in Plate 33.[12]

[12] The white back-ground was placed back of the apparatus merely for making this photograph.

Simple though it is, it is the result of numerous experiments with different arrangements of lights, diaphragms, lenses, distances, and photographic materials.

The apparatus for photographing the agar plates (natural size) consists merely of a strong (48 candle power) "Mazda" electric light enclosed in a tin can at a distance of 6 feet above the table (see upper left-hand corner of the photograph). The light escapes downward onto the table through an opening 2.5 cm. in diameter in the side of the tin can, this opening being covered with waxed cloth or paper to make it translucent. The light is turned on or off at the switch board on the table below. The entire apparatus is in a dark room. To make a photograph, a photographic plate is placed on the table directly under the light (film side up) and the agar plate to be photographed is placed directly on the photographic plate, the cover of the Petri dish removed, and the light turned on for about 5 seconds and then off again. The agar plate is covered and removed, and the photographic plate developed and fixed in the ordinary way. The picture is therefore a direct print or photograph by transmitted light rather than by reflected light. No lenses or camera are required. This method is much more satisfactory for photographing blood agar plates than is photographing with the camera by reflected light in the usual way, for in order to show hemolyzed zones and deep colonies the light must pass through the plate. The deep colonies are photographed perfectly by this simple direct method but because of refraction of light by the raised edges of the top surface colonies these appear with a double contour which if borne in mind serves to differentiate top surface colonies from bottom surface colonies which appear flat and with a single contour (Plate 6, Fig. 1). In photographing many blood agar plates half the plate was covered by a fine net so that the distinctness of the image of the net in the hemolyzed zones might indicate the degree of transparency of the zones, but later this device was abandoned as being unnecessary, the distinctness of the deep colony itself within the zone being quite as good an index of transparency (see Plates 4 and 7).

The photomicrographic apparatus consists of a wooden box about 10 x 12 x 18 inches in size. The box stands on edge as shown in Plate 33, one side, the front, being open but provided with a curtain

of black light-proof cloth. A hole in the top edge of the box is provided with a collar of the black cloth and a draw-string by means of which the collar is drawn tightly about the protuding tube of the microscope within the box. Inside the box is also a "Mazda" light (20 candle power) enclosed in a tin can like that described above and controlled from the same switchboard. The interior of the box is coated with a dull black paint. (For making Plate 33 a piece of white paper was placed in the back of the box merely for photographing the apparatus so that the interior arrangement could be seen better.) For photographing the colonies shown throughout the plates there was used an objective of very low power, no condenser, and no ocular. The agar plate containing the colony to be photographed is placed on the stage of the microscope and the image of the colony focused onto a piece of ground glass supported by the ring of a ring-stand and about 10 inches above the tube of the microscope. When the colony is in focus the light is turned off, the ground glass replaced by a photographic plate, and the light turned on again for a few seconds' exposure, after which the photographic plate is developed and fixed in the usual way. It was found necessary to slip a sleeve of black paper inside the tube of the microscope to prevent reflection from the sides of the tube.

This apparatus is particularly good for taking low power photomicrographs but I have also taken with it a fairly good photograph of encapsulated streptococci under the oil immersion objective. With low power objectives the condenser is not used but must be used with high power objectives.

Many kinds of photographic plates were tried before one that was entirely satisfactory was found. The essential qualities of the plate are (1) that it shall be slow so that the exposure can be regulated satisfactorily from the switchboard, and (2) that it shall present a great deal of detail and not too much contrast. For the photographs taken in 1915 "Wellington Special Lantern Slide Plates" were used with very good results, but in 1916 it was found that the "Wellington Ortho-process Plate" showed more detail. Some slight differences in the photographs of 1915 and those of 1916 are due to the differences in the plates used. The plate of most convenient size is $3\frac{1}{4} \times 4\frac{1}{4}$ inches. For the photomicrographs these plates are cut into four parts.

After the methods are thoroughly standardized so that the proper exposure for a blood agar plate of standard size containing a standard amount of agar and blood is known, one can photograph a plate and a colony in 2 minutes in the dark room, the exposed photographic plates being stored in light-proof boxes until a number have accumulated before being developed. The cost is about 5 cents for the plate and the first print and less than 2 cents for each duplicate print.

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TABLE I.

Tabular Description of the Principal Strains Referred to in this Work.

Designation.	Type in blood agar.	Source.	Date of isolation or possession.	Growth in bouillon.		Fermentation.						Pathogenesis for the rabbit.	Names as they might be used by various authors.
				Gross appearance.	Morphology.	Saccharose.	Lactose.	Raffinose.	Inulin.	Mannite.	Salicin.		
D-1	γ	Septic sore throat 6th day. Same case as D-1α.	Mar., 1914.	Somewhat clouded; good amount of fine flocculent sediment, disintegrated with difficulty.	Short and medium chains of fairly large round or flattened cocci.	+	−	+	−	−	+		*S. saprophyticus* (Mandelbaum). *S. anhemolyticus* (Zangemeister). *S. equinus* (Andrewes and Horder). " (Holman).
A-18 (cow)	β	Milk from single cow in suspected herd.	June, 1913.	Not clouded; abundant loose fleecy growth on bottom, easily disintegrated by shaking.	Very long rope-like chains of fairly large round or flattened cocci, often arranged in pairs.	+	+	−	−	−	−	−	*S. anginosus* (Andrewes and Horder). " (Holman).
B-18 (cow)	β	Milk from single cow in suspected herd.	May, 1913.	Well clouded; some fine flocculent sediment.	Short and medium chains of fairly large round or flattened cocci, often arranged in pairs.	+	+	−	−	−	−	−	*S. anginosus* (Andrewes and Horder). " (Holman).
A-ST	β	Peritoneal pus during epidemic of septic sore throat.	May, 1913.	Clouding variable; fine flocculent sediment.	Long chains of round elements, sometimes twisted into clumps.	+	−	−	−	+	+	+	*S. longus pathogenes seu erysipelatos* (Schottmüller). *S. pyogenes* (Rosenbach). *S. hemolyticus* (Rolly). " (Davis). *S. hemolyticus II* (Holman). *S. alactosus* (Smith and Brown).
D-AD4	β	Throat of dairyman nearly 3 wks. after clinical recovery from tonsillitis.	Mar., 1914.	Not clouded; granular sediment. Later fairly well clouded with small suspended particles; flocculent sediment.	Medium sized round cocci in moderately long tortuous chains forming tangled clumps.	+	+	−	−	−	+	+	*S. longus pathogenes seu erysipelatos* (Schottmüller). *S. pyogenes* (Rosenbach). " (Andrewes and Horder). " (Holman). *S. hemolyticus* (Rolly). " (Davis).
B-2b (cow)	β	Milk from a supposedly sound quarter of the udder of a cow known to have mastitis in another quarter.	May, 1913.	Fairly well clouded with fine suspended particles; good amount of fine flocculent sediment.	Fairly long chains and clumps of rather large round cocci, swollen elements not uncommon.	+	+	−	−	−	+	+	*S. epidemicus* (Davis). *S. mucosus* (?) (Howard and Perkins).
B-6	α′ α	Throat of convalescent dairyman. Same patient as B-7α.	May, 1913.	Moderately clouded; some fine flocculent sediment.	Rather small round or oval elements in chains of various lengths, often crooked.	+	+	+	−	−	+	−	*S. pyogenes* or *S. mitis* (?) (Andrewes and Horder). *S. pyogenes* or *S. mitis* (?) (Holman).
F-2	α′ α	Septic sore throat 9th day. Same case as F-2α.	May, 1914.	Moderately clouded; small amount of compact sediment.	Short straight chains of rather small oval elements.	+	+	+	−	−	+		*S. pyogenes* or *S. mitis* (?) (Andrewes and Horder). *S. pyogenes* or *S. mitis* (?) (Holman).
A-BL	α	Throat, during epidemic of tonsillitis.	May, 1913.	Well clouded; small amount of sediment.	Small, mostly oval or elongated elements in clumps of tangled chains.	+	+	−	−	−	+	−	*S. mitior seu viridans* (Schottmüller). *S. mitis* (Andrewes and Horder). " (Holman).
D-4	α	Septic sore throat 8th day.	Mar., 1914.	Growth rather scant; rather faintly clouded; fair amount of fine sediment.	Long straight chains of fairly large oval or elongated elements.	+	+	−	−	−	−		*S. mitior seu viridans* (Schottmüller). *S. salivarius* (?) (Andrewes and Horder). " (Holman).
B-7	α	Throat of convalescent dairyman. Same patient as B-6α′.	May, 1913.	Well clouded; little or no sediment.	Short chains of rather small round or oval elements.	+	+	+	−	−	+	−	*S. mitior seu viridans* (Schottmüller). *S. mitis* or *S. salivarius* (*variant*) (Andrewes and Horder). *S. mitis* (Holman).
E-4	α	Throat of child with sore throat, adenitis, and erysipelas.	Mar., 1914.	Well clouded; little or no sediment.	Short chains of small, mostly slightly elongated elements.	+	+	−	−	−	−		*S. mitior seu viridans* (Schottmüller). *S. salivarius* (Andrewes and Horder). " (Holman).
D-AD8$_2$	α	Throat, convalescent, 11th day.	Apr., 1914.	Not clouded; abundant fleecy growth on bottom of tube, thready when shaken.	Skeins of very long parallel chains of fair sized round and ovoid elements.	+	+	−	−	−	−		*S. mitior seu viridans* (?) (Schottmüller). *S. longissimus* (Thalmann). *S. salivarius* or *S. anginosus* (?) (Andrewes and Horder). *S. salivarius* or *S. anginosus* (?) (Holman).
D-1	α	Septic sore throat 6th day. Same case as D-1γ.	Mar., 1914.	Well clouded; small amount of fine flocculent sediment.	Short and medium chains of rather small round and elongated elements; chains often crooked or clumped.	+	+	−	−	−	+		*S. mitior seu viridans* (Schottmüller). *S. conglomeratus* (Kurth). " (Thalmann). *S. mitis* (Andrewes and Horder). " (Holman).
F-2	α	Septic sore throat 9th day. Same case as F-2α′.	May, 1914.	Slightly or moderately clouded with small suspended particles; good amount of sediment breaking up into small flakes when shaken.	Short and medium chains of rather small round and elongated elements.	+	+	+	−	−	−		*S. mitior seu viridans* (Schottmüller). *S. conglomeratus* (Kurth). " (Thalmann). *S. salivarius* (Andrewes and Horder). " (Holman).
Brig.-IIa	α	Sputum from a supposed case of influenza. Pneumonia recognized next day after this sample was taken.	Dec., 1915.	Fairly well clouded; good amount of ropy sediment.	Short and medium chains of round or slightly oval elements, the entire chain enclosed in a broad capsule.	+	+	+	+	±	+		*S. mucosus* (?) (Howard and Perkins). " (Schottmüller). *Pn.* " (Park and Williams).
Cole I 109	α	Dr. Rufus Cole, Rockefeller Institute.	May, 1915.	Well clouded; little or no sediment.	Encapsulated lanceolate diplococci.	+	+	+	+	−	+	+	Pneumococcus (Fränkel). *M. lanceolatus.* *S. pneumoniæ* (Davis, Park, and Williams, and others).
Cole II 45	α	Dr. Rufus Cole, Rockefeller Institute.	May, 1915.	Well clouded; little or no sediment.	Encapsulated lanceolate diplococci.	+	+	+	+	±	+	+	Pneumococcus (Fränkel). *M. lanceolatus.* *S. pneumoniæ* (Davis, Park, and Williams, and others).

*All the strains were Gram-positive. Only the strain of *S. mucosus* and the Pneumococci were soluble in sodium taurocholate. It is not to be understood that the strains described were considered responsible for the conditions described under "Source;" the strains tabulated were selected merely as representatives of different varieties of streptococci.

TABLE V—*Continued.*

Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
10 strains from horses of which 5 were from "*Druseeiter.*" 2 strains from human suppurations. 2 strains from the milk of cows with mastitis. 1 strain from the organs of an aborted calf.	3 parts of 2 per cent peptone water + 1 part of filtered horse serum. To 9 parts of the above added 1 part of sterile 10 per cent solution of the test substance in litmus solution. Distributed 10 cc. amounts into test-tubes and heated 2 hrs. at 58° on 3 successive days.	Arabinose, rhamnose, xylose, dextrose, levulose, galactose, mannose, lactose, saccharose, maltose, raffinose, soluble starch, dextrin, glycogen, inulin, arbutin, amygdalin, salicin, glycerol, erythrite, adonite, mannite, sorbite.	Litmus.	7 days.	All strains produced acid from dextrose, mannose, galactose, levulose, saccharose, maltose, soluble starch, dextrin, salicin, and arbutin. None attacked arabinose, xylose, erythrite, or adonite. There remained 9 substances which fermented as indicated in the following table (compiled): Organisms. Rhamnose. Lactose. Raffinose. Glycogen. Inulin. Amygdalin. Glycerol. Mannite. Sorbite. *Pferdestämmen* (5)..... 2 1 4 1 1 1 2 *Drusestämmen* (5)..... 4 2 2 2 2 2 (3) 2 Human strains (2)..... 2 2 Bovine " (3)..... 3 1 3 3 (3) 3 3 *Note.*—The figures indicate the number of strains fermenting the substances indicated. The figures in parentheses indicate strains said by the author to have been doubtful. No figure indicates that none of the strains fermented the substance. Tests in fermentation tubes showed that none of the strains produced the smallest trace of gas. The litmus was reduced in the closed arm of the tubes by some strains at times. The author concludes: "*Es haben sich aus dem Verhalten der geprüften 15 Streptokokkenstämme gegenüber 23 verschiedenen Kohlehydraten oder kohlehydratähnlichen Substanzen bei der gewählten Versuchsanordnung Anhaltspunkte für eine Charakterisierung bestimmter Streptokokkenarten oder -gruppen nicht ergeben.*"	**Maass** 1913. Zur Frage der Streptokokken-Differenzierung durch kohlehydrathaltige Nährboden.
		See under Results and Conclusions.			*S. equi* fermented dextrose, mannose, galactose, fructose, maltose, cellobiose, saccharose, dextrin, glycogen, soluble starch, salicin, and arbutin (feebly). It did not ferment sorbose, xylose, arabinose, rhamnose, glycoheptose, trehalose, formose, gentiobiose, lactose, raffinose, inulin, sorbite, mannite, dulcite, adonite, glycerol, erythrite, perseite, or amygdaline.	**Bemelmans** 1913. La spécificité des streptocoques de la gourme.
Many strains from various sources.		Dextrose, saccharose, lactose, maltose, mannite, inulin, raffinose.			Records of fermentation reactions are not given in full for all strains. No titration figures are given. Most of the pneumococcus forms and none of the streptococcus forms tabulated fermented inulin; only 1 pneumococcus is tabulated as fermenting mannite, but about half the streptococci fermented this substance. All strains fermented dextrose and nearly all fermented lactose, saccharose, and maltose. "*The results obtained* (with reference to variability) *show clearly why the classification of streptococci based on fermentative powers alone has proven to be unsatisfactory.*"	**Rosenow** 1914. Transmutations Within the Streptococcus-Pneumococcus Group.
14 strains of streptococci from joint fluid of 14 cases of acute rheumatism.		Dextrose, mannite, inulin.	Titrated, in some cases at least.		"*Three of the strains of the first group* (green producers on blood agar) *and all of the strains which produced muscle lesions ferment mannite but not inulin and produce a high acidity in dextrose broth* (*5–7 per cent*)."	**Rosenow** 1914. The Etiology of Acute Rheumatism, Articular and Muscular.
247 strains of streptococci from various human pathological conditions.	Hiss serum water. Also dextrose bouillon used. (Test substances in 10 per cent aqueous solution, "*sterilized by steaming for twenty minutes,*" and then added in the proportion of 1–9 cc. of serum water which had been previously autoclaved.)	Dextrose, saccharose, lactose, salicin, raffinose, mannite, inulin. Litmus milk.	Neutral red, in medium for all strains. One-half of strains also titrated, cold (phenolphthalein).	1 wk.	The authors divide 247 strains into 6 groups comprising no less than 51 combinations of action on the test substances including hemolysis of blood agar. Notwithstanding the authors' statements the groups apparently do not correspond with those of Andrewes and Horder. Especially noteworthy are the large number of non-fermenters, and the presence of some (21 strains) which fermented lactose or acidified milk but did not ferment dextrose. "*It is possible to make, more or less arbitrarily, divisions of streptococci from human sources, according to their fermentative properties in media containing the test substances selected by Gordon. * * The agglutination tests and complement fixation tests support the evidence yielded by cultural tests.*"	**Floyd and Wolbach** 1914. On the Differentiation of Streptococci; Preliminary Notes.

TABLE V—*Continued.*

Fermentation Reactions.

TABLE V—*Continued.*

Fermentation Reactions.

TABLE V—*Continued.*

Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
18 throat strains. 1 strain from cervical adenitis. 9 strains from milk slime.	Hiss serum water.	Dextrose, lactose, saccharose, salicin, raffinose, mannite, inulin, dulcite. Milk.	Presumably litmus. No titrations.	7 days.	All strains fermented dextrose, lactose, and saccharose; none fermented mannite, inulin, or dulcite. The strains were divided into 4 groups by their action on salicin and raffinose: Group A: Salicin +, Raffinose + " B: " −, " + " C: " +, " − " D: " −, " − Strains from 2 cows with inflamed udders and 4 throat strains fell into Group D.	**North, White, and Avery** 1914. A Septic Sore Throat Epidemic in Cortland and Homer, N. Y.
42 strains from milk. 14 strains from cows' feces. 51 strains from infected udders. 39 strains from cows' mouths.	Beef extract 0.4 per cent, peptone 1 per cent, dibasic potassium phosphate 0.5 per cent, and test substance 2 per cent. Gelatin.	Dextrose, saccharose, lactose, raffinose, starch, inulin, mannite, glycerol, adonite, dulcite. Litmus milk.	Titrated, phenolphthalein.	7 days at 30°C.	Any result above 1 per cent N acid was considered positive. " * * * *the 'metabolic gradient'* * *, in our opinion, can be correct only for the particular group under consideration."* 83 per cent of strains from the mouths of cows, and only 7, 23.5, and 37.5 per cent of those from the udders, milk, and feces respectively reduced litmus in milk. Adonite and dulcite were fermented by only 1 or 2 strains and hence were of no value. The strains studied were classified as follows: From udders Group 1.. Dextrose. + Lactose. + Saccharose. + Mannite. Occasionally. " * * *agrees with the published description of S. pyog."* Group 2.. Dextrose. + Lactose. + Saccharose. + Mannite. Usually. Glycerol. Usually. Gelatin. Often liquefied. From feces.. Dextrose. + Lactose. + Saccharose. + Raffinose. + Mannite. − Starch. Often. Inulin. Often. Gelatin. Liquefied. From mouth. Differ from feces strains in more frequently fermenting raffinose, mannite, and inulin, and in failure to ferment starch. From milk. 2 strains belonged to the feces group; *"all others to one of the two udder groups."*	**Rogers and Dahlberg** 1914. The Origin of Some of the Streptococci Found in Milk.
105 strains from various pathological processes, and from milk, normal throats, and human stools.	Unfermented veal infusion bouillon + 1 per cent of test substance.	Lactose, saccharose, salicin, raffinose, mannite, inulin. (Starch, glycerol, dextrose, arabinose.) Milk.	Titrated, phenolphthalein.	24 hrs.	*"We determined to disregard other tests and group them strictly according to their fermentation reactions,* * * ." Starch, glycerol, dextrose (fermented by all) and arabinose (fermented by none) were of no value. Using the 6 substances mentioned below the classification arrived at was as follows: A. Pathogenic type: Group 1..... + + + − − − B. Saprophytic types (*a*) Salivary..... Group 2..... + + − + − − " 3..... + + + + − − " 4..... + + − + − + " 5..... + + − − − − (*b*) Fecal..... Group 6..... + + + − + − *"We derived no useful information from our titrations that could not have been obtained by the qualitative litmus test."*	**Hopkins and Lang** 1914. Classification of Pathogenic Streptococci by Fermentation Reactions.

TABLE V—*Continued.*
Fermentation Reactions.

TABLE V—*Continued.*
Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
Many strains.	Double strength Liebig extract broth + 2 per cent test substance, sterilized fractionally and then mixed with an equal volume of filtered (unheated) 50 per cent beef serum water.		Andrade's indicator (decolorized acid fuchsin).		This medium is not coagulated by the acids produced. "*It is, I believe, fundamental in the study of the fermentation reactions of the streptococci, to have a medium in which the organisms grow well independently of the carbohydrate added. Although many of the streptococci will ferment a certain carbohydrate in broth, serum water, or serum broth, others grow so poorly in the former that they fail to attack the carbohydrate, and the result would appear negative if no further study were undertaken.*"	**Holman** 1914. A Method for Making Carbohydrate Serum Broth of Constant Composition for Use in the Study of Streptococci.
6 strains of *S. hemolyticus*. 5 strains of pneumococci. 24 strains of *S. viridans*. (All from various pathological conditions in man.)	"*Agar containing sugar-free broth from fresh meat and made neutral to litmus was used.*" "*The carbohydrates were sterilized separately for only 15 minutes in the Arnold sterilizer and added to the melted agar, * * *.*"	Saccharose, lactose, arabinose, raffinose, inulin, dextrin, mannite, salicin.	Litmus.		By repeated plating out of supposedly pure cultures the author found different individual colonies to give rise to strains with different fermentative reactions.	**Thro** 1914. Experiments on the Variability of the Fermentative Reaction of Bacteria, Especially the Streptococci.
7 strains from throats. 3 strains from suspected cow's milk. 2 strains from Chicago epidemic (1912).		Dextrose, lactose, saccharose, maltose, raffinose, mannite, inulin.			All strains were alike in fermenting dextrose, saccharose, lactose, and maltose, but not mannite, inulin, or raffinose, except for 1 throat strain which failed to ferment lactose and 1 bovine strain which failed to ferment saccharose.	**Capps and Davis** 1914. An Epidemic of Streptococcus Sore Throat in Jacksonville, Ill., Which Was Traced to the Milk of Cows Affected with Streptococcus Mastitis.
263 strains from various sources, mostly pathological conditions.	Hiss serum water + 1 per cent of test substance. Sterilized fractionally after addition of test substances.	Salicin, raffinose, mannite, inulin, (saccharose, lactose, dextrose). Milk.	Litmus.	10 days.	Recognized all the groups of Andrewes and Horder and 1 other, fermenting salicin and raffinose. "*Dextrose, lactose, and saccharose were tested with over 100 strains. With few exceptions all strains produced acid from these carbohydrates and they were consequently of no significance in differentiation. Dulcite was used with over one half of the series, giving only one positive result, a Pneumococcus mucosus. Litmus milk was employed throughout, but does not appear to give results which are sufficiently definite to be of value.*" (See also Table VIII on General Conclusions, etc.)	**Lyall** 1914. On the Classification of the Streptococci.
53 strains of streptococci from sore throats and secondary lesions in man. 12 strains from cow's milk.	Fermented veal infusion bouillon; 1 per cent N acid (infusion fermented 8 hrs. by *B. coli*, boiled, neutralized, and made into bouillon). Autoclaved 13 per cent aqueous solutions of test substances, then added 1–12 cc. of fermented bouillon.	Dextrose, maltose, saccharose, lactose, raffinose, mannite, inulin, salicin. Milk.	Titrated (phenolphthalein).	1 wk.	The α strains, the hemolytic bovine strains, and non-virulent strains generally produced more acid than the virulent β strains. All the strains fell into 8 groups according to their fermentative reactions.	**Smith and Brown** 1915. A Study of the Streptococci Isolated from Certain Presumably Milk-Borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914.

Group.	No. of Strains. α	No. of Strains. β	Dextrose.	Maltose.	Saccharose.	Lactose.	Raffinose.	Mannite.	Inulin.	Salicin.
I	4	2	+	+	+	+	+	−	−	+
II	6	33	+	+	+	+	−	−	−	+
III		4	+	+	+	−	−	+	−	+
IV		1	+	+	+	−	−	−	−	+
V	1		+	+	−	+	−	−	−	+
VI	2		+	+	+	+	+	−	−	−
VII	3	8	+	+	+	+	−	−	−	−
VIII	1		+	+	+	+	−	−	+	+

(See also Table VIII on General Conclusions, etc.)

2 strains of *S. lacticus* from ice cream and from milk.	Fermented beef infusion bouillon (infusion fermented 24 hrs. by *B. coli*, strained, boiled, peptone and salt added, neutralized with calcium carbonate). Test substances added dry and then sterilized (?).	Dextrose, saccharose, lactose, raffinose, inulin, salicin, mannite. Litmus milk.	Titrated (phenolphthalein).	3 days.	Both strains originally fermented dextrose, lactose, saccharose, and salicin. 1 of them fermented mannite also. Neither strain fermented raffinose or inulin. (See also Table VII on Mutations and Variability.)	**Helnemann** 1915. The Variability of Two Strains of *Streptococcus lacticus*.
17 strains from appendix tissue of 17 of 20 cases of appendicitis.	Ascites broth. (No details.)	Dextrose.			"*The strains of streptococci which were found to have an affinity for the appendix formed short chains, much acid, and a diffuse turbidity in ascites dextrose broth.*"	**Rosenow** 1915. The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli.

TABLE V—*Continued.*
Fermentation Reactions.

TABLE V—*Continued.*
Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
	(*a*) Plain broth + 1 per cent of test substance. (*b*) Beef serum broth sterilized at 60–70°C. on several days. (*c*) Holman's carbohydrate broth + filtered beef serum.	Lactose, saccharose, mannite, raffinose, salicin, inulin. Litmus milk.	(*a*) Litmus. (*b*) Titrated. (*c*) Andrade's indicator.		(*a*) This medium was soon abandoned "*as the organisms grew in it very slowly or not at all.*" (*b*) "*The results were so irregular and so at variance with our other experiments that we felt there was some undetected source of error.*" (*c*) "*With this medium we obtained uniform and clear cut results,* * * ." 29 green-producing strains are tabulated with reference to their action on the test substances. All but 3 acidified milk and most of them coagulated it; all but 4 fermented lactose; all but 3 fermented saccharose; about half fermented raffinose and salicin; only 1 fermented mannite; and none fermented inulin. "*From the fermentation reactions these belong to the classes S. mitis, S. salivarius, and S. fecalis of Andrewes and Horder.*"	**Hartzell and Henrici** 1915. A Study of Streptococci from Pyorrhea Alveolaris and from Apical Abscesses.
60 strains from various human pathological conditions.	"*Meat infusion broth*" with 1 per cent or 2 per cent of peptone.	Lactose, saccharose, salicin, raffinose, mannite, inulin.	Titrated (phenolphthalein).	3 days.	"*It was found in agreement with Hopkins that a broth containing 2 per cent peptone gave more uniform results,*" than did one containing 1 per cent peptone. (See also Table VIII on General Conclusions, etc. and Table VII on Mutations and Variability.)	**Kligler** 1915. A Study of the Correlation of the Agglutination and the Fermentation Reactions Among the Streptococci.
Strains of *S. viridans* from various human pathological conditions.	"*Sugar-free broth (made from fresh meat), three parts, and sterilized ascitic fluid, one part.*" Added 1 per cent of test substance, sterilized separately 20 min. at 100°.	Salicin, raffinose. (Mannite, inulin.) Milk.	Titrated (phenolphthalein).		Plated out supposedly pure cultures and tested the fermentation reactions of strains from different colonies. The only variations reported are with respect to salicin and raffinose.	**Thro** 1915. Further Experiments on the Variability of the Fermentative Reactions of Bacteria, Especially the Streptococci.
Hemolytic streptococci from sore throats, 1 strain from secondary peritonitis, and 2 from udder of cow, compared with various other hemolytic and non-hemolytic strains.					"*Serum water is unsuited for the determination of sugar fermentation. All of the type strains failed to ferment salicin using this medium, although prompt fermentation took place using serum broth or serum water containing one per cent of peptone.*" The strains reported from the epidemic at Rockville Centre fermented salicin but not raffinose or mannite. (See Table VIII on General Conclusions, etc.)	**Krumwiede and Valentine** 1915. A Bacteriological Study of an Epidemic of Septic Sore Throat.
Over 500 strains from various sources, human and animal.	"*Sugar-free broth*" plus 1 per cent of the test substances. (See under Broadhurst, 1913.) Sterilized fractionally after addition of test substances.	Saccharose, lactose, salicin, raffinose, mannite, inulin. Litmus milk.	Titrated cold (phenolphthalein).	3 days.	The fermentative reactions and other characters of over 500 strains are tabulated. The author's tables show that "*the main representatives of a species are usually from the habitat designated by Andrewes and Horder,*" * * "*plus two fermentative combinations common in my own strains.*" "*On the other hand, strains from a selected habitat are scattered through a number of species;* * * ." " * * *the combinations most frequent here are also those occurring most often in the 'family tree'* * * ." (See Table VII on Mutations and Variability.) "*A comparison of the amounts of acid formed yields little of value.*"	**Broadhurst** 1915. Environmental Studies of Streptococci, with Special Reference to the Fermentative Reactions.
					(See Table VII on Mutations and Variability.)	**Irons, Brown, and Nadler** 1916. The Localization of Streptococci in the Eye.
					Merely the following statement with regard to fermentation reactions: "*It might be said, however, that the fermentative powers of some of the strains having affinity for the appendix suggest the latter origin* (*i.e.*, udders of cows), *while those of strains producing parotitis suggest the former source* (*i.e.*, human sources)."	**Rosenow and Dunlap** 1916. An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products.
7 strains of streptococci from tonsils and pyorrheal pockets of cases of herpes zoster; 1 strain from spinal fluid.	"*Broth.*"	"*Various sugars.*"			"*The fermentative powers of 7 of these strains that were proved to have affinity for the ganglia were tested on various sugars in broth. All but 1 produced acid in saccharose; all but 2 in salicin; 3 fermented raffinose, and 2 mannite; none fermented inulin. In short, these streptococci had the features of a pneumococcus, except high virulence, capsule, and inulin fermentative powers.*"	**Rosenow and Oftedal** 1916. The Etiology and Experimental Production of Herpes Zoster.

TABLE V—*Concluded.*
Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
Résumé of 2463 strains studied by the author and others.	Unheated beef serum broth + 1 per cent test substance. (See under Holman, 1914.)	Lactose, mannite, salicin, inulin.	Andrade's indicator.	At least 1 wk.	*"Quantitative carbohydrate acid tests by titration are not as useful as the qualitative tests."* (See Table VIII on General Conclusions, etc.)	**Holman** 1916. The Classification of Streptococci.
1 strain of hemolytic streptococci (human type) from udder of cow without gross evidence of mastitis. 1 strain of human type streptococcus isolated by Davis (1912) from bovine mastitis. 1 strain of *S. epidemicus* isolated by Davis (1912) from peritoneal pus of fatal case of peritonitis.		Dextrose, lactose, saccharose, maltose, salicin.			Dextrose, lactose, saccharose, maltose, and salicin were fermented by all 3 strains mentioned.	**Mathers** 1916. Different Types of Streptococci and Their Relation to Bovine Mastitis.
79 of 85 strains of hemolytic streptococci from 328 samples of bottle milk.	*"1 per cent sugar broth."*	Glucose, lactose, maltose, saccharose, raffinose, salicin, mannite, inulin. Milk.	Titrated (results for only 14 strains recorded).	1 wk.	*"Of 79 strains of these streptococci tested on 8 sugars, all fermented dextrose, lactose and maltose, 11 failed to ferment saccharose, 65 failed to ferment mannite, 11 failed to ferment salicin, 3 fermented inulin, and 5 fermented raffinose."* *"In the fermentable carbohydrate broths the milk strains grow more profusely, acidify more quickly, with generally a higher terminal acidity,"* than do the human strains. Litmus milk was rapidly acidified and coagulated by the milk strains.	**Davis** 1916. Hemolytic Streptococci Found in Milk.
5 strains: (1) *"A strongly hemolytic streptococcus"* from a case of erysipelas; (2) *"a hemolytic strain from a scarlatinal sore throat;"* (3) *"a hemolytic streptococcus from the tonsils in a case of rheumatic fever;"* (4) *"a non-hemolytic streptococcus"* from pneumonic sputum; (5) *"a green-producing streptococcus"* from the same source as No. 3.		Dextrose, lactose, saccharose, maltose, mannite, and raffinose.			The only statement in regard to fermentation is the following: *"Organisms 1 and 5 fermented saccharose, lactose, and maltose; Organism 2 fermented lactose, mannite, raffinose, maltose, and dextrose; Organism 3 fermented saccharose, lactose, maltose, and dextrose; Organism 4 fermented maltose only."*	**Becker** 1916. The Necessity of a Standard Blood-Agar Plate for the Determination of Hemolysis by Streptococci.
Many strains of hemolytic streptococci from cases of tonsillitis, otitis media, and scarlet fever; also from normal throats and suspected milk.	Sugar-free veal infusion bouillon. Test substances autoclaved in 10 per cent aqueous solution, and 1 cc. added to 9 cc. of bouillon.	Dextrose, lactose, maltose, saccharose, mannite, salicin, inulin, and raffinose.	Titration against 0.2N NaOH, using phenolphthalein as an indicator.	7 days.	From all sources all but a few of the strains regarded as of pathological significance fermented dextrose, maltose, lactose, saccharose, and salicin but did not ferment mannite, inulin, or raffinose.	**Smillie** 1917. Studies of the Beta Hemolytic Streptococcus (Smith and Brown).
51 strains of *"Streptococcus hemolysans."* 79 strains of *"Streptococcus buccalis."* 8 strains of *"Streptococcus fecalis."* 4 strains of *"Streptococcus equinus."*	*"Two per cent peptone meat-infusion broth, 0.0–0.5+ acidity, containing one per cent of the test-carbohydrate and sterilized in flowing steam on three successive days."*	Lactose, mannite, salicin, saccharose, raffinose, and inulin.	Titration (phenolphthalein). Also observed reaction to litmus.	5 days.	*"An increase in acidity of 1.2+ or more above the initial acidity of the media was considered as indicative of fermentation. In a considerable series, as stated above, litmus was added to the remaining media in the tube, parallel results being obtained throughout."* * * *"Litmus or Andrade's decolorized acid fuchsin should be used as an indicator, titration of the acidity produced being of no additional value."* *"In the classification proposed only lactose and mannite were retained as being of differential value."*	**Blake.** 1917. The Classification of Streptococci.

TABLE V—*Concluded.*
Fermentation Reactions.

TABLE VI.

Pathogenicity and Virulence.

TABLE VI.

Pathogenicity and Virulence.

Orgaoisms.	Animals.	Inoculation. Method.	Inoculation. Amount.	Period of observation.	Results and conclusions.	Reference.
Strains isolated from the milk of cows with chronic mastitis.	2 cows, 2 goats, several pups, kids, guinea pigs, rabbits, and kittens.	Cows and goats inoculated into the milk ducts by catheter or directly into the udder by puncture. Small animals fed with infected milk; also inoculated intraperitoneally and intravenously.	1 cc. of bouillon culture.	Up to 5 mos.	Both cows and 1 goat developed mild chronic mastitis persisting in 1 cow and 1 goat for 5 mos. The other goat developed no lesions. The smaller animals developed no symptoms or lesions whatever.	**Nocard and Mollereau** 1887. Sur une mammite contagieuse des vaches laitières.
	Rabbits.	Intravenous.			*"In the course of my experiments my attention became directed to the relatively early hemolysis of the blood invariably present in rabbits dead from a rapidly fatal dose of the pneumococcus."* *"In a number of animals there was a slight hemolysis shortly before death, while the blood was crowded with pneumococci."*	**Rosenow** 1904. Studies in Pneumonia and Pneumococcus Infections.
11 strains from various pathological conditions and from normal human mouths.	Rabbits. (Mice.)				The author was unable to raise the virulence of avirulent strains by passage through rabbits and mice. (See also Table VIII on General Conclusions, etc.)	**Kerner** 1905. Experimenteller Beitrag zur Hämolyse und zur Agglutination der Streptokokken.
"S. mitis."	Gray mice.		0.5 cc. of 24 hr. bouillon culture.		Found strains of *"S. mitis"* which were pathogenic for mice, 0.5 cc. of 24 hr. bouillon culture killing them in 3–6 days.	**Fränkel** 1905. Ueber menschenpathogene Streptokokken.
22 strains from vulval, vaginal, and uterine secretions of 10 women during and 4–9 days after labor. 1 strain from a felon. 1 strain from throat (angina).	Mice, rabbits.	Actively immunized animals with each strain (4 injections) and then tested their resistance to a lethal dose of *S. pyogenes* (strain from the felon).			Succeeded in producing more or less immunity to *S. pyogenes* with 14 strains, but not with others. In these tests the 14 and other strains used for immunization were not necessarily *S. pyogenes*, but the immunity was tested in each case by injection of a lethal dose of a single strain of *S. pyogenes*. The author concludes that the 14 strains were identical with or nearly related to *S. pyogenes*.	**Natvig** 1905. Bakteriologische Verhältnisse in weiblichen Genitalsekreten.
	Mice.				Percentage of strains isolated from pathological sources: *S. equinus*, none; *S. mitis*, rarely; *S. pyogenes*, 90 per cent; *S. salivarius*, 33 per cent; *S. anginosus*, 54 per cent; *S. fecalis*, 16 per cent; pneumococcus, 34 per cent. No thorough study of pathogenesis for animals is reported. In 1 of the tables the *S. pyogenes*, *S. anginosus*, and pneumococcus groups are indicated as being pathogenic for mice, while *S. salivarius* and *S. fecalis* are indicated as non-pathogenic.	**Andrewes and Horder** 1906. A Study of the Streptococci Pathogenic for Man.
Strains of streptococci and pneumococci from 154 throats; 51 normal, 75 scarlatinal, 14 measles, 5 tonsillitis, 5 pneumonia, 4 laryngitis.	Rabbits.	Intraperitoneal.	Growth from 1 or 2 48 hr. blood agar slants.		*"Most cultures of S. pyogenes and some pneumococci isolated from scarlatinal and normal throats have some virulence for rabbits, but S. viridans has practically no virulence for these animals."* The lesions produced are not described, merely the number of animals which died or survived being given.	**Ruediger** 1906. The Streptococci from Scarlatinal and Normal Throats and from Other Sources.
	White mice.				*"It is possible to convert some but not all strains possessing the streptococcus cultural type into typical pneumococci by inoculation into white mice."*	**Buerger and Ryttenberg** 1907. Observations upon Certain Properties Acquired by the Pneumococcus in the Human Body.
1 strain from milk.	Obtained an aggressin as follows: Washed off the growth from 4 Kolle plates with 6 cc. portions of bouillon. Injected this suspension into the pleural cavity of 4 rabbits which died next day. Found about 15 cc. of clear bloody fluid in the pleural cavities which he pipetted out, sedimented, decanted, and added 0.5 per cent of phenol. Tested sterility and in experiments injected 0.1–2 cc. subcutaneously into rabbits.				Found that a certain amount of immunity could be produced by 3 injections of aggressin. If on the other hand the aggressin was injected simultaneously with live streptococci, the resistance of the animal was considerably lowered.	**Nieter** 1907. Zur Streptokokkenfrage.
40 strains of streptococci from various human infections.	Mice. (Rabbits, guinea pigs, and monkeys in some cases.)				(See Table VIII on General Conclusions, etc.)	**Zangemeister** 1909. Die Hämolyse der Streptokokken.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

Organisms.	Animals.	Inoculation. Method.	Inoculation. Amount.	Period of observation.	Results and conclusions.	Reference.
"*Modified pneumococci*" from cases of endocarditis.	Rabbits.	Intravenous.	3–15 cc. of 24 hr. bouillon, blood bouillon, or milk cultures.		" * * *endocarditis developed in five of ten animals inoculated before the special characteristics of the pneumococci were lost.*" "*Pericarditis developed in six cases,*" in 2 of which endocardial lesions were absent. " * * *of 16 rabbits injected with various strains of pneumococci after return to the usual type none developed endocarditis.*"	**Rosenow** 1909. Immunological and Experimental Studies on Pneumococcus and Staphylococcus Endocarditis.
25 streptococci and 5 pneumococci, all isolated from the blood during life.	Mice.				Obtained blood cultures during life in 90 per cent of cases of infective endocarditis. 66 per cent of these cultures were streptococci and 12 per cent were pneumococci (3 strains, *S. pyogenes*, 9 *S. salivarius*, 9 *S. anginosus*, 8 *S. fecalis*, 5 pneumococcus). At autopsy obtained streptococci in 62 per cent of 100 cases, and pneumococci in 19 per cent of cases. Concludes that "*infective endocarditis is due to the operation of streptococci of low virulence, of the types found in normal feces and in normal saliva.*" "*They are for the most part * * not pathogenic for mice.*"	**Horder** 1909. Infective Endocarditis: With an Analysis of 150 Cases and with Special Reference to the Chronic Form of the Disease.
Many strains of *S. mitior* isolated from vaginal secretions of normal puerpera and gravida. 3 laboratory strains of *S. pyogenes* for comparison with the above.					Found strains of *S. mitior* pathogenic for mice and raised their virulence by successive passages through mice until they were killed in 24 hrs. after injection.	**Konrád** 1909. Weitere Beiträge zur Vaginalstreptokokkenfrage.
					"*Virulenzprüfung an Tieren habe ich ganz unterlassen, da Tier- und Menschenpathogenität durchaus nicht parallel gehen.*"	**Sachs** 1909. Über Streptokokken-Hämolyse.
S. lacticus strains isolated from 75 per cent of 81 samples of milk and from cow dung and skin.	Mice, rabbits.				Found *S. lacticus* non-pathogenic for mice and rabbits.	**Bähr** 1910. Vorkommen und Bedeutung der Streptokokken in der Milch.
"*Modified pneumococci*" from cases of endocarditis.	Rabbits.	Intravenous, intraperitoneal, and "*directly into the heart.*" Animals often injected by 2 or all 3 methods simultaneously.	2–10 cc. of broth, ascites broth, blood broth, or blood culture. Growth from 1–4 agar or blood agar slants.		"*Injection of the bacteria, cultivated on artificial media for a considerable time, directly into the heart always produced pericarditis and usually also endocarditis.*" " * * *the production of endocarditis in animals by pneumococci without injuring the valves is closely dependent on special characteristics of the cocci and on the largeness of the dose. It was noted that while the first injection rarely caused lesions or death it rendered the animal more susceptible to subsequent inoculations,* * *.*" "*In order to produce death promptly after a second injection relatively large doses are necessary. The seemingly increased susceptibility to infection might in reality be an expression of an active immunity and death the result of an overwhelming intoxication from rapid destruction of the organisms injected.*" There is shown "*a marked increase in 'virulence' of the bacteria when cultivated in the patient's blood as compared with those grown on artificial media,* * *." "*Continuous cultivation in fresh normal blood has a similar, but not so marked an effect.*"	**Rosenow** 1910. A Study of Pneumococci from Cases of Infectious Endocarditis.
	Rabbits, guinea pigs, mice, dogs, rats.	Intraperitoneal or intrathoracic.	(See under Results and Conclusions.)		Gray mice were killed by 0.5– 4.0 cc. of bouillon culture. Rabbits " " " 20 – 30 cc. " " " Guinea pigs " " " 5 – 10 cc. " " " Dogs " " " 50 –500 cc. " " " Rats (*Mus decumanus*) were killed by 0.1 cc. or less of bouillon culture of a strain the virulence of which had been raised by passages through guinea pigs. Culture filtrates were not toxic for gray mice, rabbits, or guinea pigs.	**Pricolo** 1910. Recherches expérimentales sur le streptocoque de la gourme.
					In a series of blood cultures taken during life the author found *S. mitior seu viridans* to be the cause of a characteristic form of endocarditis which he called "*schleichende Herzklappenentzündung, Endocarditis lenta.*"	**Schottmüller** 1910. Endocarditis lenta. Zugleich ein Beitrag zur Artunterscheidung der pathogenen Streptokokken.
Many strains of *S. pyogenes*, *S. longissimus*, *S. conglomeratus*, *S. brevis*, *S. mucosus*, and pneumococcus.	Mice.				*S. longissimus* was found to be distinctly pathogenic for mice, though less so than *S. pyogenes*.	**Thalmann** 1910. Streptokokkenerkrankungen in der Armee, Einteilung der Streptokokken und ihre Bekämpfung.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

Organisms.	Animals.	Inoculation.		Period of observation.	Results and conclusions.	Reference.
		Method.	Amount.			
42 strains, mostly from mouth, throat, and joints of cases of rheumatism; a few from the heart blood and various local suppurations.	Rabbits.				Of the strains tested 15 produced fatal septicemia or pyemia with or without arthritis, 13 produced non-purulent arthritis, and 9 gave negative results.	**Beattie and Yates** 1911. Sugar Tests and Pathogenicity in the Differentiation of Streptococci.
8 strains from an epidemic of "*Grippe*" among children.	Rabbits, white mice, guinea pigs.				Quite pathogenic for white mice and rabbits, causing sepsis; non-pathogenic for guinea pigs.	**Müller and Seligmann** 1911. Klinische und bakteriologische Beobachtungen bei Säuglingsgrippe. II. Bakteriologisches von Seligmann.
Evidently many strains from various sources.					Streptococci isolated directly from the blood of human patients were often non-pathogenic for animals. The virulence of most streptococci for animals was raised by passage through animals.	**Rolly** 1912. Experimentelle bakteriologische Untersuchungen von verschiedenen Streptokokkenstämmen.
6 strains of *S. acidi lactici*. 4 strains of *S. pyogenes*. 3 strains of pneumococcus.	Mice.				*S. acidi lactici* was non-pathogenic for mice; *S. pyogenes* and *S. lanceolatus* were fatal for mice.	**Saito** 1912. Versuche zur Abgrenzung des *Streptococcus acidi lactici* von *Streptococcus pyogenes* und *Streptococcus lanceolatus*.
26 strains from bovine mastitis; 17 from dust, horse saliva, and bovine vaginitis; 1 *S. pyogenes*, 1 *S. erysipelatis*, and 1 *S. acidi lactici*.	White mice, goat.	Mice inoculated subcutaneously or intraperitoneally. Goat inoculated into the udder.	Rather large doses.		5 of the strains from bovine mastitis and the *S. erysipelatis* (Fehleisen) killed mice in 2–9 days. Mice survived inoculations with all other strains. Inoculation of a goat into the udder with streptococci isolated from bovine vaginitis resulted in a true streptococcus mastitis.	**Gminder** 1912. Untersuchungen über Mastitisstreptokokken und ihre Differenzierung von saprophytischen Streptokokken.
	Mice.				(See Table VIII on General Conclusions, etc.)	**Thalmann** 1912. *Streptococcus viridans* im Blut ohne Veränderung der Herzklappen. Zur Einteilung der Streptokokken.
Streptococci from 20 cows with mastitis compared with several from human sepsis, puerperal fever, and scarlet fever, and 1 strain of *S. lacticus*.	Mice.				Found 2 mastitis strains virulent for mice and points out that streptococci from the human throat (especially from cases of scarlet fever) may or may not be virulent for mice.	**Salus** 1912. Untersuchungen zur Hygiene der Kuhmilch.
(*a*) Fresh market milk. (*b*) Sediment from 10 cc. of milk. (*c*) Milk after incubation for 3 hrs. Strains isolated from milk, human infections, and infants' feces.	Guinea pigs.	Intraperitoneal.	(*a*) 5 cc. (*b*) Sediment from 10 cc. (*c*) 5 cc.		14 of 40 guinea pigs inoculated died after 1–11 days. Various organisms were isolated at autopsy but "*never a true S. pyogenes in long chains.*" Sterile milk produced a sterile peritonitis in guinea pigs. Phagocytic experiments *in vitro* showed the "*Virulenzzahl*" (Bürgers) of all milk streptococci to be much lower than that of streptococci from various human infections, using human serum and leukocytes. The "*Virulenzzahl*" of streptococci from infants' feces was also quite low. The "*Virulenzzahl*" of milk streptococci, using beef blood, was also low but higher than with human blood.	**Puppel** 1912. Ueber Streptokokken in der Milch und im Säuglingsstuhl.
Many strains from sore throats (Chicago epidemic, 1912) and secondary lesions.	Rabbits, guinea pigs, mice.	Intraperitoneal.	"*Small quantities.*"		Rabbits, guinea pigs, and mice were killed in 12–24 hrs.	**Davis and Rosenow** 1912. An Epidemic of Sore Throat Due to a Peculiar Streptococcus.
45 strains from throats and secondary lesions of cases of epidemic sore throat. 1 strain of ordinary hemolytic streptococci from the udder of a cow.	Rabbits, guinea pigs.	Intravenous and intraperitoneal.	"*Relatively small doses.*"		"*These organisms are very highly pathogenic for animals, killing guinea pigs in relatively small doses in from twenty-four to forty-eight hours. They are even more virulent in rabbits. Intravenous injection of small doses causes a generalized infection with death following in from twenty-four to thirty-six hours. If very small doses be given the organisms, like ordinary streptococci, tend to localize in the joints, causing multiple arthritis; also there may be infection of serous cavities generally, and local abscesses in the myocardium. Vegetative endocarditis with hemorrhages may occur but is rare.*" With regard to the strain from the cow's udder: "*One blood agar slant growth injected intravenously into a rabbit caused purulent arthritis of the left knee and death on the fourth day from septicemia. Guinea pigs succumbed usually in twenty-four hours after intraperitoneal injection of the same amount.*"	**Davis** 1912. Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

Organisms.	Animals.	Inoculation. Method.	Inoculation. Amount.	Period of observation.	Results and conclusions.	Reference.
Cocci from cases of chronic endocarditis, pure and mixed with *B. fusiformis* or streptococci. Mixed cultures from the throats of endocarditis cases.	Rabbits.	Intravenous.	Very large doses, in some cases the growth from 4–6 agar slants.		*"The results of these experiments show that the endocarditis in the rabbit which follows injection of cocci which I believe to be modified pneumococci, is due to an embolic process. There is first produced valvular hemorrhage from which vegetations develop. The localization in the endocardium of the cocci from chronic infectious endocarditis and of allied cocci from the throat, is due in large part to the presence of fine capillaries in the valves and to the peculiar mode of growth of the cocci."* *"The affinity of the endocarditis strains of cocci for the endocardium and of streptococci for joints is shown by the results of injections of mixtures of these organisms, and the exact cause of such affinity is unknown."*	**Rosenow** 1912. Experimental Infectious Endocarditis.
15 samples of milk "*slime*" from dairy separators and clarifiers, 24–72 hrs. old. Mixed cultures of the above in ascites meat broth. Strains from milk obtained from individual cows "*in sterile form.*"	(*a*) Rabbits. (*b*) Guinea pigs. (*c*) Mice.		(*a*) 6–15 cc. of thick suspension in salt solution. (*b*) 5–7 cc. of the same. (*c*) 0.5–1 cc.		"*The animals* (injected with separator slime) *usually died of streptococcemia in from 24 to 72 hours,*" apparently referring to animals of all 3 species. * * "*Intravenous injections in the rabbits gave the highest percentage of positive results. In most cases (24) streptococci in pure culture were obtained, streptococci in predominating numbers but mixed with colon bacillus in nine, and the colon bacillus pure in one. One animal yielded a pure culture of a typical pneumococcus. Nine animals survived while three developed streptococcus arthritis later.*" Of the strains isolated from milk obtained in sterile form "*two of these when injected into guinea pigs proved virulent. The milk of one cow yielded a typical virulent pneumococcus. These three cows had not had any disease of the udder at any time.*" * * "*hemolyzing streptococci in pure culture*" from the milk of "*two cows with chronic mastitis*" * * "*proved only moderately virulent for rabbits, guinea pigs, and white mice.*"	**Rosenow** 1912. A Study of Streptococci from Milk and from Epidemic Sore Throat, and the Effect of Milk on Streptococci.
1 strain "*S. epidemicus*" from milk. 1 strain from throat of carrier.	Mice.	Subcutaneous.			A mouse inoculated with the strain from milk died in 24 hrs. of peritonitis and septicemia. The strain from the throat of a carrier was not pathogenic for mice.	**Stokes and Hachtel** 1912. Septic Sore Throat. A Milk-Borne Outbreak in Baltimore, Md. Bacteriological Study of the Outbreak.
	Rabbits, guinea pigs.				(See Table VII on Mutations and Variability.) (See also Table VIII on General Conclusions, etc.)	**Davis** 1913. Interrelations in the Streptococcus Group with Special Reference to Anaphylactic Reactions.
26 strains of *S. equi* (23 laboratory strains of various ages and freshly isolated by the authors). 27 strains of *S. longus* isolated by the authors from human pathological material.	Rabbits, young dogs. (Mice.)	Intravenous.	Probably 2 cc. of serum bouillon culture.		With reference to "*der Drusecoccus,*"—"* * *; bei intravenös infizierten Kaninchen erzeugt er häufig eine typische starke fibrinöse Perikarditis, die wir beim Streptococcus longus seu erysipelatos nie beobachtet haben.*" "*Beide Streptokokkenarten sind auch für junge Hunde pathogen. Sowohl mit dem Drusecoccus, als auch mit dem Strept. longus lassen sich auf hämatogenem Wege typische Gelenkentzündungen und eine akute Enteritis erzeugen,* * *. *Nach den bisheriges spärlichen Versuchen mit dem Drusecoccus scheint jedoch dieser für junge Hunde eine grössere Pathogenität zu haben, als der S. longus.*"	**Koch and Pokschischewsky** 1913. Ueber die Artverschiedenheit des *Streptococcus longus seu erysipelatos* und des *Streptococcus equi* (Druse-Streptococcus).
	Mice.	Subcutaneous.			The formation of abscesses in the course of the lymphatics is described as being characteristic for *S. equi*.	**Bemelmans** 1913. La spécificité des streptocoques de la gourme.
A number of strains of hemolytic streptococci, *S. viridans*, *S. mucosus*, and pneumococcus from various sources.	Rabbits, guinea pigs.	Various; rabbits usually intravenous, guinea pigs usually intraperitoneal.	Various.		(See Table VII on Mutations and Variability.)	**Rosenow** 1914. Transmutations Within the Streptococcus-Pneumococcus Group.
14 strains from joint fluid of 14 cases of acute rheumatism, and "*other strains which have been made to resemble them.*"	(*a*) Rabbits. (*b*) Dog. (*c*) Monkey.	Intravenous.	(*a*) Growth from 25–75 cc. of 24 hr. ascites dextrose broth culture. (*b*) Growth from 250 cc. (*c*) Growth from 100 cc.	Up to 15 days.	The green-producing strains and those not affecting the blood agar produced arthritis, endocarditis, and pericarditis. The strains producing slight hemolysis on blood agar produced myositis, myocarditis, endocarditis, pericarditis, and arthritis; possibly also ulcer of the duodenum (dog) and of the stomach (monkey).	**Rosenow** 1914. The Etiology of Acute Rheumatism, Articular and Muscular.
20 strains from various human pathological conditions.	Rabbits.	Intravenous.	"*Cultures from Loeffler's blood serum suspended in salt solution.*" "*No attempt was made to secure a uniform dosage.*"		"*Death or recovery only were taken into consideration.* * *The results obtained are consistent with the severity of the infections from which the cultures came,* * *.*"	**Floyd and Wolbach** 1914. On the Differentiation of Streptococci. (Preliminary notes.)

TABLE VI—*Continued.*

Pathogenicity and Virulence.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

Organisms.	Animals.	Inoculation. Method.	Inoculation. Amount.	Period of observation.	Results and conclusions.	Reference.
9 strains from cases of arthritis, septicemia, scarlet fever, endocarditis, and infected finger.	Rabbits.	Subcutaneous.	1–5.5 cc. of 8–19 hr. serum bouillon cultures.		4 of the strains tested were pathogenic. " * * *several strains of streptococcus had retained a moderate degree of pathogenicity for animals, although they had been subcultured on agar for periods of from nine months to three years.*" " * * *cultures of streptococci made in the media described in this communication* (bouillon containing 15–30 per cent horse serum or 70–75 per cent rabbit serum) *appear to be most virulent at the end of ten to twelve hours' incubation,* * ." The streptococci were recovered from the heart blood and hemolysis *in vivo* was noted.	**M'Leod** 1914. Criteria of Virulence amongst Streptococci, with Some Remarks on Streptococcal Leucocidin.
2 strains of *S. pyogenes*. Source not given.	Rabbits.	Intravenous.	Various amounts of 24 hr. bouillon cultures.	At intervals from 5 min. to 30 days after inoculation or until death.	Withdrew blood samples at various intervals and made plates for counting the streptococci in the circulating blood. Found that the disease might be acute, producing death in 1–3 days, or chronic, producing death in 5–30 days. In acute cases the number of organisms in the blood is usually much larger than in the chronic cases, and is quite variable from time to time in the latter. The bacterial count was always much larger in oxalate blood than in defibrinated samples, thought to be due to mechanical removal of the bacteria by the fibrin.	**Reichstein** 1914. Ueber den Nachweis der Streptokokken im strömenden Blute.
6 strains from throats. 2 strains from milk slime.	Rabbits.	Intravenous.			All the strains tried produced suppurative arthritis or death in rabbits.	**North, White, and Avery** 1914. A Septic Sore Throat Epidemic in Cortland and Homer, N. Y.
Milk cultures and streptococcal exudate from tonsillitis case. Pure culture of hemolytic streptococcus.	A healthy cow giving about 16 quarts of milk in 24 hrs.	(*a*) Milk cultures and tonsillitis exudate smeared on the healthy teats. (*b*) Culture of hemolytic streptococcus smeared on abraded teat. (*c*) 8 cc. of milk culture of hemolytic streptococcus injected into milk duct by catheter.	8 cc.	Several wks.	(*a*) No infection by this method. (*b*) Mild mastitis without caking of the udder was produced. Streptococci persisted in the milk for over 4 wks. (*c*) Mild mastitis confined to the inoculated quarter of the udder. Large number of streptococci and leukocytes were secreted in the milk for several wks. The authors conclude that: "*Hemolytic streptococci of human origin may cause mastitis* * * *in cows.*" "*The streptococci may gain entrance through an abraded or injured surface of the teat.*" "*The mastitis may exist without physical evidence.* * * *though pus and streptococci in large numbers are being secreted in the milk.*"	**Davis and Capps** 1914. Experimental Bovine Mastitis Produced with Hemolytic Streptococci of Human Origin.
Mixture of *S. fecalis* and pneumococcus. *B. coli*.	Guinea pigs.	Intraperitoneal.	Various.	Various.	The injection of mixed cultures of *S. fecalis* and pneumococcus was followed by the recovery of only pneumococci from the blood. The injection of pure cultures of *B. coli* was followed by the recovery of streptococci from the blood. Streptococci were easily isolated from the throats and intestinal contents of normal guinea pigs and from the blood, pleura, peritoneum, etc., of guinea pigs dying "*spontaneously.*" (See Table VII on Mutations and Variability.)	**Holman** 1914. The Relative Longevity of Different Streptococci and Possible Errors in the Isolation and Differentiation of Streptococci.
12 strains (7 from throats; 3 bovine; 1 *S. epidemicus* from throat, Chicago epidemic, 1912; 1 *S. epidemicus* from milk, Chicago epidemic, 1912).	Rabbits.	Probably intravenous.	"*1–2 slant agar tubes.*"		2 strains isolated from the udders of cows produced multiple arthritis in rabbits in 3 or 4 days. All the other strains, except 1 bovine strain, are tabulated as being pathogenic for rabbits without further details.	**Capps and Davis** 1914. An Epidemic of Streptococcus Sore Throat in Jacksonville, Ill., Which Was Traced to the Milk of Cows Affected with Streptococcus Mastitis.
					(See Table III on The Use of Blood Agar.) (See Table VIII on General Conclusions, etc.)	**Davis** 1914. The Growth and Viability of Streptococci of Bovine and Human Origin in Milk and Milk Products.
28 strains of streptococci from sore throats. 10 strains from cow's milk.	Rabbits.	Intravenous.	1 cc. of 24 hr. bouillon culture.	Up to 3 mos. or until death of the animal.	18 of the strains from throats and 4 from milk were pathogenic for rabbits, producing sometimes only a rise in temperature with loss of weight for a few days and sometimes marked polyarthritis or death. (See also Table VIII on General Conclusions, etc.)	**Smith and Brown** 1915. A Study of Streptococci Isolated from Certain Presumably Milk-Borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

Organisms.	Animals.	Inoculation. Method.	Inoculation. Amount.	Period of observation.	Results and conclusions.	Reference.
2 strains of *S. lacticus* from ice cream and from milk.	Rabbits, guinea pigs.	Subcutaneous.	Growth from 1 24 hr. North's medium slant.		(See Table VII on Mutations and Variability.)	**Heinemann** 1915. The Variability of Two Strains of *Streptococcus lacticus*.
Strains of streptococci from the tissues of appendices and from the tonsils of appendicitis patients.	Half-grown rabbits.	Intravenous.	*"Very large doses."*		*"The elective affinity for the appendix of the streptococci in pure culture or in mixture with fusiform bacilli, isolated both from the tonsils at the time of, or soon after, the attack and from the appendix, is striking. Thus, the tonsillar strains produced appendicitis in nineteen of twenty-nine, the appendix strains in twenty-two of thirty, a total of forty-one of fifty-nine* (rabbits injected). *After cultivation on artificial media for a short time* (or after 1–3 animal passages) *the elective affinity is soon lost, and strains isolated from the tonsils some time after appendectomy also appear without elective affinity. The results following injections of mixtures of streptococci and colon bacilli are similar (26 of 31), although the tendency to produce lesions in the intestine and gall-bladder is much greater."* *"The results of the experiments and observations indicate that appendicitis, in the absence of foreign bodies, commonly is a hematogenous infection, secondary to some distant focus; that it develops when, for some reason or other, the organisms in the focus, usually streptococci, have acquired an elective affinity for the appendix and at the same time gain entrance into the circulation."* *"The results bear out my theory that a focus of infection is to be looked on, not only as the place of entrance of bacteria, but also as the place where they may acquire the varying affinities necessary to infect distant organs and tissues."*	**Rosenow** 1915. The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli.
Protocols of experiments with strains from 5 cases are given.	Rabbits (800–1,000 gm.).	Intravenous.	8 cc. of 24 hr. broth culture.		*"We finally adopted 8 cc. of o twenty-four hour broth culture as a standard dose; this amount does not contain enough toxic material to cause immediate symptoms, and usually kills the animal in from two to four weeks."* * * *"The pathogenicity of these streptococci varied greatly, but for the most part they were of relatively low virulence."* *"We inoculated in all twenty-four rabbits. Of these, six lived. Five died showing no gross lesions, nor microscopic changes in heart or kidneys. One died of general sepsis and two of acute toxemia. Heart lesions occurred in five, kidney lesions in seven, aortic lesions in three, and joint lesions in two."* * * *"Endocardial lesions occurred but twice in our rabbits."*	**Hartzell and Henrici** 1915. A Study of Streptococci from Pyorrhea Alveolaris and from Apical Abscesses.
9 strains from milk sediment.	Half-grown rabbits, guinea pigs.	Intravenous; intraperitoneal.	Growth from 10–45 cc. of broth culture.	Up to 8 days or longer.	4 strains *"were virulent for guinea pigs, producing a fatal peritonitis when injected intraperitoneally."* *" * * two strains of streptococci isolated from the milk, indistinguishable from each other as to form and cultural characteristics, were nevertheless quite different, as shown by intravenous injection into animals, one localizing in the muscles and joints, and the other in the gall-bladder."* Summary: *"Virulent streptococci isolated from the milk showed selective preference for certain animal structures, such as joints, muscles, gall-bladder, etc."*	**Rosenow and Moon** 1915. On an Epidemic of Sore Throat and the Virulence of Streptococci Isolated from the Milk.
2 strains of streptococci easily recognized because of their peculiar characteristics and not like any found in dogs.	Dogs, cats.	Fed.		Nearly 5 mos.	The object of the experiments was merely to recover the organisms fed and to note any changes in their characteristics which might have occurred. No pathological effects on the animals are mentioned. (See also Table VII on Mutations and Variability.)	**Broadhurst** 1915. Environmental Studies of Streptococci with Special Reference to the Fermentative Reactions.
Strains isolated at intervals from the tear sac of a patient with iridocyclitis.	Rabbits.	Intravenous.	The growth from a blood agar slant or from 5–6 cc. of ascitic dextrose bouillon culture.		Hemolytic streptococci isolated from the tear sac of a patient suffering with iridocyclitis produced typical iridocyclitis in 5 of 8 rabbits injected intravenously. *"The power of the hemolytic streptococcus in these experiments, to localize in the eye and produce iridocyclitis in rabbits was lost in a relatively short time (1) in the body of the original host (tear sac), (2) in cultures, and (3) during animal passage. This change in tissue localization seemed not to be associated with any demonstrable decrease in lethal power."* *"Attempts to obtain a return of invasive power for the tissues of the eye in strains of streptococci which had lost it, by growing the organisms in the living eye, were unsuccessful."*	**Irons, Brown, and Nadler** 1916. The Localization of Streptococci in the Eye. A Study of Experimental Iridocyclitis in Rabbits.
Over 109 strains of streptococci (73 from tonsils of normal individuals, 4 from tonsils of appendicitis patients, 9 from Steno's duct of parotitis patients, 23 from dairy products).	177 rabbits.	Intravenous.	Growth from 15–90 cc. of 24 hr. ascites dextrose broth cultures.	Animals usually killed in 48 hrs. unless dead from effects of inoculation.	There is described an epidemic of appendicitis followed soon by an epidemic of parotitis. Among the most significant figures are the following: Soon after the appendicitis epidemic 30 per cent of strains from tonsils of normal individuals, 47 per cent of strains from tonsils of appendicitis patients, and 41 per cent of strains from dairy products produced lesions in the appendices of rabbits injected; while only 10 per cent, 0 per cent, and 9 per cent respectively produced lesions of the parotid. During and soon after the parotitis epidemic 20 per cent of strains from tonsils of normal individuals, 50 per cent of strains from dairy products, and 73 per cent of strains from Steno's duct of patients with parotitis produced parotitis in rabbits; while only 6 per cent, 0 per cent, and 15 per cent respectively produced lesions of the appendix.	**Rosenow and Dunlap** 1916. An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products.

TABLE VI—*Continued.*

Pathogenicity and Virulence.

TABLE VI—*Concluded.*

Pathogenicity and Virulence.

TABLE VI—*Concluded.*

Pathogenicity and Virulence.

Organisms.	Animals.	Inoculation. Method.	Inoculation. Amount.	Period of observation.	Results and conclusions.	Reference.
11 strains of streptococci from tonsils, spinal fluid, and pyorrheal pockets of cases of herpes zoster. Mixed cultures and "*emulsions*" from tonsils often used for injection.	Rabbits, dogs. (A few guinea pigs.)	Intravenous (Occasionally subcutaneous or intraperitoneal.)	Growth from 25–90 cc. of 24 hr. ascites dextrose meat broth cultures.	Chloroformed after 1–4 days if not dead from the effects of the inoculation.	Of 61 animals injected with freshly isolated strains or infectious material 75 per cent developed herpes. "*The lesions varied from those very small, just recognizable, to others very large and marked.*" It is not stated in what percentage of animals other lesions developed, but 29 per cent developed hemorrhage of the stomach, 8 per cent ulcer of the duodenum, 10 per cent lesions in the appendix, 16 per cent in the gall-bladder, 11 per cent in joints, 11 per cent in pericardium, 11 per cent in muscles, 21 per cent in lungs, 15 per cent in eyes, etc., etc. After cultivation for 7–14 days or after 2–5 passages through animals the strains produced herpes no more frequently than various other lesions. "*It is a noteworthy fact that of the large number of animals injected with cultures from a variety of diseases other than herpes, in which lesions resembling those in man have been produced, none has exhibited typical herpes.*"	**Rosenow and Oftedal** 1916. The Etiology and Experimental Production of Herpes Zoster.
This paper is largely a review of previous work by the author and others. Apparently no new work on pathogenicity and virulence is reported.					"* * * *the streptococci are not specific in their disease production, at least as far as I have been able to determine. There is no evidence to support the view that only one type of streptococcus produces endocarditis or nephritis, or gives rise to septicemia in the puerperium, scarlet fever, or other conditions of lowered resistance.*" "*The members of the hemolytic group are commonly more virulent and pathogenic, * * than those of the viridans group.*"	**Holman** 1916. The Classification of Streptococci.
1 strain of human type (*S. epidemicus*) isolated by Davis (1912) from bovine mastitis. 1 strain of *S. lacticus*. 1 strain of *S. epidemicus* from peritoneal pus of fatal case of tonsillitis isolated by Davis (1912). 1 strain of non-pathogenic hemolytic streptococcus isolated from normal milk by Davis (1916). 1 strain of hemolytic human type of streptococcus from milk of cow without gross evidence of mastitis.	Healthy cows, rabbits.	Into the milk ducts of the cows by means of catheter. Rabbits inoculated intravenously, probably.	1–5 cc. of 36 hr. litmus milk cultures injected into milk ducts of cows. Growth from 1 blood agar slant injected into rabbits.	1 cow observed for 215 days, the other for 146 days.	The human type (*S. epidemicus*) produced in cows very severe garget which persisted in chronic atrophic form for as long as observed (215 and 146 days respectively), and produced in rabbits fatal arthritis. Referring also to the work of Davis and Capps (1914) the author concludes that mastitis produced in cows by the human type of hemolytic streptococci may be severe or the virulent streptococci "*may grow and multiply in the milk ducts without causing any visible changes in the udder.*" The strains of *S. lacticus* and the non-pathogenic hemolytic streptococcus from milk produced in the udder acute inflammation of short duration followed by complete recovery. The strain of hemolytic streptococci from the milk of the cow without gross evidence of mastitis produced arthritis in rabbits.	**Mathers** 1916. Different Types of Streptococci and Their Relation to Bovine Mastitis.
85 strains of hemolytic streptococci from 328 samples of bottle milk.	Half-grown rabbits.	Intravenous.	Growth from 2 24 hr. blood agar slants.	Permitted to live 10 days, then killed and autopsied.	"*15 strains produced lesions visible at autopsy. These involved joints and on the whole were mild.*" "*I have gained the impression from many observations that the virulence for rabbits of a given strain of hemolytic streptococci runs rather parallel with its virulence for man.*" "*From these results it is evident that all the strains in this series are relatively avirulent. None could be classed in this respect with the human types of hemolytic streptococci as found in sore throat epidemics.*"	**Davis** 1916. Hemolytic Streptococci Found in Milk.
Many strains of hemolytic streptococci from cases of tonsillitis, otitis media, and scarlet fever; also from normal throats and suspected milk.	"*The pathogenicity for mice of 0.5 cc. of a 24 hour broth culture intraperitoneally is a fair index of its pathogenicity in rabbit- Comparative results proved that in all the cases studied, a streptococcus which killed a mous in from 3 to 4 days would produce high fever and marked loss of weight in rabbits, followed u ally by polyarthritis, when 1 cc. of a 24 hour broth was injected intravenously. For the pr minary studies mice were often used. In substantiation of the more important cultures rabl were used for confirmatory evidence.*"				From all sources all but a few of the strains producing beta hemolysis and fermenting the substances described in Table V, were pathogenic for mice and rabbits.	**Smillie** 1917. Studies of the Beta Hemolytic Streptococcus (Smith and Brown).
51 strains of "*Streptococcus hemolysans.*" 79 strains of "*Streptococcus buccalis.*" 8 strains of "*Streptococcus fecalis.*" 4 strains of "*Streptococcus equinus.*"	No animal experiments reporte				"*Streptococcus hemolysans*" is "*usually parasitic and highly pathogenic, while Streptococcus viridans is either saprophytic or if associated with a pathological process is usually of relatively low virulence.*"	**Blake** 1917. The Classification of Streptococci.

TABLE II.

A Tabulation of Streptococci with Reference to Type of Appearance in Blood Agar and Fermentation Reactions.

Mannite	Lactose	Salicin.	Groups.	Type α. *S. mitior seu viridans* (Schottmüller).	Type β. *S. longus pathogenes seu erysipelatos* (Schottmüller). *S. hemolyticus* (Rolly).	Type γ. *S. saprophyticus* (Mandelbaum). *S. anhemolyticus* (Zangemeister).
		+	1	*S. mitis* (Andrewes and Horder). S&B 6 | S&B 6 | S&B 1 | | S&B 1 H&H 4 | H&H 7 | | | H&H 3 K 1 | K 8 | | K 1 | Br 50 | Br 18 | Br 1 | Br 13 | Br 28	*S. pyogenes* (Rosenbach). S&B 33 | | | | | | K 22 | | | | | | Br 38 | Br 1 | | | | | D 11 | | | | D 2 | | Sml 62 | | | | | |	K 7 | B 3 | | | | | | Br 1 | Br 4 | | Br 7 | Br 5 | Br 1 | |
+	+					
		−	2	*S. salivarius* (Andrewes and Horder). S&B 3 | S&B 2 | | | | H&H 5 | H&H 5 | | | | K 3 | | | | Br 10 | Br 9 | | Br 7	*S. anginosus* (Andrewes and Horder). S&B 8 | | | | | | K 2 | | | | | | Br 3 | | | | | | Sml 5 | | | | | |	Br 1 | | Br 1 | | |
−		+	3	*S. equinus* (Andrewes and Horder). H&H 1 | H&H 1 | | | Br 3 | Br 1 | | |	*S. equi* (Schütz). S&B 1 | | | | | | Br 1 | | | | | | Sml 1 | | | | | |	Br 1 | B 1 | | | | | |
	−					
		−	4	*S. ignavus* (Holman). H&H 2 | | | | Br 2 | Br 1 | | | Br 5 | | Br 1	*S. subacidus* (Holman). Br 1 | Br | | | | |	| | | | Br 3 | | |
		+	5	*S. fecalis* (Andrewes and Horder). H&H 1 | | | | | | | | K 3 | K 2 | | | | | | | Br 53 | Br 15 | Br 4 | Br 39 | Br 39 | | | |	*S. infrequens* (Holman). K 3 | K 1 | | | | | | | Br 7 | | | | Br 8 | | | | Sml 10 | | | | D 1 | | | |	K 3 | K 1 | | | | | | | Br 9 | Br 3 | Br 1 | Br 6 | Br 4 | | | |
	+					
		−	6	Br 3 | | | | | | | |	| | | Sml 1 | | | | |	| | | | | | | |
+						
		+	7	| | | | Br 6 | | | |	*S. alactosus* (Smith and Brown). S&B 4 | | | | | | | | | | | | | | | |	| | | | | | | |
	−					
		−	8	| | | | Br 1 | | | |	Sml 1 | | | | | | | |	| | | Br 2 | | | | |
Subgroups				.1 | .2 | .3 | .4 | .5 | .6 | .7 | .8	.1 | .2 | .3 | .4 | .5 | .6 | .7 | .8	.1 | .2 | .3 | .4 | .5 | .6 | .7 | .8
Raffinose				− + | − + | − + | − +	− + | − + | − + | − +	− + | − + | − + | − +	− + | − + | − + | − +
Inulin				− + | − +	− + | − +	− + | − +	− + | − +
Saccharose				+ | −	+ | −	+ | −

The small figures in each square represent the number of strains described by each of the following authors: S&B=Smith and Brown (1915); H&H=Hartzell and Henrici (1915); K=Kligler (1915); Br=Broadhurst (1915); D=Davis (1916); Sml=Smillie (1917); B=Brown (1917).

TABLE III.

The Use of Blood Agar.

TABLE III.

The Use of Blood Agar.

Organisms.	Medium: Blood. Species.	Medium: Blood. Amount.	Medium: Agar. Composition.	Medium: Agar. Amount.	Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
40 human and 2 equine strains.	Rabbit, defibrinated.	Enough to cloud the agar (Natvig).					An "*élégante auréole d'hémoglobine dissoute*" was formed around each colony of 39 of the human and 1 of the equine strains. These are all regarded as of one species. Distinct but fainter hemolyzed zones were formed around colonies of 1 scarlet fever strain and a "*Streptococcus der Druse der Pferde*," the former being regarded as a variant of the other human strains, the latter as a distinct species.	**Marmorek** 1902. L'Unité des streptocoques pathogènes pour l'homme. (Die Arteinheit der für Menschen pathogenen Streptokokken.)
	Human.	2 cc.	Standard agar.	5 cc.	Surface films usually, but occasionally made deep inoculations.	Up to 48 hrs. or longer.	Gave attention to macroscopic appearance only. States that "many if not all of the organisms about to be considered * * produce a certain amount of hemolysis, not visible macroscopically." "*Str. longus pathogenes seu erysipelatos*" forms gray colonies "*welche einen durchaus characteristischen kreisrunden hellen Hof um sich gebildet haben. Dieser durch völlige Resorption des Hämoglobins entstandene Hof hat einen Durchmesser von ca. 2–3 mm, je nach dem Alter der Kultur.*" "*Str. mitior seu viridans*" forms "*feine, fast farblose, später grau bis grünschwarze Punkte; am zweiten Tage haben sie sich zum Teil bis zu Kleinstecknadelkopfgrösse entwickelt.*" * * "*Die Hämolyse des Strept. m. ist so gering, dass eine makroskopisch sichtbare Resorption des Blutfarbstoffes bei Verwendung einer Blutagarmischung von etwa 2: 5 im allgemeinen nicht stattfindet.*" "*Str. mucosus*" forms "*innerhalb 24 Stunden auf dem Impfstrich ein glänzender, saftig schleimiger, grüngrauer Belag, der nach weiteren 48 Stunden eine dunklere Färbung anzunehmen pflegt, aber der Glanz, das schleimige Aussehen schwindet; der Belag trocknet auch hier. * * Makroskopisch konnte ich bei meinen Stämmen Hämolyse erst nach vielen Tagen beobachten.*" "*Der Pneumokokkus einen intensiv dunkel grünen Farbstoff bildet, dagegen führt er eine makroskopisch sichtbare Hämolyse nicht herbei.*"	**Schottmüller** 1903. Die Artunterscheidung der für den Menschen pathogenen Streptokokken durch Blutagar.
65 pneumococci. 35 streptococci.	Rabbit or human, defibrinated.	0.3–0.5 cc.	"*Plain agar.*"	5 cc.			Pneumococci form distinctly green colonies "*surrounded by a narrow zone in which the corpuscles are destroyed. This zone is never clear and transparent, but always opaque and of a distinct greenish tinge.*" "*The colonies of streptococci, on the other hand, remain small and grayish, and are surrounded by a perfectly clear transparent zone of hemolysis, the size of which is in direct proportion to their virulence.*"	**Rosenow** 1904. Studies in Pneumonia and Pneumococcus Infections.
30 strains.	Human.	(*a*) 2 parts. (*b*) 5 drops.		(*a*) 5 parts. (*b*) 5 cc.			25 strains corresponded in all respects with Schottmüller's *S. longus pathogenes*. 5 strains produced no hemolysis on blood agar (2: 5) but small clear zones on blood agar containing only 5 drops of blood in 5 cc. of agar. The colonies of these 5 strains were small dark points, but only 1 of them showed any perceptible greenish tint. The author regards these strains as *S. mitior*.	**Rieke** 1904. Beiträge zur Frage der Arteinheit der Streptokokken.
	Rabbit, guinea pig, and dog.	3–4 drops.			Surface and deep inoculation tried.	18–24 hrs.	States that surface inoculation was found to be the more satisfactory because plates inoculated deep (*i.e.*, poured) became brownish in 12–24 hrs. Typical hemolyzed zones with "*Resorption des Hämoglobin*" were 2–3 mm. in diameter. Strains non-hemolytic in blood bouillon were also non-hemolytic in blood agar.	**Kerner** 1905. Experimenteller Beitrag zur Hämolyse und zur Agglutination der Streptokokken.
22 strains from vulval, vaginal, and uterine secretions of 10 women during and 4–9 days after labor. 1 strain from a felon. 1 strain from throat (angina).	White mice, guinea pig, rabbit, beef, and human.	12 drops.	Standard meat infusion agar, neutral to phenolphthalein.	10 cc.	Streaks of several different strains on the same plate.	2 days, (possibly longer).	Appearance with various kinds of blood essentially alike. Describes 3 types of appearances on blood agar: I. (*a*) Clear colorless zones 2–3 mm. broad. (*b*) Zones colorless but clouded or hazy. II. Small colorless hazy zones surrounded by a green ring and sometimes an outer colorless hazy ring. III. No hemolysis or discoloration. All the anaerobic strains (6 of them) belonged to this group. On blood agar (2: 5) zones of Type I (*a*) apparent, but the other types were not distinguishable macroscopically. (See text for quotations.)	**Natvig** 1905. Bakteriologische Verhältnisse in weiblichen Genitalsekreten.
		Tried also blood agar 2: 5 for comparison.						
							Confirmed and approved Schottmüller's differentiation of streptococci by means of blood agar plates. (Recommends the use of litmus-lactose-nutrose-agar, according to Drigalski and Conradi for the differentiation of pneumococci, *S. viridans*, and *S. mucosus*.)	**Fränkel** 1905. Ueber menschenpathogene Streptokokken.

TABLE III—*Continued.*

The Use of Blood Agar.

Organisms.	Medium. Blood. Species.	Blood. Amount.	Agar. Composition.	Agar. Amount.	Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
Evidently several strains of *S. pyogenes* and pneumococci.	"*Fresh defibrinated rabbit blood.*"	0.3–0.4 cc.	Plain agar. Sugar-free agar (0.5 per cent sodium chloride, 1 per cent Witte's peptone, and 1.5 per cent agar).			Up to 48 hrs.	"*I have found pneumococci which produce hemolyzing colonies similar to those of S. pyogenes.*" *S. pyogenes* produces: (*a*) clear hemolyzed zones on plain agar + blood; (*b*) " " " on sugar-free agar + blood; (*c*) either no hemolysis or greenish discoloration on glucose agar + blood. Pneumococci produce: (*a*) green colonies on plain agar + blood; (*b*) may or may not produce slightly greenish colonies on sugar-free agar + blood (depending upon the kind of blood used—probably dependent upon the amount of sugar in the blood); (*c*) deep green colonies on sugar-free agar + 1 per cent glucose, lactose, or inulin + blood. Conclusion.—"*The green coloration of bacterial colonies in blood agar plates is dependent upon the production of acid and the action of this acid (probably lactic acid) on the red corpuscles.*" (See text for further quotations.)	**Ruediger** 1906. The Cause of Green Coloration of Bacterial Colonies in Blood-Agar Plates.
Strains from 154 throats; 51 normal, 75 scarlatinal, 14 measles, 5 tonsillitis, 5 pneumonia, 4 laryngitis.	Rabbit or human, defibrinated.	0.5 cc.			Deep.	24 hrs. or longer.	*S. pyogenes* produces clear zones on blood agar. *S. viridans* and related varieties produce green colonies with or without partial hemolysis. Pneumococci produce green colonies, sometimes partial hemolysis, and occasionally may be mistaken for *S. pyogenes*.	**Ruediger** 1906. The Streptococci from Scarlatinal and Normal Throats and from Other Sources.
47 streptococci. 22 pneumococci.	Human and horse.	(*a*) Few drops mixed at 100°C. (Voges' medium.) (*b*) 30 per cent mixed at 100°C. (*c*) Few drops mixed at 45°C. (*d*) 30 per cent mixed at 45°C.			Surface. 5 streptococci and 5 pneumococci deep also.		Most streptococci (75 per cent) produce more or less hemolysis on blood agar (a few drops of blood added at 45°C.), while only 5 per cent of pneumococci do so. All pneumococci (100 per cent) produce a yellow discoloration on blood agar (a few drops of blood added at 100°C.), while only 17 per cent of streptococci do so. Did not recognize Schottmüller's *S. milior seu viridans*. No green color was observed in the case of either streptococci or pneumococci.	**Boxer** 1906. Ueber das Verhalten von Streptokokken und Diplokokken auf Blutnährboden.
14 laboratory strains of streptococci from various human lesions. 1 strain isolated by the author from case of scarlet fever.	Horse and rabbit.	"*Bis zum Erzielung einer intensiv blutroten Farbung.*"			"*Stich-*" and "*Ausstrichkulturen.*"		"*10 Kulturen wiesen hämolysierende Eigenschaften auf, * **" (all from various human lesions). "*2 Kulturen, * *, wiesen Erscheinungen auf, die mit denjenigen identisch waren, welche Schottmüller der Gruppe des S. viridans zuschreibt*" (from the throat in case of rheumatism and from puerperal sepsis). "*Bei 3 Kulturen waren die Resultate unbestimmt.*" States that he had no strain of *S. mucosus*. The hemolytic strains produced similar appearances on both kinds of blood. The hemolysis was more pronounced when larger amounts of blood were employed. Deep colonies developed more hemolysis than surface colonies. Addition of glycerol to the agar was accompanied by no change in the appearance of the colonies. Colonies of *S. viridans* were at first gray, later greenish, with little or no hemolysis.	**Silberstrom** 1906. Ueber die Arteinheit der Streptokokken.
Tabulation of 700 strains classified according to fermentation reactions and with slight reference to hemolysis.	Sheep (human, rabbit, beef).	5 per cent.					"*It is evident that Schottmüller's Streptococcus pyogenes corresponds with ours but may include the hemolytic forms we have on other grounds classed as Streptococcus anginosus. His Streptococcus mitior or viridans probably corresponds in general with our Streptococcus salivarius and fæcalis, while his Streptococcus mucosus may be in part identical with the forms we have included under the pneumococci.*" Beyond the above statements and the statements that their *S. pyogenes* and *S. anginosus* groups are hemolytic, while the *S. fæcalis* group is non-hemolytic, nothing is said regarding hemolysis or appearances on blood agar.	**Andrewes and Horder** 1906. A Study of the Streptococci Pathogenic for Man.
Original paper not read.	Schottmüller's blood agar.						Quoted by Nieter (1907) as follows: "*Dass der Blutagar nicht den Erwartungen, die Schottmüller daran geknüpft hat, entspricht, dass er zwar ein schätzenswertes Hilfsmittel zur kulturellen Differentialdiagnose zwischen Streptokokken und Pneumokokken darstelle, derart, dass braune bis braun-grünliche Kolonien in diesen Nährboden bei höchstens zweitägiger Bebrütung für Pneumokokken sprechen, hämolytische Höfe dagegen, diese Diagnose ausschliessen. Als Grundlage zur Unterscheidung der verschiedenen Streptokokkenarten sei jedoch das Vorhandensein oder Fehlen der blutlösenden Fähigkeit eben so wenig wie alle sonstigen bisher herangezogenen Eigentümlichkeiten geeignet, da sie eine variable Eigenschaft bilde.*" According to Salomon (1908), "*Einen Befund von Beitzke und Rosenthal, denen ein Stamm zu verschiedenen Zeiten ungleiche Resultate gab, führt Schultze (1907) auf einen Wechsel in der Zusammensetzung der Nährboden zurück.*"	**Beitzke and Rosenthal** 1906. Zur Unterscheidung der Streptokokken mittels Blutnährboden.
							"*Hemolysis in blood agar may be caused by both pneumococci and streptococci.*" "*Although many strains* [of pneumococci] *fail to cause hemolysis in blood agar, the organisms isolated from the circulating blood frequently cause hemolysis when grown in the blood of the host.*"	**Buerger and Ryttenberg** 1907. Observations upon Certain Properties Acquired by the Pneumococcus in the Human Body.
65 strains (43 from various human pathological processes; the remainder from milk, skin, sputum, and feces).	Beef or rabbit.	1 or 2 cc.			Surface.	24 hrs.	42 strains of streptococci from pathological processes are tabulated as producing a "*heller Hof*" on blood agar, the remaining one as producing a "*kleiner heller Hof.*" All other strains produced "*kein Hof.*" No more detailed descriptions of colonies or appearances on blood agar given. The above 42 strains are designated as "*S. longus seu erysipelatos*" and all the others as "*S. mitior seu viridans.*"	**Nieter** 1907. Zur Streptokokkenfrage.

TABLE III—*Continued.*
The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
	Human, placental.	Usually 2 pts. Tried also 0.5 cc.		Usually 5 pts. Tried also 5 cc.	Surface.		Emphasizes the importance of microscopic examination of the colonies. Found that the zones of *S. mitior* colonies were made up of a collection of discolored non-hemolyzed corpuscles lying against the colony and an outer hemolyzed zone containing only corpuscle shadows. The pneumococcus and *S. mucosus* produced similar zones. *S. pathogenes* formed clear colorless zones. "*Nur zuweilen liegen einige zusammengebalte Reste von roten Blutkörperchen, sogenannte Schatten, der Kolonie auf. Niemals aber enthalten dieselben noch Blutfarbstoff. Diese Eigenschaft, die roten Blutkörperchen zur Auflösung zu bringen und deren Blutfarbstoff zu zerstören, behielt der S. pathogenes trotz monatelanges Fortzüchtung bei.*" Describes "*Streptococcus saprophyticus*" (*nov. sp.?*) as being present on all mucous membranes (mouth, nose, vagina, intestine) and as forming on blood agar "*zarten, feinen, grauweissen Kolonien*" without hemolysis or discoloration. Concludes that *S. mitior* produces a toxin which does not diffuse into the medium, as does the hemolysin, but remains bound to the coccus bodies and acts upon the blood corpuscles in contact with the colony, altering them in color and rendering them immune to the hemolysin. (See text for quotations.)	**Mandelbaum** 1907-08. Zur Streptokokkenfrage.
38 strains of *S. pyogenes*. 10 strains of *S. mucosus*. 13 strains of pneumococci. 6 strains of *S. saprophyticus*. 16 strains of "*sonstige Streptokokken*," mostly *S. viridans*.					Surface.		Only 16 of the strains of *S. pyogenes* were tried on blood agar but all were found to be hemolytic, as were also 4 strains included among "*sonstige Streptokokken*." All strains of pneumococcus and *S. mucosus* tested were found to produce more or less greenish colonies. *S. saprophyticus* produced neither hemolysis nor discoloration on blood agar. Most of the "*sonstige Streptokokken*" produced more or less greenish colonies and were evidently *S. mitior seu viridans*.	**Salomon** 1908. Zur Unterscheidung der Streptokokken durch kohlenhydrathaltige Nährboden.
Number of strains not given; evidently many strains.	Human.	2 pts.		5 pts.	Surface.		Found streptococci in vaginal secretion of 46 of 136 normal gravida and puerpera (33 per cent), all of *S. viridans* type. Found hemolytic streptococci in cases of severe endometritis (number not given) and in 10 cases of septicemia and peritonitis. Found an intermediary type of streptococci in puerperal parametritis.	**Fromme** 1908. Klinische und bakteriologische Studien zum Puerperalfieber.
Number of strains not given; evidently many strains.	Human, placental, defibrinated. Rabbit, for comparison in a few cases.	2 pts.		5 pts.			No hemolytic streptococci found in the vaginal secretions of 200 normal gravida, but found hemolytic streptococci in 53 of 175 cases of puerperal fever. In 8 milder cases only "non-hemolytic" streptococci were found. These strains produced on blood agar more or less greenish discoloration and some produced more or less hemolysis, always clouded and greenish, however. Intermediary types were found in convalescent cases. Minor differences in appearance on human or rabbit blood agar are noted.	**Heynemann** 1908. Die Bedeutung der hämolytischen Streptokokken für die puerperale Infection.
From vaginal and uterine secretions of 30 normal puerpera. Strains obtained with unusual precautions. Also 31 strains from various pathological conditions.	Human (probably).	2 pts.		5 pts.	Surface.		Ante partum only non-hemolytic streptococci were found in the vaginal secretions. Post partum there were found in the uterine cavity no streptococci in 14 cases, hemolytic streptococci in 8 cases, and non-hemolytic streptococci in 8 cases. They regard *S. mucosus* as a distinct type but could observe no constant morphological or cultural differences between *S. longus* (*erysipelatos*) and *S. viridans* (*mitior*) because there were intermediate forms. In general, however, the former produced on blood agar clear hemolyzed zones several mm. broad, while the latter produced a green discoloration.	**Lüdke and Polano** 1909. Ueber Hämolyse der Streptokokken.
Presumably strains from various sources.	Human, hog, calf, beef, horse, rabbit, hen, and pigeon.	10 per cent = optimum.	Standard glycerol agar.	10 cc., making a layer of agar 3–4 mm. thick.	Surface and deep.		Too much blood in the plate or too thick a layer of medium results in slow hemolysis; too little blood, in indefinite zones. Describes clear transparent zones (2–3 mm. "*im Radius*") formed by "*gewöhnliche Streptokokken*" on blood agar plates inoculated either in the depths (*i.e.*, in fluid condition) or by surface streaks. "*Der gleiche Hof tritt dann auch auf, wenn man unter Luftabschluss (überschichtet) züchtet.*" Microscopically no corpuscle shadows were seen within the hemolyzed zones. If the plates are sown too thickly with streptococci the hemolyzed zones are not characteristic since (1) the individual colonies are hindered in their development, and (2) "*eine Säuerung des Agars sofort die Platte braun verfärbt.* With regard to the use of different kinds of blood,—"*Nie fiel eine Blutart negativ aus, wenn andere positiv waren.*" As the result of various experiments (see text) the author concludes that "*Die Aufhellung ensteht: 1 dadurch, dass Blutfarbstoff aus dem betroffenen Bezirk austritt und sich durch Diffusion im umgebenden Agar verteilt*; * ."	**Zangemeister** 1909. Die Hämolyse der Streptokokken.

TABLE III—*Continued.*
The Use of Blood Agar.

Organisms.	Medium. Blood. Species.	Blood. Amount.	Agar. Composition	Agar. Amount.	Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
Several strains of "*modified pneumococci*" from cases of endocarditis.							The "*modified pneumococci*" "*always produced more or less greenish discoloration around the colony on blood agar plates but never a zone of hemolysis.*"	**Rosenow** 1909. Immunological and Experimental Studies on Pneumococcus and Staphylococcus Endocarditis.
Strains from blood, pus, throats, and blister fluid of 88 cases of scarlet fever, measles, and diphtheria.	Horse.	1 cc.	Standard agar and the following modified agar: (*a*) peptone water agar; (*b*) sugar-free agar ("*made from broth with colon bacillus added and sterilized*"); (*c*) glucose agar; (*d*) glycerol agar; (*e*) beerwort agar.		Deep (inoculated blood and then mixed with melted agar).	18–24 hrs.	By "*hemolyzing streptococcus*" is meant one "*which gives a more or less definite area of hemolysis about each colony when plated out on blood agar.*" "*In our laboratory all the hemolyzing strains and also the green ones, when tested and retested in glucose agar blood plates, gave green colonies.*"	**Anthony** 1909. Some Characteristics of the Streptococci Found in Scarlet Fever.
Many strains of *S. mitior* isolated from vaginal secretions of normal puerpera and gravida. 3 laboratory strains of *S. pyogenes* for comparison with the above.					"*Blutagarausstrich.*"		Replating only the most variable fishings repeatedly on standard blood agar and the use of specially modified blood agar revealed "*marked variation* [in hemolysis] *up and down the scale.*"	**Konrád** 1909. Weitere Beiträge zur Vaginal-Streptokokkenfrage.
15 strains of *S. longus hemolyticus.* 4 strains of *S. brevis non-hemolyticus* (mostly) from cases of puerperal sepsis 1 strain of *Staph. aureus.*	Human corpuscles.	2.5 cc.		8–10 cc.	Surface and deep.	Observations after 24, 48, and 72 hrs.	Found streptococci in the vaginal secretions and lochia respectively of 17 of 60 gravida and of 40 of 100 normal puerpera. All strains were of the *S. viridans* type. Describes "*glashellen Hof*" (3–4 mm. broad) formed by the 3 strains of *S. longus* which he studied for comparison with his *S. mitior* (*viridans*) strains. With regard to the colonies of *S. mitior* on blood agar: "*Nur wenn die Platten noch auf weitere 6–8 Stunden bebrutete und sie dann bei Zimmertemperatur aufbewahrte, begann nach 48–50 Stunden ein kleiner hämolytischer Hof sich auszubilden, der in weiteren 24 Stunden ziemlich hell und an 2–3 mm. breit wurde. Aber die Farbe und Aussehen der Kolonien war von denen des Streptococcus longus ganz verschieden, graubraun in verschiedenen Schattierungen, und wenn man die Platten mikroskopisch untersuchte, so fand man Mandelbaums Angabe bestätigt: die dunkle Färbung der Kolonien rührte wirklich von den unter den Kolonien liegenden ungelosten braunrot-grauweaun gefärbten Blutkörperchen her.*" "*Bei der Beurteilung der Platten hämolyse schloss ich nicht ganz Schottmüller an und nannte Hämolyse die glashelle Aufhellung im Umkreis von etwa 1 bis 2 mm. um die fast stets kleinbleibende, nie konfluierende Kolonie herum. Auf stärker bluthaltigen Platten (3 cc.: 8 cc. z. B.) wird die Aufhellung überhaupt nicht glashell, sondern mattgelb, * * .*" Most surface colonies of *S. aureus* were hemolytic, but the deep colonies were not.	**Sachs** 1909. Ueber Streptokokken-Hämolyse.
Cultures from lochia of 44 febrile puerpera and 56 afebrile cases.	Human.	2 pts.		5 pts.	At first employed only surface inoculation, but later inoculated the blood before mixing it with the fluid agar.	24 hrs. and longer.	Similar results were obtained on weakly alkaline blood agar and on dextrose blood agar plates. Found deep inoculation (*i.e.*, into fluid agar) more satisfactory than surface streaks or films. Found "*typisch hämolysirende Streptokokken*" in the lochia of 31 of 44 febrile puerpera and in the lochia of 38 of 56 normal puerpera, also in the vaginal secretion of 3 of 20 normal gravida. "*Unter typischer Hämolyse verstehe ich nur die vollständige Aufhellung der Umgebung der Kolonie, so dass auch mikroskopisch von einer Structur der rothen Blutkörperchen nichts mehr zu sehen ist. In 12–24 Stunden kann die helle Hof, bei nicht zu dicht beschickter Platte, die Grösse von 2 mm. erreichen (Reaction I nach Natvig).*" Certain "*nicht hämolysirenden Streptokokken*" produce no hemolysis in 24 hrs. but after 2 or 3 days incubation produce zones which microscopically are seen still to contain some unhemolyzed corpuscles. In the refrigerator the zones of the typical hemolytic streptococci did not change while those of the atypical strains became larger. (See text for quotations and further description.)	**Sigwart** 1909. Untersuchungen über die Hämolyse der Streptokokken in der Schwangerschaft und im Wochensbett.
S. lacticus found in 75 per cent of 81 samples of milk and also in cow dung and on skin.							*S. lacticus* produces no hemolysis on blood agar plates.	**Bähr** 1910. Vorkommen und Bedeutung der Streptokokken in der Milch.

TABLE III—*Continued.*

The Use of Blood Agar.

Organisms.	Medium. Blood. Species.	Blood. Amount.	Agar. Composition.	Agar. Amount.	Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
Cultures from lochia of febrile and normal puerpera.							Found hemolytic streptococci in the lochial secretions of 11–15 per cent of normal afebrile puerpera, and of 32 per cent of febrile cases. The streptococci found in both sets of cases were regarded as identical.	**Fabre and Bourret** 1910. Quelques notions nouvelles sur les streptococcies des suites de couches.
7 strains of *S. erysipelatos.* 3 "*Darmstreptokokken.*" 1 *S. mitior.* 1 *S. mucosus.* 4 pneumococci.	Horse.	2 pts.		5 pts.	Surface.		*S. erysipelatos* produces strong hemolysis in 12–20 hrs., the colonies themselves being gray. "*Darmstreptokokken*" produce an abundant greenish growth without any trace of hemolysis. Conclusion: The biological and especially the hemolytic characters of streptococci are far too variable to form the basis of differentiation ("*Artverschiedenheit*"). (See also Table VII on Mutations and Variability.)	**Hoesslı** 1910. Das Verhalten der Streptokokken gegenüber Plasma und Serum und ihre Umzuchtung.
S. pyogenes from 74 suppurations, 37 cases of angina, 6 of 15 normal throats, and 7 of 8 scarlatinal throats. *S. longissimus:* 23 strains from normal throats, 41 from throats with recent, chronic, or mild lesions. *S. conglomeratus* A: from 11 normal throats and 3 cases of angina. *S. conglomeratus* B: 14 strains, all but 2 from pathological throats. *S. brevis:* strains from intestinal canal, normal throats, heart blood, and lesions in which other bacteria were present also. *S. mucosus.* Pneumococci.	Human or animal (species?).	5 large drops.	Meat infusion agar, slightly alkaline to litmus.	10 cc.	Surface.	20–24 hrs.	*S. pyogenes* strains isolated from all the sources mentioned were alike and produced round grayish white granular colonies with large, clear, hemolyzed zones. *S. longissimus* formed flat irregular clear to brownish colonies with either no hemolysis or greenish partly hemolyzed zones with collection of corpuscles next to the colony. *S. conglomeratus* A grew on blood agar like *S. longissimus.* *S. conglomeratus* B formed small, dark, round, discrete, crateriform colonies, often wrinkled; sometimes almost as hemolytic as *S. pyogenes* and sometimes producing little or no hemolysis; 2 strains produced green colonies. *S. brevis* formed delicate homogeneous or finely granular colonies, usually with more or less green color but non-hemolytic. *S. mucosus* and pneumococci always produced greenish discoloration and never hemolysis on blood agar. The author mentions intermediary forms between *S. brevis* and *S. conglomeratus* B. (See quotations in text.)	**Thalmann** 1910. Streptokokkenerkrankungen in der Armee, Einteilung der Streptokokken und ihre Bekämpfung.
	Horse, rabbit, beef, goat, hog, guinea pig.						*S. equi* was found to form "*deutliche Resorptionshöfe*" around the colonies on blood agar made with any of the bloods tried.	**Laabs** 1910. Vergleichende Untersuchungen über den *Streptococcus equi* und andere pathogene Streptokokken.
	Schottmüller's blood agar.						On blood agar *S. equi* produced clear hemolyzed zones 2–4 mm. in diameter. (See also Table VIII on General Conclusions, etc.)	**Pricolo** 1910. Recherches expérimentales sur le streptocoque de la gourme.
"*Die dabei zur Verwendung gelangten Streptokokkenstämme (zumeist S. vulgaris) waren sämtlich menschenpathogener Natur, aus dem Blute von Sepsiskranken gezüchtet.*"	Human, probably.						Hemolytic streptococci ("*S. vulgaris*") produced hemolyzed zones on laked blood agar as well as on the ordinary blood agar plates. Hence the author concludes that there is "*eine Zerstörung von Blutfarbstoff durch die Streptokokken*" as well as a laking of the corpuscles. *S. viridans* produced larger and darker green colonies on laked blood agar than on ordinary blood agar. (See quotations in text.)	**Loening** 1910. Ueber Unterschiede in der Streptokokken gegenüber zur Geltung kommenden bakterizidin Wirksamkeit des menschlichen Blutes, insonderheit des Zitratblutes.
8 strains of "*modified pneumococci*" from cases of endocarditis. 3 strains of pneumococci from pneumonia cases. 1 *S. viridans.* 1 *S. pyogenes.* 1 *M. rheumaticus.*							*S. pyogenes* produced hemolysis on blood agar plates. All other strains produced more or less green color but no hemolysis. "*The production of green by the endocarditis cocci on blood agar when first isolated is always less than in those from pneumonia.*"	**Rosenow** 1910. A Study of Pneumococci from Cases of Infectious Endocarditis.

TABLE III—*Continued.*

The Use of Blood Agar.

TABLE III—*Continued.*

The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
Evidently many strains.							Distinguishes the following three groups of capsule-free streptococci according to their appearances on blood agar: "*1. Die einen erzeugen in der Umgebung ihrer Kultur einen ausgeprägten Aufhellungshof, von dem ich gezeigt habe, dass er lediglich durch Farbstoffaustritt aus den roten Blutkörperchen—nicht oder nur unwesentlich durch Resorption des Hämoglobins—bedingt wird ('hämolytische' Streptokokken);* "*2. Andere wachsen auf der Blutagarplatte, ohne den Nährboden zu verändern; ('S. anhaemolyticus vulgaris');* "*3. Wieder andere bilden zwar ebenfalls keinen Aufhellungshof, verfärben aber das Blutagar in nächster Nähe der Kultur grünlich ('Streptococcus viridans').*"	**Zangemeister** 1910. Ueber die Verbreitung der Streptokokken im Hinblick auf ihre Infektiosität und ihre hämolytische Eigenschaft.
Streptococci from 100 cases of angina and from 25 of 100 cases of influenza.							The author states that the streptococci (*S. pyogenes*) from cases of angina show considerable difference in their hemolytic powers, but as a rule are less hemolytic than those from suppurations of the body surface. From 4 of 100 cases of angina he found in large numbers and in almost pure culture streptococci which resembled *S. pyogenes* except that they produced neither hemolysis nor discoloration on blood agar plates. These strains are regarded as varieties of *S. pyogenes* by the author, however. Found intermediary varieties between *S. longissimus* and *S. conglomeratus* as previously described. "*Streptococcus longissimus und conglomeratus A mit ihren Uebergangsformen gehören also zu einer Streptokokkenunterart zusammen.*" (See also Table VII on Mutations and Variability.)	**Thalmann** 1911. Weitere Mitteilungen über Streptokokken, inbesondere über pyogene Streptokokken bei Erkrankungen der Atmungsorgane und deren Komplikationen.
8 strains of streptococci isolated at autopsy during an epidemic of "*grippe*" among children.	Rabbit.						Isolated a streptococcus ("*Grippestreptococcus*") from 8 cases of "*grippe*" among children, at autopsy. The growth on blood agar is described as being quite characteristic and different from that of other streptococci. It formed rather large flat light brown colonies which appeared dry even on the first day. The medium in the neighborhood of the colonies of freshly isolated cultures was discolored brownish. Hemolysis was usually quite weak and not increased by animal passage of the strains.	**Muller and Seligmann** 1911. Klinische und bakteriologische Beobachtungen bei Säuglingsgrippe. II. Bakteriologisches,—von Seligmann.
10 strains of *S. pathogenes* from throats and joints. 6 strains of *S. milior* from joint, throat, empyema, and bronchitis. 6 strains of *S. saprophyticus* from sputum.	Human, placental.	0.5 cc.	Standard agar.	5 cc.		Up to 48 hrs.	Approves Mendelbaum's differentiation of streptococci as (1) *S. pyogenes*, (2) *S. milior*, and (3) *S. saprophylicus*. "The colonies of *S. pathogenes* and *S. milior* may appear alike macroscopically on the blood agar plate. Microscopic examination is necessary for their differentiation" (Translation).	**LeBlanc** 1912. Zur Artenfrage der Streptokokken.
Evidently many strains from various sources; number not given.							Found classification based upon appearance of cultures in bouillon, length of chain, etc. unsatisfactory because these features were variable, but found character of growth on blood agar quite constant and satisfactory for classification as follows: (1) *S. hemolyticus*, (2) *S. anhemolyticus*, (3) *S. viridans*, (4) *S. mucosus*.	**Rolly** 1912. Experimentelle bakteriologische Untersuchungen von verschiedenen Streptokokkenstämmen.
40 hemolytic streptococci (sepsis, scarlet fever, suppurations, and angina).	Rabbit.						All streptococci which produced hemolysis on blood agar plates were found to produce a filterable hemotoxin in rabbit serum bouillon.	**Braun** 1912. Ueber das Streptolysin.
10 strains of *S. acidi lactici* from bovine and human milk. 6 strains of *S. pyogenes* from various suppurations. 5 strains of pneumococci from sputum.	Rabbit, hog, sheep, beef, calf.	2 pts.	Meat extract peptone agar.	3 pts.	Surface.	24 and 48 hrs.	The appearance on blood agar was constant for any given species of blood but somewhat different for different species of blood. This was apparent principally among strains of *S. acidi lactici*, none of which hemolyzed rabbit or calf blood but all of which produced slight hemolysis of beef blood, and two of which hemolyzed hog blood. *S. lanceolatus* and *S. pyogenes* are tabulated as having produced considerable hemolysis of all the bloods tried. "*Die schönsten hämolytischen vollkommen hellen, scharf abgegrenzten und mittelmässig breiten Höfe bekam ich von Streptococcus pyogenes, bei lanceolatus waren die Höfe schmal, aber scharf. Bei acidi lactici fehlte eine Hämolyse zuweilen, wenn sie vorhande war, so war sie meist ausgebreitet, diffus, aber oft nicht vollständig. Ich glaube nicht, dass man pyogenes und acidi lactici mit voller Sicherheit durch Hämolyse trennen kann. Scharf war der Unterschied von lanceolatus und acidi lactici.*" Noted the appearance of double or multiple concentric rings or zones around some strains of acidi lactici and pneumococci. (See quotation in text.)	**Salto** 1912. Versuche zur Abgrenzung des *Streptococcus. acidi lactici* von *Streptococcus pyogenes* und *Streptococcus lanceolatus*.
3 strains from (1) "*Handphlegmone,*" (2) "*Zungeninfiltrat,*" (3) lung of guinea pig.	Sheep, (beef, veal, and hog blood for comparison).	5 per cent.			Surface.		When first isolated "*Der Blutfarbstoff war nicht nur unter dem Impfstrich, sondern auch noch in breiten Hof gelöst.*" (See Table VII on Mutations and Variability.) Concludes that "*Die Hämolyse ist als differentialdiagnostisches Mittel für Streptokokken ungeeignet, da sie eine viel zu inkonstante Grösse ist und ohne erkennbare Ursache verschwinden und wieder auftretten kann.*"	**Jaffé** 1912. Beobachtunge bei blutlösenden und bei gramnegativen Streptokokken.

TABLE III—*Continued.*

The Use of Blood Agar.

TABLE III—*Continued.*
The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
							Hemolysis was much reduced in blood agar containing sugar. On sugar blood agar hemolytic streptococci produced small hemolyzed zones surrounded by broad grayish green outer zones without hemolysis which the author attributes to the action of the acids formed. He does not think the acids were responsible for the reduction of hemolysis but rather that presence of sugar in the medium resulted in a suppression of the "carnivorous metabolism" and the adoption of an "herbivorous metabolism."	**Kuhn** 1912. Einflus von Zucker auf Hämolyse und Virulenz.
36 strains: 26 from bovine mastitis, 3 from stable air, 1 *S. acidi lactici*, 1 from saliva of horse, 3 from bovine vaginitis, 1 *S. pyogenes* (Rosenbach), and 1 *S. erysipelatos* (Fehleisen).	Beef or goat, fresh, not defibrinated.	2 pts.		5 pts., 7–10 cc. in each Petri dish.	Surface, probably.	24 hrs.	*S. pyogenes*, *S. erysipelatos*, and 4 strains from bovine mastitis are indicated as being hemolytic, producing clear colorless zones 2–3 mm. in diameter in 24 hrs. 3 other mastitis strains and the strains from stable dust and horse saliva are marked ±. All other strains were non-hemolytic. Colonies on blood agar were described as gray, brown, or grayish brown. "*Nur die wenigsten Milchstreptokokken zeigen diese hämolytische Eigenschaft. Die meisten bilden kein Hämolysin und nehmen eine Mittelstellung zwischen dem S. mitior und dem S. mucosus ein. Die Mehrzahl ist aber dem ersteren beizuordnen.*"	**Gminder** 1912. Untersuchungen über Mastitisstreptokokken und ihre Differenzierung von saprophytischen Streptokokken.
	"*Schottmüller-Agar.*"						Was unsuccessful (in a limited number of experiments) in differentiating mastitis streptococci from human strains culturally. Mastitis streptococci usually resembled "*S. mitior seu viridans und S. mucosus*" on "*Schottmüller-Agar.*"	**Ernst** 1912. Eine Entgegnung zu A. Gminders Arbeit.
	Human.	5 large drops.	Meat infusion agar.	5–10 cc.			"*Die pyogenen Streptokokken treten durch ihren grossen hellen Hof deutlich hervor; der Streptococcus viridans zeigt Grünfärbung und lässt sich vorhandener Hämolyse mikroskopisch dadurch trennen dass die Hämolyse ringförmigen Charakter hat; er hält den Blutfarbstoff der roten Blutkörperchen direkt um die Kolonie so fest, dass selbst da, wo Viridans-Kolonieen sich im Resorptionshofe des Pyogenes entwickeln, diese ihre direkt aufgelagernden roten Blutkörperchen unverändert erhalten. Der Pneumococcus bildet sehr charakteristische Kolonieen, bei denen der Rand wie ein verdickter Ring hervortritt; es zeigt nur selten und dann geringe ringförmige Hämolyse.*" (See also Table VIII on General Conclusions, etc.)	**Thalmann** 1912. *Streptococcus viridans* im Blut ohne Veränderung der Herzklappen. Zur Einteilung der Streptokokken.
13 strains of various degrees of hemolytic activity from vaginal secretion and puerperal sepsis. 1 strain of *S. viridans* and a non-hemolytic streptococcus for comparison.			Agar containing 0, 1.5, and 4 per cent dextrose; also glycerol agar.		Surface.		Hemolysis was most extensive on glycerol blood agar, almost as great on blood agar without dextrose, less on 1.5 per cent dextrose blood agar, and least or often negative on 4 per cent dextrose blood agar. The *S. viridans* strain produced less greenish discoloration on 4 per cent dextrose blood agar than on dextrose-free blood agar. Brownish discoloration accompanied growth on dextrose blood agar in most cases.	**Benthin** 1912. Beiträge zur Hämolysefrage der Streptokokken.
(*a*) 20 strains from various human infections. (*b*) 54 strains from milk of normal cows. (*c*) 18 strains from cows with mastitis. (*d*) 48 strains from market milk. (*e*) Strains from feces of all but one of 31 normal bottle-fed infants and 30 with intestinal disorders.	Goat, also beef, rabbit, and human to some extent.			10 cc.	Surface.		No detailed descriptions of appearances on blood agar are given, but the author evidently uses the term *hemolytic* to describe even those strains which produce even a trace of hemolysis. (*a*) Of the strains from various human infections 1 hemolyzed human blood only; the others hemolyzed all the bloods tried. (*b*) Of the strains from milk of normal cows almost half produced only a trace of hemolysis of human blood, but a fair amount of hemolysis of beef, goat, and rabbit blood; 9 hemolyzed beef or goat blood only; 16 hemolyzed none of the bloods. (*c*) Of the mastitis strains only 1 produced strong hemolysis of human blood. (*d*) Of the strains from market milk 24 were non-hemolytic; 15 showed a tendency to hemolyze; and 9 were green. (*e*) All the streptococci from infants' stools were either non-hemolytic or produced only a trace of hemolysis on human blood agar.	**Puppel** 1912. Ueber Streptokokken in der Milch und im Säuglingsstuhl.
35 strains from milk.							The paper points out that *S. lacticus* can be differentiated from *S. pyogenes* by means of blood agar plates. *S. pyogenes* produces small colonies surrounded by a large zone of hemolysis, whereas *S. lacticus* produces green or grayish colonies with very little or no hemolysis.	**Ruediger** 1912. A Study of Thirty-Five Strains of Streptococci Isolated from Samples of Milk.

TABLE III—*Continued.*

The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
Strains from throats (septic sore throats) and various complications.							*"The colonies on blood agar plates are larger and more moist than those of the common hemolytic streptococcus (Streptococcus pyogenes); at times there may be a faint greenish tint on transmitted light. The zone of hemolysis is relatively narrow and begins immediately around the colony, and its outer margin is often indistinct. The growth on the surface of blood agar slants is abundant, raised, and moist, but does not present the mucoid appearance of the Streptococcus mucosus."* *"Morphologically in the smears from the throat and the exudates, it occurs in short chains and often in pairs. The cocci, * * , are spherical, but appear in twos in the chain. They are strongly Gram-positive and are surrounded by a definite capsule. The capsular substance is less abundant than that surrounding the pneumococci, does not indent between the pairs, as is the case in the latter, and is soon lost on artificial cultivation."*	**Davis and Rosenow** 1912. An Epidemic of Sore Throat Due to a Peculiar Streptococcus (Chicago Epidemic 1912).
45 strains from throats and secondary lesions of cases of epidemic sore throat. 1 strain of ordinary hemolytic streptococci from udder of cow.					Probably deep since he speaks of growth on the upper and under surfaces of plates. No deep colonies described, however.		Practically all the strains from the various complications and some of those from throats had certain peculiarities by which they were readily differentiated from ordinary streptococci. On blood agar plates *"The colonies are larger and more moist than those of the ordinary type (S. pyogenes). They are raised and tend to spread on the upper or under surface of the media. A distinct zone of hemolysis is always present, which is clear with often a faint greenish tint when viewed by transmitted light. This zone is usually but not always narrower than that produced by the ordinary Streptococcus hemolyticus. It begins immediately about the colony and its outer margin may be indistinct. All the blood-corpuscles in this clear zone have been dissolved."* *"On blood agar slants the growth is abundant, raised and moist but does not have the mucoid appearance of the Streptococcus mucosus. There is much less tendency for the colonies to remain discrete than is the case with ordinary Streptococcus pyogenes."* *" * * , hemolytic streptococci, apparently of the ordinary type, were common, especially in the throat and tonsils. These streptococci do not have a capsule; the colonies on blood agar possess wide clear zones of hemolysis; they tend to spread less on the surfaces, and are smaller and less moist than the organism above described."*	**Davis** 1912. Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat (Chicago Epidemic 1912).
Strains of streptococci from cases of epidemic sore throat and from *"separator slime."* Also strains of *S. pyogenes*.							*"The difference in the character of the growth of the streptococci* (from separator slime) *on blood agar plates before and after animal passage was striking, hemolyzing colonies only being present on the plates from the blood of the animals and almost exclusively non-hemolyzing colonies on the plates made directly from the suspensions."* The ordinary hemolytic streptococcus (*S. pyogenes*) is described as producing small colonies surrounded by wide zones of hemolysis on blood agar. The streptococci obtained from animals inoculated with *"separator slime"* resembled those obtained from cases of epidemic sore throat and produced a more abundant growth and less hemolysis on blood agar than *S. pyogenes*.	**Rosenow** 1912. A Study of Streptococci from Milk and from Epidemic Sore Throat, and the Effect of Milk on Streptococci.
Strains of *S. epidemicus:* 1 from milk, 2 from sore throats, 1 from throat of carrier, and 3 from secondary peritonitis and adenitis.	Rabbit.						*"On blood agar plates the colonies were moist, somewhat larger than those of Streptococcus pyogenes, had a tendency toward spreading, and were surrounded by clear zones of hemolysis with a greenish tinge by transmitted light."* *"It therefore corresponds in the essential cultural characteristics and in its morphology to the streptococcus epidemicus* (Davis)." The above description is of the strain isolated from milk, but the other strains are described as being like it.	**Stokes and Hachtel** 1912. Septic Sore Throat. A Milk-Borne Epidemic in Baltimore, Md. Bacteriological Study of the Outbreak.
							State that pneumococci are non-hemolytic on blood agar or blood bouillon but that they produce a dark green discoloration on blood agar.	**Neufeld and Händel** 1912. Pneumokokken (In Kolle and Wassermann's Handbuch, 2nd edition).
Strains from vaginal and lochial secretion (before, during, and after labor) of 72 or more normal and 24 or more febrile women, of whom died.	Sheep or guinea pig, defibrinated.	5 per cent.			Surface.	Anaerobic and aerobic, 72 hrs.	Staphylococci were the most numerous of the organisms found, the streptococci and diphtheroids coming next in order of occurrence. The staphylococci and streptococci are merely tabulated as hemolytic or non-hemolytic. No detailed descriptions of appearances on blood agar are given.	**Sitzenfrey and Vatnick** 1913. Zur Frage der prognostischen und praktischen Verwertung bakteriologischer Befunde bei puerperalen Prozessen.
92 strains from milk and the human throat (normal?).	Rabbit.	*"A few drops."*			Surface.	24 hrs.	17 of the strains were hemolytic, of which 10 strains were from milk and 7 from throats. No detailed descriptions of appearances on blood agar are given.	**Stowell, Hilliard, and Schlesinger** 1913. A Statistical Study of the Streptococci from Milk and from the Human Throat.
	Human and horse.						*S. hemolyticus, S. epidemicus, S. mucosus, S. pneumoniæ,* and *S. viridans* merely tabulated with reference to amount of hemolysis on blood agar. (See Table VIII on General Conclusions, etc., and Table VII on Mutations and Variability.)	**Davis** 1913. Interrelations in the Streptococcus Group with Special Reference to Anaphylactic Reactions.

TABLE III—*Continued.*

The Use of Blood Agar.

TABLE III—*Continued.*
The Use of Blood Agar.

Organisms.	Medium. Blood. Species.	Blood. Amount.	Agar. Composition.	Agar. Amount.	Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
26 strains of *S. equi* (23 laboratory strains of various ages and 3 freshly isolated by the author). 27 strains of *S. longus* isolated by the authors from human pathological material.	Rabbit. (Rabbits were inoculated intravenously with 2 cc. of serum bouillon culture, and in 1 hr. 2 cc. of blood were drawn from the ear vein, mixed with agar at 45°, and poured into a Petri dish).	2 cc.	Beef infusion, Witte peptone, agar.	14 cc.	Deep. (See under Blood.)	24 hrs. or longer.	Speaking of *S. equi*, "*Nach 24 Stunden hat sich als-dann um jede einzelne Kolonie innerhalb des Agars die typische, etwa 2 mm. breite glashelle Zone gebildet.*" "*Der Rand des hämolytischen Hofes einer Streptococcus longus-Kolonie zeigt nicht die scharfe Begrenzung wie der des Drusecoccus. Im allgemeinen ist der hämolytische Hof einer Drusekolonie um ein geringes grösser wie beim Streptococcus longus. Der wichtigste Unterschied besteht jedoch darin, dass die hämolytische Zone beim Streptococcus longus nach 24 Stunden stationär bleibt, selbst bei weiterem Verweilen im Brutschrank, während der Hof des Drusecoccus sich in den folgenden Tagen sowohl bei Zimmer-. als auch bei Brut-temperatur, langsam vergrössert, wodurch die Höfe benachbarter Kolonien häufig zusammenfliessen. Auf diese Weise hellt sich häufig eine mit dem Druse-Streptococcus bewachsene Blutplatte vollkommen auf. Meist ist auch der Farbenton der Blutplatten beider Arten ein anderer. Der Blutfarbstoff erleidet beim Drusecoccus kaum eine Veränderung, während die Farbe der Blutplatte beim Streptococcus longus, zumal beim Vorhandensein zahlreicher Kolonien, einen schmutzig braunroten Farbenton annimmt (wahrscheinlich durch Bildung von Methämoglobin).*"	**Koch and Pokschischewsky** 1913. Uber die Artverschiedenheit des *Streptococcus longus seu erysipelatos* und des *Streptococcus equi* (Druse-Streptococcus).
							The formation of peculiar excresences or droplet-like colonies on coagulated blood serum or blood agar is described as being characteristic for *S. equi*. The question of hemolysis is dismissed with the following remark: "*On ne réussit pas plus par hémolyse à établir une différence entre les streptocoques de la gourme et d'autres. La propriété de l'hémolyse semble pouvoir être acquise par le milieu de culture.*"	**Bemelmans** 1913. La spécificité des streptocoques de la gourme.
247 strains from a great variety of pathological processes.	"*Freshly prepared blood agar plates were used to demonstrate hemolysis.*" No other details.						"*In the test for hemolysis about five per cent of the cultures showed the production of a greenish zone about the colonies.*" " * * * *several cultures secured from the severest general infections have not been hemolytic.*" In the tables all cultures are simply marked + or − with regard to hemolysis. (See also Table VIII on General Conclusions, etc.)	**Floyd and Wolbach** 1914. On the Differentiation of Streptococci. (Preliminary Notes).
Studied many strains from various sources and of various types.	Human, defibrinated.	0.5 cc.	Standard agar prepared from Liebig extract and Witte peptone.	7 cc.	Surface.		Generally describes the colonies on blood agar simply as "*hemolyzing*," "*hazy or slightly hemolyzing*," "*green*," "*grayish*," "*non-hemolyzing*," *etc.*, but in describing the transformations of pneumococci to streptococci he distinguishes between the "*clear zone of hemolysis that began immediately outside the colony itself*" of the streptococcus, and the colony of the partially transformed pneumococcus in which "*the hemolysis was outside of a zone in which the corpuscles were intact.*"	**Rosenow** 1914. Transmutations within the Streptococcus-Pneumococcus Group.
14 strains of streptococci from the joint fluid of 14 cases of acute rheumatism.							5 strains "*produced green on blood agar.*" 6 strains "*produced a slight but hazy hemolysis.*" 3 strains "*produced small grayish colonies * * * without perceptibly affecting the medium.*"	**Rosenow** 1914. The Etiology of Acute Rheumatism, Articular and Muscular.
9 strains: from cases of arthritis, septicemia, scarlet fever, endocarditis, and infected finger.	Rabbit, for all strains. Human, for 2 strains.						The amount of hemolysis on blood agar (3 separate tests, interval not stated) is described merely as "*marked*," "*moderate*," "*distinct*," "*slight*," *or* "*none.*" As tabulated, the results of the 3 tests for each strain varied from "*marked*" to "*moderate*" or between "*none*" and "*slight.*" All strains produced "*marked*," "*moderate*," or "*distinct*" hemolysis in some of the tests. Also determined the "*hemolytic index*" of each strain in serum bouillon by the special method described by the author in 1912. (See Table IV on The Use of Blood Bouillon and Other Fluid Media.) It is concluded "*That for the quantitative and qualitative estimation of hemolysin production by streptococci the blood agar plate is unreliable and should give place to titration of the M. H. D. (minimum hemolytic dose) of a culture of streptococci in fluid media.*"	**M'Leod** 1914. Criteria of Virulence amongst Streptococci, with Some Remarks on Streptococcal Leucocidin.
2 strains of *S. pyogenes;* sources not given.	Oxalated blood of infected rabbit.	2 cc. of oxalate solution + blood, 3:1.	4 per cent glycerol agar.		Deep, the blood itself being infected.	24 hrs.	The author gives no descriptions of appearances on blood agar. The results are merely recorded as + or − (hemolytic or non-hemolytic). (See also Table VII on Mutations and Variability.)	**Relchstein** 1914. Ueber den Nachweis der Streptokokken im strömenden Blute.
18 strains from throats. 1 from cervical gland, and 9 from milk slime.	Rabbit.				"*Conradi method.*"		On blood agar all strains produced "*green*" or "*faint green*" colonies. Only the one from a cervical gland produced any hemolysis. Most of the others produced "*methemoglobin brown*" zones.	**North, White, and Avery** 1914. A Septic Sore Throat Epidemic in Cortland and Homer, N. Y.

TABLE III—*Continued.*

The Use of Blood Agar.

Organisms.	Medium. Blood. Species.	Blood. Amount.	Agar. Composition.	Agar. Amount.	Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
105 strains from various pathological processes, and from milk. normal throats, and human stools.	Human.				Surface.	1–3 days.	*"Some of the strains, notably the mannite fermenters, produced no lysis. Others obtained from septicemias produced a wide zone in less than twenty-four hours. Between these two extremes, however, were all gradations. Many of the pneumococci and strains of the 'viridans' type from endocarditis produced a narrow zone in two to three days. The hemolysis depended also on the depth of the medium and the amount of blood it contained, for in an unevenly mixed blood plate it was sometimes observable in the thin, but not in the thicker portions. * * It is difficult, then, to draw a line sharply between hemolyzing and non-hemolyzing types. The property is present to some extent in most streptococci and while in general it is more pronounced in the more virulent type, the differences are essentially quantitative and therefore not altogether satisfactory as the basis of classification."*	**Hopkins and Lang** 1914. Classification of Pathogenic Streptococci by Fermentation Reactions.
164 strains of various organisms (cholera, other vibrios, typhoid, anthrax, *proteus, pyocyaneus*, etc.) including 27 streptococci and 20 staphylococci.	Sheep, also human, beef, and rabbit to some extent.	5 per cent.			Surface.	Up to 48 hrs.	Recognizes 2 types of hemolysis on blood agar plates: 1) *"Hämopepsie,"* *"Bildung eines durchsichtigen Hofes um die einzelnen Kolonieen auf der Platte;"* 2) *"Hämoglobinopepsie,"* *"Aufhellung der Blutplatte in der Umgebung der Kolonieen, so dass die Blutplatte transparent und schwach grünlich gefärbt, dagegen nicht durchsichtig erscheint."* The streptococci studied were either non-hemolytic or produced *"Hämopepsie."* The staphylococci studied all produced *"Hämopepsie."* Some of the other organisms studied produced *"Hämoglobinopepsie."* (See also Table IV on Blood Bouillon, etc., and discussion in text.)	**Baerthlein** 1914. Ueber Blutveränderung durch Bakterien.
6 strains of *S hemolyticus*. 5 strains of pneumococci. 24 strains of *S. viridans*.							*"All the strains were tried in blood agar and by this means were separated into three groups, that is, those producing green pigment, those producing hemolysis, and those producing pneumococci. All were considered pneumococci that had well-developed capsules, that fermented inulin, that were green in blood agar, and that were dissolved by bile."* No detailed descriptions given.	**Thro** 1914. Experiments on the Variability of the Fermentative Reaction of Bacteria, Especially the Streptococci.
12 strains studied, (7 from throats, 3 from udders of cows, and 1 human and 1 bovine strain from Chicago epidemic, 1912, for comparison).							Of the strains reported all except one (a bovine strain) were hemolytic. 4 of the throat strains and 2 isolated from the udders of cows resembled the *S. epidemicus* from the Chicago epidemic (1912), producing a narrow zone of hemolysis. *"The other two* (throat strains) *resemble the ordinary hemolytic streptococcus."* (See also Table VIII on General Conclusions, etc.)	**Capps and Davis** 1914. An Epidemic of Streptococcus Sore Throat in Jacksonville, Ill., Which was Traced to the Milk of Cows Affected with Streptococcus Mastitis.
2 strains studied, 1 from blood of fatal case (*S. epidemicus*) during Chicago epidemic, 1912; 1 from cow with inflamed udder, Chicago 1912.	Human.					24 hrs.	Most of the paper is devoted to the subject indicated by the title. *"So far as we know, all the streptococci causing epidemics of septic sore throat have been of the hemolytic variety."* *"Those organisms are called hemolytic which cause a distinct clear zone of hemolysis about the colonies when incubated at 37°C. for 24 hours Many streptococci may produce a narrow greenish, grayish, or brownish zone in which, especially after two or more days at times, some clearing of the media may occur. Such strains, however, are not considered true hemolyzers and are referred to as non-hemolytic."* *"So far as we know at present, any appreciable number of hemolytic streptococci found in cow's milk obtained carefully in sterile tubes directly from the cow's udder always means a diseased condition."*	**Davis** 1914. The Growth and Viability of Streptococci of Bovine and Human Origin in Milk and Milk Products.
"There were sixteen acute alveolar abscesses, eighteen subacute or chronic abscesses, and eight teeth with diseased root-canals from which material was received for bacteriologic examination."	*"Blood agar slants."* (No other details.)				Surface.	Aerobic and anaerobic.	*"Within twenty-four hours one will usually see a fine granular growth over the surface of the aerobic blood agar slant. If one examines this closer the growth is seen as fine greenish colonies. The water of condensation contains chains of these organisms which are usually Gram-positive. There may also be a similar growth in the anaerobic tube but one can not distinguish the green color. At the end of forty-eight hours, however, there is quite a change in the appearance of the anaerobic growth; it is very luxuriant, grayish-white in color and the colonies have a great tendency to fuse. On opening this tube one notices immediately a very offensive odor."* *" * * we found the predominating organism to be a streptococcus. We have obtained many graded variations from a hemolytic streptococcus with a wide zone of hemolysis in the acute abscesses, to a Streptococcus viridans in the chronic, and just recently one in which a Streptococcus mucosus was the predominating organism. These * will in many instances grow as well anaerobically as they will aerobically."*	**Gilmer and Moody** 1914. A Study of the Bacteriology of Alveolar Abscess and Infected Root Canals.
53 strains of streptococci from sore throats and secondary lesions in man. 12 strains from cow's milk.	Horse.	⅔ cc.	Standard veal infusion agar (Witte peptone). Reaction = ± 1 per cent N acid to phenolphthalein.	12 cc.	Deep.	48 hrs	The authors distinguish 2 types of appearance in blood agar plates: *"Type α"*) " * * *a somewhat greenish discoloration and partial hemolysis of the blood corpuscles immediately surrounding the colony forming a rather indefinitely bounded zone one to two millimeters in diameter and surrounded by a second narrow, clearer, not discolored, partially hemolyzed zone. * * Under the microscope many of the corpuscles* (in the inner discolored zones) *were seen to be present but obviously discolored, the discoloration differing a good deal in intensity for different strains of streptococci. The corpuscles remaining in the outer (clearer) zones were much fewer and never discolored."* *"Type β"*) " * * *sharply defined, clear, transparent, completely hemolyzed, colorless zones two to four millimeters in diameter. Under the microscope no corpuscles were seen to remain within the zones."*	**Smith and Brown** 1915. A Study of Streptococci Isolated from Certain Presumably Milk-Borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914.

TABLE III—*Continued.*

The Use of Blood Agar.

TABLE III—*Continued.*

The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
Streptococci from 150 cases of pyorrhea alveolaris and apical abscesses.	Sheep, defibrinated, or rabbit, whole.	10 per cent.	Agar plates or slants.		Surface, probably.		"*In every case but two the colonies on blood agar were surrounded by a green halo. In the two exceptions, the colonies appeared as small gray dots without any change in the surrounding blood agar. In no case were hemolytic streptococci obtained;* * *."	**Hartzell and Henrici** 1915. A Study of Streptococci from Pyorrhea Alveolaris and from Apical Abscesses.
2 strains of *S. lacticus* from ice cream and milk respectively.	Human or goat, defibrinated.	5 per cent.	Beef infusion agar.		Surface.		Both strains, originally non-hemolytic, acquired to some extent the power to hemolyze human and goat blood after animal passages.	**Heinemann** 1915. The Variability of Two Strains of *Streptococcus lacticus*.
Strains of streptococci isolated from the tissues of the appendix in 17 of 20 cases of appendicitis.							"*The strains of streptococci which were found to have an affinity for the appendix formed short chains, much acid and a diffuse turbidity in ascites dextrose broth, but no clumps and, with but two exceptions, produced a moderate amount of green on blood agar plates.*"	**Rosenow** 1915. The Bacteriology of Appendicitis and its Production by Intravenous Injection of Streptococci.
60 strains from various human infections.	Rabbit, defibrinated.	1 cc.		10 cc.	Surface.		"*The results were recorded under three heads: H, hemolysis; G, green colonies with or without slight hemolysis; and N, gray or brown colonies showing no action on hemoglobin.*" "*An interesting observation, the constancy of which was not determined, is the fact that many of the raffinose fermenters gave green colonies accompanied by a slight zone of hemolysis, while the mannite fermenters usually gave a green to a greenish brown colony without any hemolysis. This reaction was merely noted and no significance can be claimed for it.*"	**Kligler** 1915. A Study of the Correlation of the Agglutination and the Fermentation Reactions Among the Streptococci.
9 strains from milk sediment. A number of strains from sore throats.							"*The streptococci isolated from the throats produced on blood agar plates rather large, moist colonies, surrounded by narrow, ill-defined, hazy zones of hemolysis, corresponding closely in these characteristics with the organisms isolated from the milk, and indistinguishable from the milk organisms after animal passage.*" "* * *, *the organisms from milk resembled the rheumatic strains* (previously studied?) *culturally and morphologically.*"	**Rosenow and Moon** 1915. On an Epidemic of Sore Throat and the Virulence of Streptococci Isolated from the Milk.
Strains of *S. viridans* from various human lesions.	Human.						"*When tested in human blood agar, some of the strains were distinctly green without any suggestion of hemolysis. Some grew as minute, green colonies with a narrow clear zone.* * * *Two strains of the short-chained type, obtained from urine, formed dark brown colonies.*" "*In blood plates, if the blood and agar were unevenly distributed, a slight variation in the production of hemolysis and green pigment took place. In areas of the plate where the agar was of greater depth and there was more blood, the colonies were green, while in the areas where the agar was of less thickness, a slight zone of hemolysis was shown.*"	**Thro** 1915. Further Experiments on the Variability of the Fermentative Reaction of Bacteria, Especially the Streptococci.
Many strains from various sources.	Horse.	1 cc. to each plate.	(Presumably meat infusion agar.)		Surface.	Up to 3 days.	No descriptions of appearances on blood agar are given except such as are indicated by the use of the following terms: "*Hemolysis,*" "*Green,*" "*No color,*" "*Haze,*" "*G-hemolysis,*" "*G-haze,*" "*Haze-hemolysis,*" "*No color-haze,*" "*Brown,*" etc. The strains from various sources produced appearances indicated by the foregoing terms as follows: Human throat (43 strains); feces (31); and blood, pathological conditions, etc. (52): 14 = "*Hemolysis*" (none from throat); the others = "*G-hemolysis,*" "*Haze-hemolysis,*" "*Brown-hemolysis,*" "*Haze,*" "*Green,*" "*G-haze,*" "*No color,*" etc. Cow's milk (20): all "*Green*" or "*G-haze.*" Cat throat (51); esophagus and stomach (31); intestine (54); and feces (11). 2 (from throat) = "*Haze-hemolysis;*" all others = "*Green,*" "*Haze,*" "*G-haze,*" or "*No color.*" Dog throat (80); esophagus and stomach (64); intestine (37); and feces (38). 43 = "*Hemolysis*" (from esophagus and stomach, intestine, and throat); others = "*Green,*" "*G-hemolysis,*" "*G-haze,*" "*Haze,*" "*No color,*" etc. Pigeon, esophagus to intestine (10): 2 = "*Hemolysis;*" others = "*Green,*" "*Haze,*" or "*? color.*" Hen, stomach and intestine (6): 1 = "*Hemolysis ?,*" others = "*Haze*" and "*No color.*" Horse feces (6): all = "*Green.*"	**Broadhurst** 1915. Environmental Studies of Streptococci, with Special Reference to the Fermentative Reactions.
Hemolytic streptococci from sore throats, 1 strain from secondary peritonitis, and 2 from udder of cow. Compared with other hemolytic and non-hemolytic strains.					Surface and deep.		"*Typical hemolysis may not be present on surface streaked blood plates, although it may develop after longer incubation.*" "*Compared with the streaked plates, the poured plates showed an apparent higher percentage of hemolyzing types and more important, typical wide zones of hemolysis. Although the streaked plates are more convenient, it is evident from this that they may give confusing results.*" (See Table VII on Mutations and Variability.)	**Krumwiede and Valentine** 1915. A Bacteriological Study of an Epidemic of Septic Sore Throat.

TABLE III—*Continued.*

The Use of Blood Agar.

TABLE III—*Continued.*

The Use of Blood Agar.

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The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
							(See Table VII on Mutations and Variability.)	**Irons, Brown, and Nadler** 1916. The Localization of Streptococci in the Eye.
Many strains of streptococci; from tonsils of normal individuals and appendicitis patients, from Steno's duct in patients with parotitis, from various dairy products, and from removed appendices.							The authors describe an epidemic of appendicitis followed by an epidemic of parotitis. The organisms responsible for the appendicitis were apparently "*green-producing streptococci.*" Cultures from samples of butter obtained during the epidemic of parotitis "*showed a large number of 2 types of colonies of streptococci: the one produced distinct green colonies on blood agar; the other produced smaller, grayish, elevated, round colonies surrounded by a narrow hazy zone of hemolysis.*" "*The cultures from the dairy products showed a preponderance of non-hemolyzing, short-chain-producing streptococci, often in almost pure form and in enormous numbers. Slightly hemolyzing streptococci were found occasionally.*"	**Rosenow and Dunlap** 1916. An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products.
11 strains of streptococci from tonsils and pyorrheal pockets of cases of herpes zoster.							The streptococci believed to cause herpes zoster formed "*moist green-producing colonies of a non-encapsulated, gram-staining, short-chained, often lanceolate streptococcus.*"	**Rosenow and Oftedal** 1916. The Etiology and Experimental Production of Herpes Zoster.
Résumé of work done on many strains of various kinds, studied by the author and others in this and previously reported papers.	Human, defibrinated.	5 per cent.	"*Plain agar (0.6 plus).*"	"*Poured to a depth of about 2 mm.*"	"*Surface rubbed.*"		"*The hemolytic streptococci liberate the hemoglobin, both on solid and in fluid media. * * It appears to me probable that the organism grown on ordinary blood agar liberates the hemoglobin * * and then either destroys it or uses it as a food material. * * The colonies * * show no evidence of having in them any of the altered forms of hemoglobin. * * the clear zone surrounding the colony appears devoid of any trace of hemoglobin, and if hemolyzed blood be used in the agar, a distinct clear zone develops about the growth.*" "*The non-hemolytic forms growing on blood agar produce either no hemolysis, or more commonly, a pale bluish green to green, followed by brown, and finally a deep dirty brown, almost black color. * * A curious phenomenon of these green-producing streptococci is the apparent storing of some of the altered hemoglobin in the colony itself. * * It is probable that in all these green producers a certain amount of hemolysis actually occurs, although in the vast majority of them it is not visible.*" "*Acid production is not necessary for the production of the green color, because in the colonies of B. lactis aerogenes, an actively fermenting non-hemolyzing organism, green is not produced.*" However, "*in the case of the hemolytic streptococci, the hemolysin of which is absent or inactive on dextrose blood agar, the organisms form the green coloration in much the same manner as the non-hemolytic green producers.*" (See text for additional quotations.)	**Holman** 1916. The Classification of Streptococci.
1 strain of hemolytic streptococci (human type) from udder of cow without gross evidence of mastitis. 1 strain of human type isolated by Davis (1912) from bovine mastitis. 1 strain of *S. epidemicus* isolated by Davis (1912) from peritonitis following tonsillitis.	Human.						The streptococci of the human type isolated from one of the cows "*grew as small, translucent, slightly moist, grayish-white colonies on blood agar plates with a clear transparent hemolytic zone from 2 to 4 mm. in diameter; * * .*" The strains from mastitis (isolated by Davis, 1912) produced "*small dry gray colonies with a sharply defined zone of hemolysis from 4 to 6 mm. in diameter.*" The colonies of the streptococcus from peritoneal pus were "*large, moist grayish-white, and spreading, often on the surface almost covering the clear hemolytic areas around them.*"	**Mathers** 1916. Different Types of Streptococci and Their Relation to Bovine Mastitis.
85 strains of β hemolytic streptococci from 328 samples of bottle milk.	Human.					48 hrs.	"*Only those streptococci were selected the colonies of which were surrounded by a distinct clear zone of hemolysis on human blood agar plates* (Type β, Smith and Brown). *The feebly hemolytic streptococci (Type α) were often noted in the milk, but were disregarded. * * .*" "*Though all these strains belong to the hemolytic group the degree and character of hemolysis in all instances may not be uniform. The strains, on the basis of hemolysis, have arbitrarily been arranged in 4 groups as follows: 1st, wide clear hemolytic circle, from 2 to 4 or more mm. in diameter; 2nd, clear hemolytic circle from 1 to 3 mm. across, with diffuse and indefinite margin; 3rd, small clear hemolytic circle from 1 to 2 mm. across with sharp margin; 4th, gray-green or hazy hemolytic circle from 1 to 1.5 mm. across, hemolysis often incomplete in circle. * * This grouping demonstrates that there is a more or less gradual transition from 1 type to the other, and indicates, as was observed, that a strain may change to some extent its type of hemolysis.*"	**Davis** 1916. Hemolytic Streptococci Found in Milk.

TABLE III—*Concluded.*

The Use of Blood Agar.

Organisms.	Medium.				Method of inoculation.	Period of incubation.	Results and conclusions.	Reference.
	Blood.		Agar.					
	Species.	Amount.	Composition.	Amount.				
5 strains: (1) "*A strongly hemolytic streptococcus*" from a case of erysipelas; (2) "*a hemolytic strain from a scarlatinal sore throat;*" (3) "*a hemolytic streptococcus from the tonsils in a case of rheumatic fever;*" (4) "*a non-hemolytic streptococcus*" from pneumonic sputum; (5) "*a green-producing streptococcus*" from the same source as No. 3.	Sheep, goat, horse, rabbit, and human.	1 cc. defibrinated, or 2 cc. citrated (blood and citrate solution āā), "*unless otherwise mentioned.*" 3, 6, 9, or 12 drops used for special experiments.	"* *all the agar used was of one lot; a portion was made 1 per cent alkaline with sodium hydrate, another portion 1 per cent acid with hydrochloric acid, and a third portion of standard agar was made up with 1 per cent dextrose.*"	6 cc.	Surface.	24, 48, and 96 hrs.	" * * *no hemolysis was produced by any of the organisms in alkaline blood agar.*" "*Hemolysis was distinctly inhibited on dextrose blood-agar,*" and greenish discoloration was enhanced for some strains. In acid agar the results were quite different for different strains; *e. g.*, strain No. 1 produced "*an indistinct zone on the acid medium,*" while "*with Organism No. 3 the results were the reverse.*" With different kinds of blood the results were very different for different strains, some hemolyzing one blood and some another. The hemolytic streptococci produced the largest and clearest zones on blood agar containing the least amounts of blood. Crowding of colonies on the plate resulted differently for different strains, causing more or less hemolysis. As a standard method it is recommended that streptococci be streaked on defibrinated human blood agar (1:6) and observed after incubation for 24 hrs.	**Becker** 1916. The Necessity of a Standard Blood-agar Plate for the Determination of Hemolysis by Streptococci.
Many strains of hemolytic streptococci from cases of tonsillitis, otitis media, and scarlet fever; also from normal throats and suspected milk.	Horse (guinea pig, rabbit, and dog).	1 cc.	Veal infusion Witte peptone agar. Reaction "*+ 0.8 –1.0.*"	10 cc.	Deep.	Up to 48 hrs.	The paper is largely epidemiological. Several epidemics of sore throat are studied, the infection being traced to milk in some cases and to contact in others. "*The beta hemolytic streptococcus of Smith and Brown on horse-blood-agar plates forms a small lanceolate colony in the depths of the media. Around the colony is a clear-cut zone of hemolysis, from 2 to 4 mm. in diameter, at the end of from 24 to 48 hours. Under the low power of the microscope complete disappearance of the outline of the red cells is noted. The border of the hemolytic zone is rather abruptly limited by the dense mass of red cells.*" "*It is important to remember that the blood of different animals produces different types of hemolysis. Guinea-pig blood is particularly unsatisfactory. Dog blood produces a brownish discoloration. Washed corpuscles give a different type of hemolysis from that of whole blood, with narrower and less well defined borders.*" (See Table VIII on General Conclusions, etc.)	**Smillie** 1917. Studies of the Beta Hemolytic Streptococcus (Smith and Brown).
51 strains of "*Streptococcus hemolysans.*" 91 strains of "*Streptococcus viridans.*" (All from pathological sources.)	Human or rabbit, defibrinated.	1.5 cc.	"*Plain agar (0.3+ to 0.0+).*" (Probably meat infusion agar.)	15 cc.	Deep.	24 hrs.	Distinguishes hemolytic, green-producing, and indifferent colonies on blood agar, but believes that the latter two should be grouped together as "*Streptococcus viridans.*" (See Table VIII on General Conclusions, etc.)	**Blake** 1917. The Classification of Streptococci.

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5 strains: (1) "*A strongly hemolytic streptococcus*" from a case of erysipelas; (2) "*a hemolytic strain from a scarlatinal sore throat;*" (3) "*a hemolytic streptococcus from the tonsils in a case of rheumatic fever;*" (4) "*a non-hemolytic streptococcus*" from pneumonic sputum; (5) "*a green-producing streptococcus*" from the same source as No. 3.	Sheep, goat, horse, rabbit, and human.	1 cc. defibrinated, or 2 cc. citrated (blood and citrate solution āā), "*unless otherwise mentioned.*" 3, 6, 9, or 12 drops used for special experiments.	"* *all the agar used was of one lot; a portion was made 1 per cent alkaline with sodium hydrate, another portion 1 per cent acid with hydrochloric acid, and a third portion of standard agar was made up with 1 per cent dextrose.*"	6 cc.	Surface.	24, 48, and 96 hrs.	"* * *no hemolysis was produced by any of the organisms in alkaline blood agar.*" "*Hemolysis was distinctly inhibited on dextrose blood-agar,*" and greenish discoloration was enhanced for some strains. In acid agar the results were quite different for different strains; *e. g.*, strain No. 1 produced "*an indistinct zone on the acid medium,*" while "*with Organism No. 3 the results were the reverse.*" With different kinds of blood the results were very different for different strains, some hemolyzing one blood and some another. The hemolytic streptococci produced the largest and clearest zones on blood agar containing the least amounts of blood. Crowding of colonies on the plate resulted differently for different strains, causing more or less hemolysis. As a standard method it is recommended that streptococci be streaked on defibrinated human blood agar (1:6) and observed after incubation for 24 hrs.	**Becker** 1916. The Necessity of a Standard Blood-agar Plate for the Determination of Hemolysis by Streptococci.
Many strains of hemolytic streptococci from cases of tonsillitis, otitis media, and scarlet fever; also from normal throats and suspected milk.	Horse (guinea pig, rabbit, and dog).	1 cc.	Veal infusion Witte peptone agar. Reaction "+ *0.8–1.0.*"	10 cc.	Deep.	Up to 48 hrs.	The paper is largely epidemiological. Several epidemics of sore throat are studied, the infection being traced to milk in some cases and to contact in others. "*The beta hemolytic streptococcus of Smith and Brown on horse-blood-agar plates forms a small lanceolate colony in the depths of the media. Around the colony is a clear-cut zone of hemolysis, from 2 to 4 mm. in diameter, at the end of from 24 to 48 hours. Under the low power of the microscope complete disappearance of the outline of the red cells is noted. The border of the hemolytic zone is rather abruptly limited by the dense mass of red cells.*" "*It is important to remember that the blood of different animals produces different types of hemolysis. Guinea-pig blood is particularly unsatisfactory. Dog blood produces a brownish discoloration. Washed corpuscles give a different type of hemolysis from that of whole blood, with narrower and less well defined borders.*" (See Table VIII on General Conclusions, etc.)	**Smillie** 1917. Studies of the Beta Hemolytic Streptococcus (Smith and Brown).
51 strains of "*Streptococcus hemolysans.*" 91 strains of "*Streptococcus viridans.*" (All from pathological sources.)	Human or rabbit, defibrinated.	1.5 cc.	"*Plain agar* (*0.3*+ to *0.0*+)." (Probably meat infusion agar.)	15 cc.	Deep.	24 hrs.	Distinguishes hemolytic, green-producing, and indifferent colonies on blood agar, but believes that the latter two should be grouped together as "*Streptococcus viridans.*" (See Table VIII on General Conclusions, etc.)	**Blake** 1917. The Classification of Streptococci.

TABLE III—*Concluded.*

The Use of Blood Agar.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

Organisms.	Blood.		Fluid media.		Period of incubation.	Results and conclusions.	Reference.
	Species.	Amount.	Composition.	Amount.			
30 strains, of which 25 were *S. longus pathogenes*. The other 5 were thought to be *S. mitior* by the author. "*Zur Zeit steht leider kein Streptokokkenstamm zur Verfügung, der als S. mitior angesehen werden kann, um seine Wirkung auf Blutbouillon mit der des S. longus zu vergleichen.*"	Human.	10 drops.		10 cc.	Several days.	All strains of *S. longus pathogenes* produced at first laking and a burgundy red color, but later (after several days) the color changed to brownish red. Occasional shaking of the tubes or incubation of the cultures in shallow layers in Erlenmeyer flasks hastened the appearance of the brownish color. Killed bouillon cultures (60° for 1 hr.) or sterile filtrates caused no apparent change in blood added to them. Spectroscopically the change from burgundy red to brownish red was found to be a change from oxyhemoglobin to methemoglobin. The author concludes that "*Diese Umwandlung des Oxyhämoglobins in neutrales Methämoglobin ist eine Folge der physiologischen Funktionen der Streptokokken, sie ist geknüpft an den Lebensprozess derselben.*" The author points out that the brownish red discoloration of blood bouillon described by Schottmüller as characteristic of *S. mitior* is produced also by *S. longus pathogenes*, and therefore "*Es kan deshalb von der Schottmüller angegebene Unterschied in der Funktion des Streptococcus longus und Streptococcus mitior nicht bestätigt werden.*"	**Rieke** 1904. Beiträge zur Frage der Arteinheit der Streptokokken.
16 strains from various human lesions, normal mouths, and intestines.	Rabbit. (Human, guinea pig, dog, beef, and frog for comparison.) Used only washed corpuscles.	A few drops.	(*a*) "*Weakly alkaline meat infusion peptone bouillon.*" (*b*) Heated (½ hr. at 55°) rabbit, dog, or beef serum plus a couple of drops of whole blood.	10 cc.	(*a*) Up to 3–4 days without shaking. (*b*) 24 hrs.	The author measured the amount of hemolysis by the height to which the layer of released hemoglobin rose in the undisturbed tube of blood bouillon. Strains pathogenic for animals were generally hemolytic. Hemolysis began in 6–8 hrs. after inoculation of the medium, producing a burgundy red layer beginning at the bottom of the tube. After 3–4 days the hemoglobin became brownish. Dog blood was most easily hemolyzed; human and frog blood were least readily hemolyzed. The addition of 2 per cent instead of 0.5 per cent sodium chloride to the bouillon inhibited hemolysis to some extent. The hemolytic activity of bouillon cultures was destroyed by heating for 30 min. at 55°C. Hemolytic filtrates were obtained from cultures in medium (*b*). These filtrates were more weakly hemolytic than the cultures and were inactivated in 15 min. at 55°C.	**Kerner** 1905. Experimenteller Beitrag zur Hämolyse und zur Agglutination der Streptokokken.
24 strains: 22 from vulval, vaginal, and uterine discharges of 10 women during and 4–9 days after labor; 1 from a felon; 1 from a sore throat.	Rabbit, beef, human.	12 drops.		10 cc.		In "alkaline bouillon" (neutral to phenolphthalein) only the most hemolytic strains (type Ia on blood agar) produced hemolysis. In "neutral bouillon" (2 per cent N acid to phenolphthalein) all aerobic strains produced complete hemolysis in 2–5 days, with burgundy red color, later turning to brown. The anaerobic strains produced hemolysis with burgundy red discoloration but no brownish discoloration, though they produced good amounts of acid. Finding the brownish discoloration of blood to be produced by free acid and oxygen the author concludes that, since acid production and hemolysis occur together in cultures in "neutral" bouillon, there is probably a causal connection between them. Being unable to obtain hemolytic filtrates from neutral bouillon cultures, however, he concludes that the acid is much more hemolytic in a nascent state.	**Natvig** 1905. Bakteriologische Verhältnisse in weiblichen Genitalsekreten.
6 streptococci. 6 pneumococci.	Human (possibly others).		Blood bouillon heated at 100°C. Blood bouillon, unheated.			Heated blood bouillon was unaltered by either streptococci or pneumococci. Unheated blood bouillon was laked and turned burgundy red by the streptococci; under the influence of the pneumococci the blood became at first dark red and then green (precipitate). Spectroscopic examination showed the burgundy red color to be due to oxyhemoglobin, brown discoloration due to methemoglobin, and the greenish precipitate was found to be an albuminous precipitate the color of which was due not to pigment but to an optical effect.	**Boxer** 1906. Ueber das Verhalten von Streptokokken und Diplokokken auf Blutnährboden.
14 laboratory strains of streptococci from various human lesions. 1 strain from the blood of a scarlet fever case.	Horse, rabbit.					Either kind of blood in bouillon was completely laked by the strains which were hemolytic on blood agar, leaving a sediment of corpuscle shadows. The strains which were non-hemolytic on blood agar produced no laking of blood in bouillon, the blood corpuscles settling on the bottom of the tube unaltered.	**Silberstrom** 1906 Ueber die Arteinheit der Streptokokken.
	The method of procuring the hemolytic filtrates and of testing them was supposedly the same as described by the author in 1903.					In addition to the results described in 1903 the author gives the following: Streptolysin "*is intimately associated with the globulins of the serum in which it is produced and has not yet been separated from them. It can be separated quantitatively from the serum albumin by saturation with magnesium sulphate at 35°C. or by half-saturation with ammonium sulphate, and it seems not unlikely that it is a globulin.*" "*It cannot be extracted from the serum with alcohol, ether, or chloroform.*" "*Attempts to produce a specific antistreptolysin have not been successful.*"	**Ruediger** 1907. A Study of the Nature of Streptolysin.
65 strains (43 from various human pathological processes; the remainder from milk, skin, sputum, and feces).	Beef or rabbit.					The 43 strains from pathological processes are all recorded as being hemolytic in blood bouillon; all others were non-hemolytic.	**Nieter** 1907. Zur Streptokokkenfrage.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

Organisms.	Blood.		Fluid media.		Period of incubation.	Results and conclusions.	Reference.
	Species.	Amount.	Composition.	Amount.			
	Human.	"*Several drops.*"				*S. pathogenes* was found to lake the blood and change the color to burgundy red, becoming brownish in 8–10 days. Corpuscle shadows and bacterial cells were found in the sediment. *S. mitior* produced no laking but a brownish discoloration of the corpuscles. If these discolored corpuscles were sedimented and placed in fresh bouillon inoculated with *S. pathogenes* or hemolytic staphylococci, they were readily laked, but not by *S. mitior*. *S. saprophyticus* darkened the blood in bouillon but did not lake it. The author concludes that in blood bouillon cultures of *S. mitior* all the corpuscles are in contact with the streptococci and are therefore "immunized" against hemolysis by *S. mitior*, but, since they can still be hemolyzed by *S. pathogenes* or staphylococci, the hemolysin of *S. mitior* must be different from that of *S. pathogenes*.	**Mandelbaum** 1907–08. Zur Streptokokkenfrage.
Many strains from vaginal and uterine secretions, and from various pathological processes.	Used blood bouillon according to the method of Kerner.					No special results were recorded and so they were presumably in agreement with those obtained on blood agar.	**Lüdke and Polano** 1909. Ueber Hämolyse der Streptokokken.
Presumably strains from various sources.					24 hrs.	"*Impft man die Streptokokken in Blutbouillon, so sieht man nach 24 Stunden den unteren Teil der Bouillon durchsichtig weinrot, während in Kontrollröhrchen (oder bei nicht hämolytischen Streptokokken) die Blutkörperchen in der Kuppe des Reagenzröhrchens als kleiner, schwartzroter Bodensatz liegen und die Bouillon darüber völlig ungefärbt bleibt.*" The wine-red color was found to be due to reduced hemoglobin (spectroscopic analysis) formed by the streptococci. Sterile blood bouillon controls remained blood-red. The author states that it is important to note that (1) the bouillon must be isotonic with the blood, and "*Es ist * * * zu achten, dass (2) wird die Säuerung der Bouillon verhindert (z. B. durch Zusatz pulverisierter Kreide, Marmorstaub); sonst findet eine unter Umständen störende Braunfärbung statt (Methämoglobin).*"	**Zangemeister** 1909. Die Hämolyse der Streptokokken.
15 strains of *S. longus hemolyticus*. 4 strains of *S. brevis non-hemolyticus*. (Mostly from cases of puerperal sepsis.)	Human corpuscles, washed.	1 cc. of 5 per cent suspension.	Various amounts of bouillon cultures were diluted to 1 cc. with salt solution.		2 hrs. at 37° and 20–22 hrs. in the refrigerator.	All strains of *S. longus hemolyticus* produced hemolysis in strongly alkaline bouillon, weakly alkaline bouillon, calcium carbonate bouillon, sugar bouillon, and lactic acid bouillon. The author concludes that hemolysis is not a function of the acid produced since hemolysis is not inhibited by growth in media containing calcium carbonate and is not augmented by growth in dextrose bouillon nor by acidification of the bouillon (lactic acid). Since acid formation may arrest the growth of the culture, however, hemolysis is to this extent dependent upon it. No hemolysin was found in filtrates or centrifugates. The author concludes that hemolysis by streptococci is dependent upon growth in presence of the blood and that heat or acid production inhibits hemolysis only by killing the streptococci or preventing their growth. Schlesinger's "*Hämolysoid*" experiment could not be confirmed. (See also Table VIII on General Conclusions, etc.)	**Sachs** 1909. Über Streptokokken-Hämolyse.
40 strains of hemolytic streptococci from various human pathological conditions.	Fresh normal rabbit serum was diluted 1: 10 with standard bouillon, filtered, and heated 30 min. at 60°C. It was heavily inoculated with a fresh streptococcus culture and incubated 8–10 hrs. The culture was filtered through a Reichel or Chamberland filter and the hemolytic activity of the filtrate tested by mixing 0.5 cc. of 5 per cent suspension of rabbit corpuscles with 1.5 cc. of various dilutions of the filtrate.					Streptococci which produce hemolysis on blood agar plates produce in rabbit serum bouillon in 8–10 hrs. an abundant hemotoxin which is filterable. It is destroyed usually in 30 min. at 60°C. or in 6 hrs. at 37°. It is very resistant to strong acid (HCl) and alkali. The results of anaerobic cultivation do not differ from those of aerobic cultivation. Acid formation and hemolysin production are not identical since a large amount of acid may be accompanied by no hemolytic activity. The filtrates of 10 hr. cultures of some streptococci are toxic for rabbits but not for mice or guinea pigs. The toxin is not identical with the hemolysin. The blood corpuscles from animals of different species have different susceptibilities towards the streptolysin. Most susceptible are the corpuscles from those animals which are most susceptible to streptococcus infection (rabbit, mouse, man). Sheep and guinea pigs are least susceptible. Normal rabbit, guinea pig, horse, and human sera contain antilysin. The immunization of rabbits by the injection of streptolysin has resulted in no increase in the antilysin in their sera.	**Braun** 1912. Ueber das Streptolysin.
						The hemolytic activity was much reduced in blood bouillon containing sugar. (See Table VII on Mutations and Variability.) (See also Table III on The Use of Blood Agar.)	**Kuhn** 1912. Einfluss von Zucker auf Hämolyse und Virulenz.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

Organisms.	Blood.		Fluid media.		Period of incubation.	Results and conclusions.	Reference.
	Species.	Amount.	Composition.	Amount.			
26 strains from bovine mastitis. 10 strains from various other sources.	Beef or goat, defibrinated.	8 drops.	Ordinary weakly alkaline bouillon, presumably meat infusion bouillon.	10 cc.	12–24 hrs.	(1) Strongly hemolytic strains rendered the boullion almost clear and burgundy red. (2) Weakly hemolytic strains left a brownish red sediment of corpuscles covered by a clear burgundy red layer with colorless bouillon above. (3) Non-hemolytic strains left a sediment of brownish or reddish corpuscles with no discoloration of the supernatant bouillon.	**Gminder** 1912. Untersuchungen über Mastitis streptokokken und ihre Differenzierung von saprophytischen Streptokokken.
Determined the "*M. H. D.*" of several strains of hemolytic streptococci and selected one for further experiment.	Ox blood corpuscles, washed.	1 cc. of 5 per cent suspension.	To 1 cc. portions the corpuscle suspension are added graduated amounts of culture in distinctly alkaline (to litmus) bouillon plus 15–20 per cent of horse serum (previously heated to 56° for 1 hr.).		2 hrs. at 37°C.	Measured hemolytic activity by determining the "*minimum hemolytic dose*" ("*M. H. D.*"); *i.e.*, the smallest amount of culture or filtrate which produced complete hemolysis of 1 cc. of 5 per cent suspension of washed ox blood corpuscles in 2 hrs. at 37°C. The "*hemolytic index*" is defined as the reciprocal of the "*M. H. D.*;" *i.e.*, the number of cubic centimeters of 5 per cent corpuscle suspension which would be completely hemolyzed by 1 cc. of culture in 2 hrs. at 37°C. The author concludes that virulence and hemolytic power are closely associated amongst pathogenic streptococci. Filtrates were obtained which had about one-third the hemolytic activity of the cultures. The hemolysin entered into combination with the corpuscles in 2 hrs. at 0°C. but did not produce hemolysis at this temperature. It was inactivated in 30 min. at 55°C. Schlesinger's "*Hämolysoid*" experiment could not be verified. The normal sera of man, horse, rabbit, and guinea pig were found to be antihemolytic to a certain extent, but no immune antihemolysin was produced by the injection of rabbits with hemolytic filtrates.	**M'Leod** 1912. On the Hemolysin Produced by Pathogenic Streptococci, and on the Existence of Antihemolysin in the Sera of Normal and Immunized Animals.
Most experiments were made with 2 "*strongly hemolytic strains*," though others were tested also.	The best medium for hemolysin production was found to be standard bouillon (weakly alkaline to litmus) plus 40–50 per cent of horse serum (inactivated 56° for 30 min.). Next best was bouillon plus 33 per cent of inactivated ascitic fluid. Less efficient was bouillon plus 10 per cent of inactivated rabbit serum. Standard bouillon alone produced very little hemolysin. Inconstant results were obtained with 1 per cent dextrose bouillon. The hemolytic activity was tested by adding 8 cc. of 1–2 per cent suspension of washed corpuscles to 2 cc. of culture or filtrate in various dilutions. The mixtures were incubated 2 hrs. at 37° and refrigerated over night. Horse blood corpuscles were used for most experiments, though those of many other animals were also tried.					The author used cultures for most of his experiments, though hemolytic filtrates also were obtained. The hemolytic activity of cultures reached a maximum in 7–18 hrs., more rapidly in aerobic than in anaerobic cultures. The hemolysin could be extracted by ether. A hemolytic filtrate was inactivated in 6 min. at 75°C., 30 min. at 67°, 70 min. at 60°, and in 20–40 hrs. at 37°. The hemolytic ether extract resisted 100°C. for 10 min. The addition of 5 per cent of peptone or 30 per cent inactivated serum or ascitic fluid to the test mixture (hemolytic culture and corpuscles) resulted in a considerable (sometimes 300 per cent) increase in hemolytic activity. The author adopts the explanation of Walbum (working with staphylolysin) that a "*prolysin*" is formed by the bacterial cell and is activated (forming hemolysin) by something in the peptone, serum, or ascitic fluid added to the medium. Antistreptolysin was not detected in normal human, horse, beef, sheep, goat, dog, pig, rabbit, guinea pig, or pigeon serum. The author was unable to produce an immune antistreptolysin by injection of the goat or guinea pig.	**von Hellens** 1913. Untersuchungen über Streptolysin.
	The methods were the same as those employed by the auth in 1912.					(See Table VIII on General Conclusions, etc.)	**M'Leod** 1914. Criteria of Virulence amongst Streptococci, with Some Remarks on Streptococcal Leucocidin.
164 strains of various organisms (cholera, other vibrios, typhoid, anthrax, *proteus*, *pyocyaneus*, etc.) including 27 strains of streptococci and 20 staphylococci.	Sheep corpuscles, presumably washed.	5 cc. of 5 per cent suspension.	The corpuscle suspension was mixed with 1 cc. of a solution containing ½ loop of culture material.		48 hrs. at 37°C.	In addition to "*Hämopepsie*" and "*Hämoglobinopepsie*" observed in blood agar or blood gelatin only, the author describes "true hemolysis;" *i.e.*, the release of unaltered hemoglobin from the corpuscles, the stromata remaining intact, a phenomenon observed in fluid media only. The author states that he employed bacterial suspensions in salt solution rather than in bouillon because different lots of bouillon were apt to vary and because some bouillons were in themselves hemolytic. (See also Table III on The Use of Blood Agar.)	**Baerthlein** 1914. Ueber Blutveränderung durch Bakterien.
18 throat strains. 1 strain from cervical adenitis. 9 strains from milk slime.	Sheep and human corpuscles, washed.	1 cc. of 5 per cent suspension.	Added to the corpuscle suspension 0.5 cc. of 1hr. ascitic broth culture.		2 hrs. at 37° and over night in the refrigerator.	"*This method is considered by the authors as yielding more definite evidence of hemolytic action than that obtained by observing the colonies on blood agar.*" Only 1 strain, that from a cervical gland, produced hemolysis in the washed corpuscle suspension. It belonged to Group C (see Table V on Fermentation Reactions). 3 other strains of Group C produced "*no color*" in the corpuscle suspension; all the other strains produced "*methemoglobin brown.*" No differences are mentioned in the results obtained by the use of the 2 kinds of blood corpuscles used in these suspensions.	**North, White, and Avery** 1914. A Septic Sore Throat Epidemic in Cortland and Homer, N. Y.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

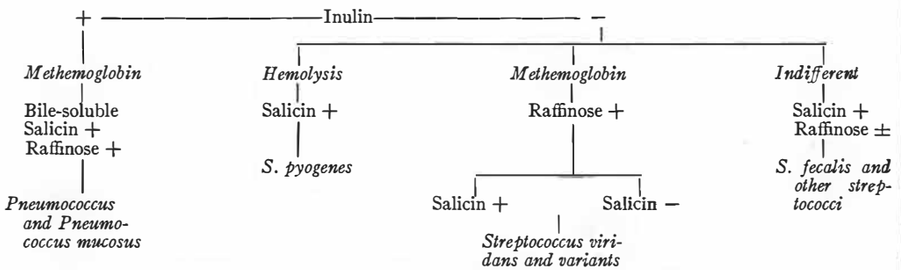
Organisms.	Blood.		Fluid media.		Period of incubation.	Results and conclusions.	Reference.
	Species.	Amount.	Composition.	Amount.			
263 strains from various sources, mostly pathological conditions.	Sheep corpuscles, washed.	1 cc. of 5 per cent suspension in salt solution.	Added to the corpuscle suspension "*usually*" 1.5 cc. of 18 hr. calcium carbonate ascitic broth culture (ascitic fluid 1 part, peptone bouillon, + 3, 5 parts).		1 hr. at 37.5°C.	The author describes three distinct reactions with the blood corpuscles: (1) "*Hemolytic*," a complete laking of the corpuscles; (2) "*Methemoglobin producers*," producing a change in color of the unlaked corpuscles from bright red to dark brown due to the transformation of oxyhemoglobin to methemoglobin; (3) "*Indifferent*," no change in the corpuscles. On the basis of the above reactions and the fermentation of inulin, salicin, and raffinose the following scheme of classification is proposed:  Cultures in 2 per cent peptone bouillon were more hemolytic than those in 1 per cent peptone bouillon. The hemolytic activity was more or less inhibited by 1 per cent fermentable sugar or by over 2 per cent of salt. Centrifugates were less hemolytic than whole cultures. Hemolytic filtrates could not be obtained through a Berkefeld N filter. The hemolytic activity of cultures was destroyed in 30 min. at 56°C. Normal sera of man, guinea pig, rabbit, sheep, and ox were more or less antihemolytic.	**Lyall** 1914. On the Classification of the Streptococci. Observations on Hemolysin Production by the Streptococci.
Evidently a number of strains of pneumococci; no details regarding them.	"*Solutions of the bodies of pneumococci obtained by disso*		*g them in*		From 5 min. to 3 hrs. at 37°C.	"*While the power to produce hemolysis in culture medium is not possessed by pneumococci, or, if so, to a very slight degree, observations which we have made indicate that the bodies of pneumococci contain a substance or substances, which when set free are actively hemolytic, and that the serum of animals immunized to the bacteria or to the bacterial substance has increased power of neutralizing this lytic poison.*" "*The substance on which this hemolytic property depends is very labile, much of its activity is lost on passing through a filter, and it is destroyed by the action of trypsin.*" "*It may therefore be concluded that the bodies of pneumococci contain a toxin that is hemolytic for red blood corpuscles. This substance is not simply a product of autolysis but undoubtedly exists preformed in the bacterial cell. However, it is not given up to the surrounding fluid as long as the bodies of the bacteria are intact. It may therefore be considered a hemolytic endotoxin.*"	**Cole** 1914. Pneumococcus Hemotoxin.

TABLE IV—*Continued.*

The Use of Blood Bouillon and Other Fluid Media.

TABLE IV—*Concluded.*

The Use of Blood Bouillon and Other Fluid Media.

Organisms.	Blood. Kind.	Blood. Amount.	Fluid media. Composition.	Fluid media. Amount.	Period of incubation.	Results and conclusions.	Reference.
Evidently a number of strains of pneumococci; no details regarding them.	To 1 cc. of various dilutions of the bouillon culture, bacterial emulsion, sterile bouillon, filtrate, or other material to be tested was added 1 cc. of salt solution (plain or containing sugars, etc.) and 0.5 cc. of sheep blood corpuscle suspension or hemoglobin solution.				From a few min. to 24 hrs. at 37°C.	"*When pneumococci are grown in media containing blood or hemoglobin, the red color of the latter is changed to a greenish brown,* * *" (on blood agar or in blood bouillon). "*This change is undoubtedly due to the formation of methemoglobin.*" "*This reaction occurs only when the pneumococci are living; it is not induced by the culture fluid or by extracts of the bacteria.*" "*Boiling a broth culture, or even heating it to 56°C. for one half hour, before addition of blood prevents the reaction from occurring.*" "*The reaction does not occur when hemoglobin is added to an emulsion of washed pneumococci in salt solution. However, if minute traces of dextrose be added to such a mixture, the reaction quickly occurs. The dextrose may be replaced by any one of a number of other sugars, and also by certain other organic substances* (peptone, egg albumin), *if the latter are added in large amounts.*" "*The reaction does not occur in the absence of oxygen.*" "*From the work of others it is probable that the formation of methemoglobin is always a reaction of oxidation. In the formation of methemoglobin by reducing agents, the latter are first oxidized, this occurring better in the presence of oxyhemoglobin.*" "*According to this interpretation the formation of methemoglobin by pneumococci occurs as a result of reduction and oxidative processes occurring in the neighborhood of the bacteria* * *, *and without producing substances capable of isolation.*"	**Cole** 1914. The Production of Methemoglobin by Pneumococci.
29 strains of streptococci from various human infections (endocarditis, tonsillitis, alveolar abscess, scarlet fever, rheumatism, pneumonia, pyemia, diphtheria).	Graduated amounts of 12 hr. ascitic bouillon cultures were made up to 1 cc. with salt solution and then mixed with 1 cc. of washed sheep corpuscles. "*The smallest amount of culture which caused complete transformation of oxyhemoglobin to methemoglobin was taken as a measure of the activity of a given strain and may be spoken of as the methemoglobin titre of that strain.*"				Up to 2 hrs. at 37°C.	This paper is practically a repetition of Cole's paper on "The Production of Methemoglobin by Pneumococci," except that strains of *S. viridans* are used instead of pneumococci. The same results as those of Cole are obtained throughout. In addition, however, the following observation is significant: "* *there seems to be no relationship between the carbohydrate cleavage power of a strain of streptococcus and its ability to form methemoglobin in the presence of various carbohydrates. Of six strains which formed methemoglobin in the presence of inulin, three fermented the sugar, three did not; and of five strains causing methemoglobin formation in the presence of mannite, only one fermented that substance.*" "*Since methemoglobin may be formed by streptococci in the presence of sugars which the organisms do not ferment, it seems improbable that the reaction depends upon intermediate or end products formed by the action of the streptococci on the nutrient substance present*" (*e.g.*, carbon dioxide or acids).	**Blake** 1916. The Formation of Methemoglobin by *Streptococcus viridans.*
51 strains of "*Streptococcus hemolysans.*" 91 strains of "*Streptococcus viridans.*" (All from pathological sources.)	"* * *about one hundred strains were tested by adding one cubic centimeter of eighteen hour ascitic-broth cultures to one cubic centimeter of a five per cent suspension of washed sheep corpuscles in small test-tubes and incubating for two hours at 37°C.*"					"*Under these conditions* (with *S. viridans*) *methemoglobin formation has always occurred, and no trace of hemolysis has been demonstrable. On the other hand, hemolytic streptococci under the same conditions have consistently caused complete hemolysis without methemoglobin formation in from ten to thirty minutes.*"	**Blake** **1917.** The Classification of Streptococci.

TABLE IV—*Concluded.*

The Use of Blood Bouillon and Other Fluid Media.

TABLE V.

Fermentation Reactions.

TABLE V.

Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
Strains of streptococci isolated from the milk of cows with chronic mastitis.	Meat infusion bouillon, with and without peptone, salt, or calcium carbonate.	Dextrose, lactose, saccharose, mannite, glycerol. 2–5 per cent.			In calcium carbonate bouillon cultures the sugars added were rapidly consumed. Saccharose, like dextrose, lactose, and mannite, was rapidly fermented but at no time during the fermentation of saccharose could a positive reaction with Fehling's solution be obtained. Noting that cultures in sugar broth died out rather quickly whereas if calcium carbonate was added they remained viable often for several months, the authors conclude that the death of the cultures is brought about by the acids formed.	**Nocard and Mollereau** 1887. Sur une mammite contagieuse des vaches laitières.
28 strains of streptococci. 9 strains of pneumococci.	Serum water medium, (1 pt. beef serum; 2 pts. water; + 1 per cent of test substance), sterilized at 100°C. for 10 min. 3 successive days.	Dextrose, levulose, galactose, maltose, saccharose, lactose, dextrin, starch, glycogen, and inulin.	Fermentation of the substance is indicated by coagulation of the serum.	3–8 days.	Streptococci ferment dextrose, levulose, and galactose readily; saccharose, lactose, and maltose less readily; dextrin, starch, and glycogen after several days or not at all; and inulin not at all. Pneumococci fermented all the substances including inulin.	**Hiss** 1902. A Contribution to the Physiological Differentiation of Pneumococcus and Streptococcus, and to Methods of Staining Capsules.
21 strains; 19 of them from vulval, vaginal, and uterine secretions of 10 women during and 4–9 days after labor; 1 from a felon; 1 from angina. 3 of the strains from the women were anaerobic.	Standard meat infusion bouillon.	Dextrose (sterilized in the autoclave after being added to the bouillon).	Titration (phenolphthalein).		In standard bouillon (without dextrose) the acid production of different strains varied within quite narrow limits. In dextrose bouillon the acid production appeared to be limited by the acid tolerance of the strains, the final titrations varying from 3.3–5.9 per cent N acid. The author states that acid production begins very early and reaches a maximum in 2 days, and that in dextrose bouillon the bacteria die in 1–3 days after the maximum acidity has been attained.	**Natvig** 1905. Bakteriologische Verhältnisse in weiblichen Genitalsekreten.
300 strains from normal sputum. 300 strains from normal human stools. 20 strains from various lesions in man. 2 strains from outside sources.	*"Ordinary beef broth freed from sugar by cultivating B. coli therein for 3 days at 37°C. and then sterilized, filtered, rendered slightly alkaline and tinted with litmus."*	1 per cent of saccharose, lactose, raffinose, inulin, salicin, coniferin, or mannite. Milk.	Litmus.	3 days.	Tabulated the strains with regard to source and to their reaction towards the 9 test substances (including also the reduction of neutral red in bouillon anaerobically). Believes these tests afford a definite basis on which to classify the streptococci, and hopes it may be useful in connection with serum therapy by enabling one to choose a suitable antistreptococcus serum for the case in hand.	**Gordon** 1905. A Ready Method of Differentiating Streptococci and Some Results Already Obtained by its Application.
Tabulation of 701 strains including those of Gordon and Houston (from horse dung, air, human feces, saliva, throats, various lesions, etc.)	Gordon's sugar-free broth or Liebig extract broth (?).	Saccharose, lactose, raffinose, inulin, salicin, coniferin, or mannite. Milk.	Litmus.		Of the 701 strains tabulated about 200 represent the *"Type Forms"* characterized in the following table; all the other strains were *"Variants"* with regard to action on one or more of the test substances. (For table of *"Type Forms"* see Table VIII on General Conclusions, etc.)	**Andrewes and Horder** 1906. A Study of the Streptococci Pathogenic for Man.
124 strains of *S. pyogenes* from various lesions.	Modified Hiss serum water medium, containing 2 per cent peptone, 2 per cent carbohydrate, and 25 per cent beef serum or ascitic fluid. Sterilized fractionally before adding serum. Serum heated 30 min. at 65–70°C. and then filtered.	Dextrin, inulin, lactose, saccharose, maltose, raffinose, rhamnose, salicin, mannite, and dulcite.			*"All strains fermented dextrin, lactose, maltose, saccharose, and salicin, but none fermented inulin, raffinose, rhamnose, and dulcit."* 63 strains fermented mannite; 61 did not. The action on mannite had no relation to the type of infection but all strains isolated from the same individual behaved alike towards mannite.	**Ruediger** 1906 The Streptococci from Scarlatinal and Normal Throats and from Other Sources.
65 strains (43 from various human pathological processes; remainder from milk, sputum, skin, and feces).	Bouillon (no details) + 1 per cent test substance. Used also dextrose- and lactose-Barsiekow media.	Dextrose, levulose, maltose, lactose, saccharose, raffinose, and salicin. Milk.	No mention of acid production or any means of detecting it except in case of litmus dextrose bouillon, litmus lactose bouillon, and the Barsiekow media.		*"Von meinen sämtlichen Stämmen, welche ich in Gärungsröhrchen mit diesen verschiedenen Zuckerarten zusammen beobachte, zeigte nicht ein einziger auch nur eine Spur von Gärung."* All strains produced acid (red) in litmus dextrose bouillon and dextrose Barsiekow medium, but showed negative or late positive reactions in litmus lactose bouillon or lactose Barsiekow medium. Concludes that he has been unable to confirm Gordon's method of differentiation (*i.e., "Gärungsvermögen in Bouillon zu 1 Prozent mit Saccharose, Laktose, etc."*).	**Nieter** 1907. Zur Streptokokkenfrage.

TABLE V—*Continued.*

Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
33 strains of streptococci from various pathological conditions. 1 strain from milk. 80 strains of pneumococci. 8 strains of *S. mucosus.*	(*a*) Sugar-free broth of the following reaction 0.8 per cent acid, 0.5 per cent acid, neutral, 0.5 per cent alkaline. (*b*) Sugar-free broth (0.5 per cent acid) + ⅓ volume of ascitic fluid. (*c*) Beef serum and water. (No further details.) To the above media added 1 per cent of test substances. Sterilized fractionally, 3 days.	Dextrose, levulose, galactose, arabinose, rhamnose, saccharose, lactose, dextrin, inulin, mannite, dulcite.	Litmus.	4 days.	Sugar-free broth (0.5 per cent acid) plus ⅓ volume of ascitic fluid was found to be the most suitable medium for fermentation tests. "*Beef serum water (Hanna, 1898; Hiss, 1904) although a good culture medium, was found unreliable. The results in it were variable* * * *.*" The other media tried also gave less satisfactory results. In broth without ascitic fluid a number of strains failed to attack mannite, lactose, saccharose, or dextrin. Of the 34 strains of streptococci (not including *S. mucosus*) all fermented dextrose, levulose, galactose, maltose, and dextrin, but varied with respect to saccharose, lactose, mannite, and inulin, the largest group (19 strains) fermenting saccharose and lactose but not mannite and inulin. "*The serum (ascitic) media are even more important for the pneumococcus than for streptococci.*" 15 strains of pneumococcus and 8 strains of *S. mucosus* gave identical fermentation reactions, as follows: (+) dextrose, levulose, galactose, maltose, saccharose, lactose, inulin, and dextrin. (−) mannite, dulcite, arabinose, and rhamnose. Of 65 strains of pneumococcus tested with inulin only, "*it was found that a number of them failed to produce acid in certain generations. All the organisms, however, fermented inulin at some time or other of their life history.*" "*Chemical tests of this kind should be made only in the media which are most favorable for the growth of the organisms.*"	**Buerger** 1907. The Differentiation of Streptococci by Means of Fermentative Tests.
					The authors accept the capsule stain described by one of them (Buerger, 1907) as diagnostic for the distinguishing of pneumococci from streptococci. "*The fermentation of inulin is of limited value in the differential diagnosis between pneumococci and streptococci. Pneumococci may lose the power to produce acid in this carbohydrate either temporarily or permanently.*" "*The precipitation of glucose serum agar although more frequently a property of streptococci, may also be produced by the action of pneumococci.*"	**Buerger and Ryttenberg** 1907. Observations upon Certain Properties Acquired by the Pneumococcus in the Human Body.
38 strains of *S. pyogenes.* 10 strains of *S. mucosus.* 13 strains of pneumococcus. 6 strains of *S. saprophyticus.* 16 strains of "*sonstige Streptokokken,*" mostly *S. mitior* evidently.	Litmus ascites agar + 1 per cent of test substance, streaked with culture. A few strains were also tested in bouillon + 1 per cent glycerol, dextrose, starch, mannite, and raffinose.	Dextrose, levulose, galactose, arabinose, mannose, maltose, saccharose, lactose, raffinose, glycerol, mannite, isodulcite, dulcite, adonite, erythrite, dextrin, starch, inulin. (Sterilized in 10 per cent solutions in water and then added to agar or bouillon.)	Litmus in agar. Bouillon cultures titrated (phenolphthalein).	Agar observed 3 days or longer. Bouillon cultures incubated 40 hrs.	Glycerol, starch, mannite, raffinose, and arabinose in agar were found to be of differential value. Members of the *S. pyogenes* group fermented starch almost always, and arabinose rarely. They varied with respect to the other substances. Starch was fermented by none of the other groups. Pneumococci and many strains in the *S. mucosus* group fermented none of the substances in agar. The *S. saprophyticus* group had the same fermentation reactions as the *S. pyogenes* group. There was nothing characteristic about the fermentation reactions of the group of "*Sonnstige Streptokokken,*" evidently a miscellaneous group. In carbohydrate bouillon some of the pneumococci did produce acid from dextrose, starch, raffinose, and in one or two cases from mannite and glycerol. Most of those tested and also the *S. mucosus* were capable of producing alkali when the test substances were not fermented. In no case did *S. pyogenes* produce any alkali.	**Salomon** 1908. Zur Unterscheidung der Streptokokken durch kohlenhydrathaltige Nährboden.
"*Modified pneumococci*" from cases of endocarditis.					"*Most of the strains soon after isolation fermented inulin to a mild degree, a property which all soon lost.*"	**Rosenow** 1909. Immunological and Experimental Studies on Pneumococcus and Staphylococcus Endocarditis.
15 strains of *S. longus haemolyticus.* 4 strains of *S. brevis non-haemolyticus.* (Most strains from cases of puerperal sepsis.)	1 per cent dextrose bouillon; reaction = 1 per cent N acid to litmus.	Dextrose only.	Titrated against 0.01 N acid or alkali using litmus paper as indicator.	Various.	Considers only the question of the relation of acid production to hemolysis. (See Table VIII on General Conclusions, etc.)	**Sachs** 1909. Uber Streptokokken-Hämolyse.
302 strains of streptococci from 49 samples of fresh human, bovine, and equine feces.	Liebig extract, Witte peptone, bouillon + 1 per cent of test substance.	Dextrose, lactose, raffinose, mannite.	Titrated cold (phenolphthalein).	72 hrs.	"*There are three points of difference which seem to deserve investigation. First, the presence of streptococci forming over 3.5 per cent of acid in dextrose broth would seem in general to be characteristic of human stools. Second, raffinose-fermenting forms (S. salivarius) appear to be more abundant in bovine than in human feces. Third, and of most importance, mannit-fermenting streptococci (S. fecalis), which make up about one-quarter of the human streptococci, are very rare in the feces of the horse and cow.*" According to the authors' tables most equine strains attack dextrose only. In material polluted with streptococci, therefore, the number of lactose and raffinose fermenters should help to distinguish between human or bovine strains and equine strains; the number of mannite fermenters should help to distinguish between human strains and equine or bovine strains.	**Winslow and Palmer** 1910. A Comparative Study of Intestinal Streptococci from the Horse, the Cow, and Man.

TABLE V—*Continued.*

Fermentation Reactions.

TABLE V—*Continued.*
Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
					The author states that he was unable to differentiate *S. equi* from other streptococci by means of sugar bouillon.	**Laabs** 1910. Vergleichende Untersuchungen über den *Streptococcus equi* und andere pathogene Streptokokken.
	Bouillon. Gelatin.	Mannite, lactose, levulose, inulin, salicin, 1 per cent.			Milk was never coagulated by *S. equi*. No gas was formed from any of the test substances in bouillon or in gelatin. Acid production is not mentioned by the author.	**Pricolo** 1910. Recherches expérimentales sur le streptocoque de la gourme.
S. lacticus from 75 per cent of 81 milk samples and from cow dung and skin. *S. pyogenes* found in 2 samples, 1 from cow with mastitis, the other from market milk.					*S. lacticus* produces a large amount of acid and coagulates milk in 24 hrs., even in the presence of calcium carbonate. (See also Table VIII on General Conclusions, etc.)	**Bähr** 1910. Vorkommen und Bedeutung der Streptokokken in der Milch.
8 strains of "*modified pneumococci*" from cases of endocarditis. 3 strains of pneumococci from pneumonia. 1 *S. viridans*. 1 *S. pyogenes*. 1 *M. rheumaticus*.		Saccharose, lactose, raffinose, inulin, salicin, mannite. Milk.			*"Pneumococci from the sputum in pneumonia ferment inulin more strongly than those isolated from the blood, while pneumococci from cases of endocarditis ferment this carbohydrate still less and sometimes not at all."* "The author's tables show that the fermentation reactions of the various strains of endocarditis cocci and pneumococci differed among themselves with regard to most of the test substances.	**Rosenow** 1910. A Study of Pneumococci from Cases of Infectious Endocarditis.
42 strains, mostly from mouth, throat, and joints of cases of rheumatism, a few from the heart blood and local lesions.	Houston's medium (distilled water containing 1 per cent lemco, 1 per cent peptone, 0.1 per cent sodium bicarbonate and litmus).	Lactose, saccharose, mannite, salicin, raffinose, inulin. Milk.	Litmus.		Strains classified according to Andrewes and Horder: 3 *S. pyogenes* and its variants. 23 *S. salivarius* and its variants. 6 *S. fecalis* and its variants. 4 *S. anginosus* and its variants. 6 not corresponding to any of these types or variants. 3 strains tested at intervals during subculture varied with respect to fermentation of inulin. Of 5 passed through rabbits 3 varied with respect to one or more substances. Many strains which showed no fermentation of inulin in 3 or 5 days did ferment it in 8 days and could be trained to ferment it readily. *"From this work we can not suggest any definite method for differentiating between the various strains of streptococci we have used in our experiments, and in our hands 'Gordon's tests' have proved quite unreliable for this purpose."*	**Beattie and Yates** 1911. Sugar Tests and Pathogenicity in the Differentiation of Streptococci.
8 strains of streptococci isolated from human subjects. 1 strain from horse dung.	Gordon's sugar-free broth was used at first, but later and for most experiments used media prepared from lemco.	Salicin, lactose, saccharose, raffinose, mannite, inulin. Milk.	Litmus, probably.		Strains were tested at intervals of a few mos. to 2 yrs. Found fermentation reactions inconstant. (See Table VII on Mutations and Variability.) *"It follows that the method of identifying varieties of streptococci by means of the series of test media which have been employed in these experiments rests on no fixed or specific differences in the organisms themselves. The differences observed cannot in any sense be regarded as permanent, but would seem to be due to merely temporary changes in the metabolism of the organisms concerned."*	**Walker** 1911. On Variation and Adaptation in Bacteria, Illustrated by Observations upon Streptococci, with Special Reference to the Value of Fermentation Tests as Applied to These Organisms.
10 strains of *S. acidi lactici* from human and bovine milk. 6 strains of *S. pyogenes* from various suppurations. 5 strains of pneumococci from sputum.	Meat extract bouillon.	Dextrose, lactose, mannite, inulin (a few with raffinose and arabinose).	Litmus added to cultures; also titrated.		All strains fermented dextrose and lactose, most acid being produced by *S. acidi lactici* and least by *S. pyogenes*. Mannite was fermented by about ½ of the *S. acidi lactici* strains and 1 of the *S. lanceolatus* strains, the others producing only 0.2–0.3 per cent of acid. No acid was produced by *S. pyogenes* in mannite. In inulin *S. acidi lactici* produced up to 0.7 per cent acid (except 1 strain which in its 2nd test produced 6 per cent acid). *S. lanceolatus* produced up to 0.6 per cent acid, and *S. pyogenes* not more than 0.1 per cent acid from inulin. The author apparently regards even the smallest amount of acid production as a positive test, however.	**Saito** 1912. Versuche zur Abgrenzung des *Streptococcus acidi lactici* von *Streptococcus pyogenes* und *Streptococcus lanceolatus*.
26 strains of streptococci from bovine mastitis. 10 strains from other sources.	(1) "*Bouillon*" (not stated whether sugar-free or not). (2) Barsiekow media.	Dextrose, lactose, mannite. Milk.	Litmus.		Milk was coagulated by all strains except 5 from stable dust and bovine vaginitis. Dextrose was fermented by all strains. Mannite was fermented by none. Lactose was fermented by all strains except 6 from horse saliva, stable dust, and bovine vaginitis.	**Gminder** 1912. Untersuchungen über Mastitisstreptokokken und ihre Differenzierung von saprophytischen Streptokokken.

TABLE V—*Continued.*

Fermentation Reactions.

Organisms.	Media. Composition.	Media. Test substances.	Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
Many strains from sore throats and complications.		Inulin. Milk.			*"Milk is always acidified and sometimes coagulated. Inulin is not fermented."*	**Davis and Rosenow** 1912. An Epidemic of Sore Throat Due to a Peculiar Streptococcus.
100 strains of streptococci isolated from 100 samples of supposedly normal dairy milk.	*"Sugar-free broth,"* (method of preparation not given) + 1 per cent of test substance. Average reaction of controls = 1.6 per cent N acid to phenolphthalein. Method of sterilization not given.	Saccharose, lactose, salicin, raffinose, mannite, inulin. Milk.	Titrated, (phenolphthalein). Biometric method. Intermodal point = 1.5 per cent N acid above control.	3 days.	Excluding coagulation of milk, reduction of neutral red, and morphology from the differential criteria, the 100 strains fell into 20 fermentative groups, not more than 15 belonging to any one group. The groups are not named. The tests excluded were found unsatisfactory and inconstant. *"The milk streptococci are characterized by unusually high fermentative powers"* and *"are most like the human strains; there is less likeness between the milk and the bovine strains; they show practically no resemblance to the equine strains."*	**Broadhurst** 1912. A Biometrical Study of Milk Streptococci.
202 strains from human and bovine feces (Winslow and Palmer). 101 strains from milk (Broadhurst). 65 strains from normal and diseased throats (Hilliard). 17 strains from various sources (Ballinger).	This paper is a summarization of previous work by the author and others, and involves no new experimental work.				Attaches special importance to the titration of the reaction of cultures as carried out by American observers as opposed to the qualitative results obtained by the English observers by the use of litmus media. Notes an order of availability (*"Metabolic Gradient"*—Howe) among the test substances used, the order being: dextrose—lactose—salicin—saccharose—mannite—inulin—raffinose. *"If any member of the series is fermented, the chances are that those ahead of it will be fermented also. If any member is not attacked, the chances are that those behind it will not be attacked either."* " * * *the size of the molecule appears to be the main factor involved."*	**Winslow** 1912. The Classification of the Streptococci by their Action upon Carbohydrates and Related Organic Media. (A Summary.)
55 strains of streptococci from normal throats, and 15 from diseased throats. 16 strains from milk.	*"1 per cent carbohydrate broth."* No other details.	Dextrose, lactose, maltose, saccharose, raffinose, mannite. Milk.	Titrated.	72 hrs.	*"Streptococci from the human throat and from fresh milk very generally ferment one or more of the sugars, dextrose, lactose, maltose, and saccharose, attacking them most readily in the order named. They do not generally ferment raffinose or mannite."* (See also Table VIII on General Conclusions, etc.)	**Stowell and Hilliard** 1912. A Comparison of the Streptococci from Milk and from the Human Throat.
92 strains from horse, human, rabbit, and guinea pig feces, dust, milk, throats, and pathological conditions.	Hiss litmus serum water and *"peptone solution colored with litmus."*	Saccharose, lactose, raffinose, inulin, salicin, sorbite.	Litmus. Also titrated acid formed in some cases.		Experienced difficulty in keeping strains alive. Found the titration of acid formed in cultures of no particular value or advantage over use of litmus as indicator. Dextrose, levulose, galactose, and dextrin were fermented by all the streptococci and are therefore considered of no differential value. Most of the groups of Andrewes and Horder were found. Found fermentation reactions usually constant, but observed some irregularities. (See Table VII on Mutations and Variability.) Concludes that the fermentation reactions, like other methods that have been suggested, are unsatisfactory as a means of classification of the streptococci.	**Bergey** 1912. Differentiation of Cultures of Streptococcus.
45 strains from throats and secondary lesions of cases of epidemic sore throat. 1 strain of ordinary hemolytic streptococcus from the udder of a cow.					*"On sugars, milk, and other mediums these two varieties of streptococci grow practically alike"* (*i.e.*, the peculiar type of streptococcus here described and considered responsible for the epidemic, later (1913) called *S. epidemicus;* and the ordinary *S. hemolyticus*). *"Milk is always acidified and usually coagulated. These cocci ferment glucose, lactose, saccharose, dextrin, and maltose, but not inulin, mannite or raffinose."*	**Davis** 1912. Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat.
1 strain of *S. epidemicus* from milk. 2 strains of *S. epidemicus* from sore throats. 1 strain from throat of carrier. 3 strains from secondary peritonitis and adenitis.	*"Bouillon."*	Dextrose, maltose, lactose, saccharose, dextrin, raffinose, mannite, inulin.			*"When first isolated this streptococcus fermented dextrose, maltose, lactose, saccharose, and dextrin bouillon, but failed to ferment inulin, raffinose, and mannite bouillon; however, after several months of laboratory existence the organism now ferments raffinose broth. It was not very soluble in bile. It therefore corresponds in the essential cultural characteristics and in its morphology to the streptococcus epidemicus."* The above description applies to the strain from milk, but the others are described as being like it except that the strain from the carrier and one strain from adenitis failed to ferment raffinose.	**Stokes and Hachtel** 1912. Septic Sore Throat. A Milk-Borne Outbreak in Baltimore, Md. Bacteriological Study of the Outbreak.

TABLE V—*Continued.*

Fermentation Reactions.

TABLE V—*Continued.*

Fermentation Reactions.

Organisms.	Media.		Indicator or titration.	Period of incubation.	Results and conclusions.	Reference.
	Composition.	Test substances.				
240 strains of streptococci from milk and the human throat (normal ?).	Liebig extract bouillon + 1 per cent of test substance. Sterilized at 100°C. on 3 successive days.	Dextrose, lactose, saccharose, salicin, raffinose, inulin, mannite.			Mannite is concluded to be of no differential significance since it was fermented by only 2 "*aberrant*" strains. The streptococci are arranged into 9 groups according to their action on the remaining 6 substances. (See also Table VIII on General Conclusions, etc.)	**Stowell, Hilliard, and Schlesinger** 1913. A Statistical Study of the Streptococci from Milk and from the Human Throat.
		Inulin.			"*Sugar fermentation with the streptococcus group has on the whole not been trustworthy or satisfactory on account of inconsistency of results.*" By consideration of a number of characters 5 groups of streptococci are differentiated: *S. hemolyticus*, *S. epidemicus*, *S. mucosus*, *S. pneumoniæ*, and *S. viridans*. The only fermentation considered is that with inulin, *S. hemolyticus*, and *S. epidemicus* being non-fermenters of inulin. (See Table VIII on General Conclusions, etc.)	**Davis** 1913. Interrelations in the Streptococcus Group with Special Reference to Anaphylactic Reactions.
12 strains.	Comparison of results with: (1) Sugar-free meat infusion bouillon (fermented by *B. coli* 7 days at 37°C. and then filtered through Berkefeld). (2) Meat extract bouillon. Both sterilized at 100°C. 30 min. on 2 days after adding 1 per cent sugar.	Saccharose, lactose, salicin, raffinose, mannite, inulin.	Titrated (phenolphthalein).	3 days.	"*The fermentative activities of streptococci vary greatly with the use of meat or of meat extract in making the special media. * * the differences are not only most marked (often reaching 200 to 300 per cent), but they are remarkably uniform in amount and direction.*" "*Qualitative results, estimated with litmus as an indicator, are not comparable with quantitative ones, with phenolphthalein as an indicator, if meat extract is used in making the special media. * * in meat extract media, where the final acidity record often drops down to 0.7–1.0, litmus with its higher neutral point gives no indication of the possible powers of the strain under investigation.*"	**Broadhurst** 1913. Effect of Meat and Meat Extract Media upon the Fermentative Activity of Streptococci.
Fecal streptococci; 123 human, 129 horse, and 97 bovine.	Beef extract bouillon. Reaction = 0.5 per cent N acid to phenolphthalein. Added 1 per cent of test substance.	Dextrose, lactose, saccharose, raffinose, mannite, inulin, salicin.	Titrated cold (phenolphthalein).	3 days.	Results are tabulated and compared with those of Winslow and of Houston; in general agreement. "*Streptococci that ferment mannite are found abundantly only in human feces.*" 65 per cent of streptococci from human feces fermented mannite, as opposed to only 2 or 3 per cent from equine and bovine feces. "*No strains which fermented raffinose were isoloted from human feces and only 12 per cent of those obtained from horse manure attacked this sugar. On the other hand, 73 per cent of the streptococci from cow dung fermented raffinose.*" "*Streptococci fermenting lactose are comparatively rare in horse dung.*" 4 strains are reported which fermented lactose and raffinose or lactose only, but not dextrose.	**Fuller and Armstrong** 1913. The Differentiation of Fecal Streptococci by their Fermentative Reactions in Carbohydrate Media.
26 strains of *S. equi* (23 laboratory strains of various ages, and 3 freshly isolated by the authors). 27 strains of *S. longus* isolated by the authors from human pathological material.	(*a*) Horse serum bouillon, the serum being sterilized by heating 1 hr. at 60° on 3 successive days. (*b*) "*Chapoteaut-Aszites-agar.*" To 9 cc. of either medium there was added 1 cc. of a 10 per cent sugar solution.	Raffinose, saccharose, lactose, maltose, galactose, mannose, levulose, dextrose, mannite, dulcite, sorbite. (10 per cent in litmus solution, sterilized by steaming 2 or 3 mins. on 3 successive days.)	Litmus. Titrations also made with litmus as an indicator.	1–10 days.	10 strains of *S. equi* and 10 of *S. longus* were tested against all the substances. Dextrose, levulose, mannose, galactose, maltose, and saccharose were fermented by all of them, raffinose by none. The remaining 4 substances were fermented by the number of strains indicated in the table. All strains (53) were titrated after growth in mannite, dextrose, and levulose serum bouillon. The maximum acidity was attained in 48 hrs. "*Der Streptococcus longus bildet starke Säure im Mannit und rötet blaue Lakmus-Mannit-nährboden, während der Streptococcus equi die blaue Farbe sehr wenig oder gar nicht verändert; dieser Unterschied lässt sich praktisch zur Differentialdiagnosis beider Arten verwenden.*" "*Der Druse-Streptococcus ist also gegen die von ihm selbst gebildete Skure viel empfindlicher als der Streptococcus longus; der erstere besitzt überhaupt eine geringere Widerstandsfähigkeit gegen schädigende Einflüsse, als der letztere.*"	**Koch and Pokschischewsky** 1913. Ueber die Artverschiedenheit des *Streptococcus longus seu erysipelatos* und des *Streptococcus equi* (Druse-streptococcus).

	Lactose.		Mannite.		Dulcite.		Sorbite.	
	+	−	+	−	+	−	+	−
S. equi.....	4	6	0	10	0	10	4	6
S. longus.....	7	3	10	0	2	8	2	8

TABLE V—*Continued.*

Fermentation Reactions.

TABLE IV.

The Use of Blood Bouillon and Other Fluid Media.

TABLE IV.

The Use of Blood Bouillon and Other Fluid Media.

Organisms.	Blood.		Fluid media.		Period of incubation.	Results and conclusions.	Reference.
	Species.	Amount.	Composition.	Amount.			
	Pure rabbit serum heated 30 min. at 55°C. was inoculated with 2 or 3 drops of heart blood from a rabbit killed by inoculation with streptococci. The culture was incubated for about 10 hrs., diluted with an equal amount of salt solution, and then filtered through a porcelain filter. To various amounts of filtrate 1 drop of defibrinated rabbit blood was added. Results were read after incubation for 2 hrs. at 37°. (Beef, sheep, guinea pig, dog, human, and horse blood were also tried.)					Hemolytic filtrates were obtained. The hemolysin was non-dialyzable and was inactivated by heating at 70°C. for 2 hrs. or 55° for 10 hrs. Hemolysis was retarded by the addition of salt to the filtrate. The filtrates were non-toxic for rabbits or sheep but injection of rabbits failed to produce an immune anti-hemolysin.	**Besredka** 1901. De l'hémolysine streptococcique.
17 strains of streptococci (5 from *"Druseerkrankung von Pferde,"* others from various human infections.	Rabbit, defibrinated.	A small amount.	Horse meat bouillon containing 0.5 per cent salt, 0.5 per cent peptone, and 0.1 per cent dextrose. Reaction alkaline to litmus but acid to phenolphthalein.		24 hrs.	The blood was completely laked by cultures of virulent streptococci in 12–24 hrs. The author was unable to obtain hemolytic filtrates though they are said to have been toxic.	**Aronson** 1901. Untersuchungen über Streptokokken und Antistreptokokken-serum.
40 strains of human origin. 2 equine strains.	Rabbit, defibrinated.	*"A little."*	*"Peptone bouillon."*			All strains produced laking and burgundy red color in blood bouillon. A scarlet fever strain and one equine strain (*"Streptococcus der Druse"*) were less hemolytic than the others. (See also Table VIII on General Conclusions, etc.)	**Marmorek** 1902. L'Unité des streptocoques pathogènes pour l'homme.
A number of pathogenic and non-pathogenic strains from various sources.	A series of tubes of ordinary weakly alkaline bouillon was inoculated with streptococci. A tube was removed each day for a hemolysin test. Hemolytic activity was tested by adding 1 drop of defibrinated rabbit blood to 5 cc. of culture. The mixture was incubated 2 hrs. and refrigerated over night.					Filtrates gave inconstant results, though centrifugates of hemolytic cultures were hemolytic. The hemolytic streptococci were generally those from pathological sources. The hemolytic activity of cultures was destroyed in 15 min. at 60°C. or in 8 days at 37°. In 2 hrs. at 0°C. no hemolysis was produced by cultures, but after such an exposure the washed corpuscles from the mixture underwent hemolysis in 1 hr. at 37°C. The author concludes that at 0° there was a combination of hemolysin with corpuscles. A drop of blood exposed (2 hrs. at 37°) to an old (8 days) hemolytically inactive culture was washed in salt solution and found to be resistant to hemolysis by a young active culture. The author concludes that the corpuscles were saturated wth *"Hämolysoid"* and hence incapable of hemolysis.	**Schlesinger** 1903. Experimentelle Untersuchungen über das Hämolysin der Streptokokken.
The strain employed was isolated from a retroperitoneal abscess in a guinea pig which had been inoculated intraperitoneally with a fungus.	*"Mix 1 part of human serum with 2 parts of rabbit serum, heat the mixture to 56°C. for 30 minutes, inoculate this with a young virulent culture of streptococci, incubate for 20 to 22 hours, dilute with an equal volume of physiologic salt solution, and pass through a porcelain filter."* Tested the action of filtrates on corpuscles of man, rabbit, dog, goat, sheep, hog, guinea pig, chicken, ox, and horse.					*"One drop of a thick suspension of these corpuscles, washed, is completely hemolysed by 1 cc. of streptocolysin in 1½ hours"* at 37°. Washed corpuscles were more easily hemolyzed than unwashed because of the presence of *"antihemolysin"* in the serum. The corpuscles of man and the rabbit were found least resistant while those of the chicken, ox, and horse were most resistant. The hemolysin is said to be an organic substance, destroyed in 2 hrs. at 70°C., deteriorating rapidly at 37° and gradually at room temperature, but keeping for a much longer time in the refrigerator. *"It is destroyed by peptic digestion." "It is non-dialysable."* *"It is composed of a haptophore and a toxophore group, which are firmly bound together. The haptophore group may be neutralized with chicken serum and the toxophore group is destroyed by zinc chlorid."* *"The sera of some animals contain antistreptocolysin."* *"A weak solution of formaldehyde has antihemolytic properties."* *"The filtered cultures of a virulent streptococcus, in heated serum, are toxic for rabbits."*	**Ruediger** 1903. The Production and Nature of Streptocolysin.
	Human.					*"Während meist der S. longus dem Blutfarbstoff allmählich eine karminrote Nuancierung verleiht, * * wird Blutbouillon, in der sich das Blut bald nach dem Zusatz scharf am Boden von der klaren Bouillon abgesetzt, durch den S. mitior in eine gleichmässig braunrot Flüssigkeit verwandelt."* With regard to the pneumococcus, *"Blutbouillon zeigt ebenfalls schon nach 24 Stunden Aufenthalt bei 37° eine deutliche Grünfärbung."*	**Schottmüller** 1903. Die Artunterscheidung der für Menschen pathogenen Streptokokken durch Blutagar.

TABLE VII.

Mutations and Variability.

Results and conclusions.	Reference.
Thought he had changed *S. longus* into *S. brevis* by passages through mice (intraperitoneal injections).	**Waldvogel** 1894. Ueber das Wachsthum des *Streptococcus longus* in Bouillon.
The hemolytic activity of stock cultures remained unaltered for several mos. Successive passages of strains through series of white mice resulted in increased virulence and hemolytic activity.	**Schlesinger** 1903. Experimentelle Untersuchungen über das Hämolysin der Streptokokken.
" * * *it has been found that* * * *the pneumococcus when grown in the serum, both natural and pneumonic, soon loses its hemolytic power.*"	**Rosenow** 1904. Studies in Pneumonia and Pneumococcus Infections.
3 of the 5 strains which the author regarded as *S. mitior* (see Table III on The Use of Blood Agar) were soon lost and the other 2 after 5 mos. cultivation acquired the characters of typical *S. longus pathogenes.*	**Rieke** 1904. Beiträge zur Frage der Arteinheit der Streptokokken.
The hemolytic activity of pathogenic strains of streptococci was increased by animal passage (rabbits). The hemolytic activity was decreased by passage through sugar bouillon.	**Kerner** 1905. Experimenteller Beitrag zur Hämolyse und zur Agglutination der Streptokokken.
Reports that 1 strain of streptococci during several passages through mice showed variations as to virulence and hemolytic activity, being originally hemolytic and virulent hut finally losing both characters.	**Natvig** 1905. Bakteriologische Verhältnisse in weiblichen Genitalsekreten.
Found no change in his test reactions during cultivation for periods up to a fortnight. Passed 11 strains through mice; 9 were unchanged, 1 acquired the ability to ferment salicin, 1 lost the power to produce green fluorescence in neutral red broth.	**Gordon** 1905. A Ready Method of Differentiating Streptococci and Some Results Already Obtained by Its Application.
With regard to the constancy of the fermentation reactions of *S. pyogenes*, "*Five strains were tested 3 times, at intervals of 2 to 3 months, and the results were always the same.*" " * * *the secretion of hemolysin by bacteria is a variable character. It is very pronounced in some strains of streptococcus, rather feeble in others, and may be absent entirely in others. I have two strains of streptococcus which have lost this property entirely by being grown in glucose broth for about two years. They now produce brownish colonies in plain blood agar plates and greenish colonies in plates of glucose agar and blood.*"	**Ruediger** 1906. The Streptococci from Scarlatinal and Normal Throats and from Other Sources.
Cited by Mandelbaum (1907–08) as having claimed to transform *S. pathogenes* into *S. mitior* by prolonged cultivation, and *S. mitior* into *S. pathogenes* by animal passage and cultivation.	**Beitzke and Rosenthal** 1906. Zur Unterscheidung der Streptokokken mittels Blutnährboden.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
According to Salomon (1908), "*Einen Befund von Beitzke und Rosenthal, denen ein Stamm zu verschiedenen Zeiten ungleiche Resultate gab, führt Schultze* (1907) *auf einen Wechsel in der Zusammensetzung der Nährboden zurück.*"	**Beitzke and Rosenthal**—*Continued.* 1906.
"*It is important to note that the fermentative properties described suffered no change from generation to generation*" over a period of from 6–8 mos.	**Buerger** 1907. The Differentiation of Streptococci by Means of Fermentative Tests.
The authors accepted the capsule stain described by Buerger as diagnostic for the differentiation of pneumococci from streptococci. "*Although not a single cultural property is differential between the streptococcus and the pneumococcus, we may speak of a streptococcus and a pneumococcus cultural type, in order to facilitate further discussions. The streptococcus cultural type would include the following: non-fermentation of inulin, precipitation of glucose serum agar, and hemolysis in blood media; the pneumococcus cultural type, fermentation of inulin, non-precipitation, and failure to produce hemolysis.*" "*Pneumococci may acquire certain unusual cultural properties in human blood or in human exudates (in vivo). The power to ferment inulin may become lost, and the ability to cause precipitation in glucose serum agar may be gained. In other words a substitution of the common cultural features of pyogenic streptococci may take place; the organisms then resemble the 'streptococcus cultural type.'*" "*It is possible to convert some but not oll strains possessing the streptococcus cultural type into typical pneumococci by inoculation into white mice.*" "*The tendency of pneumococci of the streptococcus cultural type as well as those which have been converted to the normal variety, seems to be toward a gradual degeneration which manifests itself in the assumption of permanent streptococcic features. Such organisms can then no longer be differentiated from streptococci.*" "*Two months after isolation all the atypical organisms had apparently acquired fixed streptococcus cultural characteristics and repeated animal inoculation failed to bring about a change.*"	**Buerger and Ryttenberg** 1907. Observations upon Certain Properties Acquired by the Pneumococcus in the Human Body.
Found the hemolytic characters of streptococci unchanged by heat or by cultivation on plain agar or blood agar for 3–6 mos. By prolonged cultivation on blood agar strains of *S. mitior* which originally produced visible zones in 3–5 days were trained to produce them in 2–3 days and might be visible even in 24 hrs., but these zones did not equal those of *S. pathogenes* in size or clearness.	**Mandelbaum** 1907–08. Zur Streptokokkenfrage.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
The author had never witnessed the transformation of one of the following fundamental types to another: *S. pathogenes*, *S. mitior*, *S. saprophylicus*.	**Mandelbaum**— *Continued.* 1907–08.
Says that the hemolytic character of streptococci isolated from cases of severe endometritis was soon lost in artificial cultivation. He regarded the pathogenic and hemolytic characters of streptococci as extremely lahile and easily modified by artificial cultivation and therefore emphasized the importance of investigating strains bacteriologically when freshly isolated. The streptococci of moderate hemolytic power found in cases of parametritis were regarded as hemolytic streptococci modified within the body.	**Fromme** 1908. Klinische und bakteriologische Studien zum Puerperalfieber.
The author was unable to change hemolytic to non-hemolytic or green-producing streptococci or *vice versa* by cultural methods. He suspects, however, that some of the saprophytic non-hemolytic streptococci normally present in the vagina may, in their struggle with the living hody, acquire virulence and hemolytic power.	**Heynemann** 1908. Die Bedeutung der hämolytischen Streptokokken für die puerperale Infection.
The hemolytic character of streptococci was reduced by artificial cultivation and increased by animal passage, but in only 1 case did the authors observe an actual change from the *longus* to the *mitior* type or *vice versa*. They give no further details regarding this observation.	**Lüdke and Polano** 1909. Ueber Hämolyse der Streptokokken.
Although some of the strains of *S. mitior* became more hemolytic as a result of animal passages, yet "*Eine Hämolysierungsfähigkeit kann man ihm wohl anzüchten, aber dieselbe geht stets langsam vor sich und erreicht selbst in 48 Stunden nicht diesen Grad der Hämolyse, den der Streptococcus longus in der ersten Kultur innerhalb von 16–18 Stunden erzeugt.*"	**Konrád** 1909. Weitere Beiträge zur Vaginalstreptokokkenfrage.
The author states that the hemolytic characters of streptococci are generally quite constant and "*Fortzüchten auf künstlichen hämoglobinfreien Nährboden veränderte an hämolytischen Stämmen ebensowenig, wie das Züchten nichthämolytischer Streptokokken auf Blutnährboden.*" The author does, however, describe the following variations as having been ohserved by him: (*a*) "** * ein Stamm—der hochvirulent für Mäuse war—während fortgesetzter Tierpassagen seine hämolytische Kraft reduzierte!*" (*b*) "*Ferner habe ich * * den Uebergang eines nichthämolytischen Streptococcus in einen exquisit hämolytischen an zwei Wöchnerinnen einwandfrei beobachtet.*" "*Auf der andern Seite besteht auf künstlichen Nährboden für den einzelnen Stamm selbst graduell*	**Zangemeister** 1909. Die Hämolyse der Streptokokken.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
eine Konstanz, welche so weit geht, dass man einzelne Stämme aus der Art und Grösse ihres hämolytischen Hofes ohne weiteres erkennen und unter anderen hämolytischen Streptokokken gelegentlich herausfinden kann."	**Zangemeister**—*Continued.* 1909.
"Jedenfalls kann ich nach meinen Beobachtungen mit Bestimmtkeit sagen, dass die einmal vorhandene Fähigkeit eines Streptococcus, eine typische Hämolyse hervorzurufen, eine für den betreffenden Streptococcus constante Eigenschaft ist, die sich nicht alteriren lässt durch kleine Abänderungen der Untersuchungsmethoden, sondern die Hämolyse tritt ein, wie und wo der Streptococcus auf Blutagar angeht, sei es im Ausstrich oder in der Aussaat, sei es in der Tiefe oder an den Oberfläsche. Auch das Alter der Streptococcus ändert an der Hämolyse nichts; ich habe Stämme wochenlang fortgezüchtet, sie haben ihr Wachstum in Bouillon geändert, sie wuchsen Anfangs in klarer Bouillon in grossen Flocken, dann üpig und diffus die Bouillon trübend, zuletzt ganz kümmerlich mit leichter Trübung nur noch bei täglicher Ueberimpfung, sie konnten im Brutschrank weitergezüchtet sein oder wochenlang auf Eis stehen, sie konnten von einer tödlichen Peritonitis stammen oder von einer gesunden Wöchnerin: auf Blutagar gebracht—zeigten sie wieder dieselbe Hämolyse, wie wenn sie frisch vom Menschen gewonnen wären."	**Sigwart** 1909. Untersuchungen über die Hämolyse der Streptokokken in der Schwangerschaft und im Wochenbett.
"Hemolysis is a constant property of some strains of streptococci." (Translation.) The author's strains underwent no change in hemolytic or other cultural characters during 6 mos. of artificial cultivation.	**Sachs** 1909. Ueber Streptokokken-Hämolyse.
With regard to so called *"modified pneumococci"* from cases of endocarditis, *"Most of the strains soon after isolation fermented inulin to a mild degree, a property which all soon lost."* *"The stained specimen gave diplococcus forms and chains."* *"By cultivation on artificial media all the strains change gradually into typical lanceolate diplococci, often encapsulated, growing as typical pneumococci in broth and on blood agar slants. On animal inoculation this modification occurred abruptly. It has been impossible to so modify strains of S. viridans which these organisms much resemble."*	**Rosenow** 1909. Immunological and Experimental Studies on Pneumococcus and Staphylococcus Endocarditis.
"In subsequent platings, as in the case of fishings from the original throat cultures, there was a marked variation up and down the scale. Hemolyzing colonies varied in hemolysis, became slightly hemolyzing, doubtful, green, or showed no green nor hemolysis. Slightly hemolyzing colonies became hemolyzing, remained slightly hemolyzing, or were doubtful, green,	**Anthony** 1909. Some Characteristics of the Streptococci Found in Scarlet Fever.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
or showed no green nor hemolysis. Green colonies remained green for the most part, but a few returned to hemolyzing, slightly hemolyzing colonies, or were doubtful." *"Although the power of hemolyzing streptococci to cause hemolysis seems to be a slightly variable quality * * , yet there is a distinct tendency in this power to continue, * * for in the retest the majority of colonies were hemolyzing."*	**Anthony**—*Continued.* 1909.
The author conducted experiments with 3 hemolytic and 2 green-producing non-hemolytic strains of *"S. mitis."* Dilute vaginal secretion and undiluted saliva were sterilized by heating for 3–15 hrs. at 58° or 56°C. respectively. Milk was treated with *"Perhydrol (Merck)"* which was then inactivated by the addition of the *"Katalase, Hepin"* said to have been recently discovered by Much and Römer. There resulted *"eine vollkommen bakterien- und perhydrolfreie Milch mit Rohmilcheigenschaften."* By soaking for 24 hrs. in the vaginal secretion, 1 hemolytic strain was changed into a non-hemolytic green-producer; 1 strain of *S. mitis* became strongly hemolytic. After 24 hrs. in the saliva, 1 hemolytic strain formed green colonies but remained hemolytic; another hemolytic strain came to form *"breite, lehmfarbene Rasen ohne Hämolyse."* After 24 hrs. soaking in the milk, 1 *mitis* strain became strongly hemolytic; 1 hemolytic strain produced large greenish colonies but remained hemolytic; another hemolytic strain produced large white colonies but remained hemolytic. *"Zusammenfassung: 1. Es gelang durch Einbringen von Streptokokkenstämmen in Vaginalsekret, Milch oder Speichel, hämolytische in nichthämolytische umzuwandeln und umgekehrt."*	**Zöppritz** 1909. Ueber Streptokokkenversuche.
With regard to the claims of others that the various groups of streptococci described by him (1903) are not constant in their properties, he states that except for quantitative variations (*e.g.*, in the amount of hemolysis) he has never seen such variations. *"Wohl sind mir ausnahmsweise Stämme von Erysipelstreptokokken, welche unter gewissen Umständen wenig oder gar nicht hämolysierten, vorgekommen, aber Farbstoff bildelen sie nicht. Und umgekehrt trifft man gelegentlich auf einen Streptococcus mitior, der zu Zeiten hämolysiert, aber trotzdem erzeugt er Farbstoff, der erkennbar ist, so lang der hämolytische Hof nicht vorhanden ist."* He admits that there may be intermediary forms but believes that they can nevertheless be differentiated culturally especially by reference to their pathological effects.	**Schottmüller** 1910. Endocarditis lenta. Zugleich ein Beitrag zur Artunterscheidung der pathogenen Streptokokken.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
Hemolytic streptococci carried along on artificial media gradually, though slowly, lost hemolytic power. Many observations led the author to believe that in their struggle with the living tissues streptococci might become hemolytic and again become non-hemolytic when growing as saprophytes. The following appear especially significant: Wounds becoming accidentally infected were found to harbor at first only non-hemolytic streptococci, then weakly hemolytic, and finally strongly hemolytic strains in pure culture. "*Ich bemerke dazu, dass es sich in solchen Fällen nicht etwa um die Verdrängung einen nichthämolytischen handelt*—(since in such cases at some time during the infection both hemolytic and non-hemolytic forms would be found)—, *sondern dass sich im Sekret dann stets eine Reinkultur von Streptokokken findet, die in gleicher Weise zunächst gar nicht, dann schwach und dann ausgesprochen hämolysieren.*" "*Die völlige Beschränkung der hämolytischen Streptokokken auf Infektionsgebiete und deren Umgebung war nun aber nur dann erklärlich, wenn die hämolytischen Streptokokken, ehe sie ins Wundgebiet kommen, in der Regel anhämolytisch sind, und wenn sie andererseits ihre Hämolyse wieder verlieren, sobald sie aus dem Infektionsbereich herauskommen.*" Hemolytic streptococci were smeared between the shoulders of normal women. In some cases the streptococci were all dead in about 3 days; in others only non-hemolytic streptococci were recovered. Referring also to the results of Zöppritz (1909) who altered the hemolytic characters of streptococci by soaking them in vaginal secretion, milk, and saliva, he says: "*Es scheinen also besonders gewisse normale Körpersekrete die Fähigkeit zu haben, den Streptokokken die Hämolyse zu nehmen.*"	**Zangemeister** 1910. Ueber die Verbreitung der Streptokokken im Hinblick auf ihre Infektiosität und ihre hämolytische Eigenschaft.
Quoted by Hoessli (1910) as believing that *S. mitis* could be changed into *S. erysipelatos* and *vice versa* in the living body.	**Much** ?
By various passages of strains through horse blood plasma and serum, (1) 1 of the strains of "*Darmkokken*" became hemolytic and in all other respects like *S. erysipelatos;* (2) there was obtained from *S. mitior* a variant which produced neither hemolysis nor greenish discoloration and also a variant which produced in 48 hrs. a "*beginnende Hämolyse*" around the green colonies and from 1 of these colonies a strain which produced "*starke Hämolyse*" in 16–24 hrs.; (3) the *S. mucosus* strain after prolonged cultivation on horse blood agar plates acquired the ability to produce a small amount of hemolysis; (4) a pneumococcus after 6 transplants on blood agar showed "*ziemlich starke Hämolyse*" apparent after 48 hrs. incubation, but by passage through serum the hemolytic character was again lost.	**Hoessli** 1910. Das Verhalten der Streptokokken gegenüber Plasma und Serum and ihre Umzüchtung.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
The author emphasizes the importance of classifying streptococci when first isolated since, he states, prolonged cultivation on artificial media brings about changes in the characters of pathogenic germs (*e.g.*, the gonococcus, meningococcus, and cholera vibrios).	**Thalmann** 1910. Streptokokkenerkrankungen in der Armee, Einteilung der Streptokokken und ihre Bekämpfung.
"*The endocarditis cocci usually ferment inulin slowly when first isolated; a short time later this power may be increased, only to disappear entirely after prolonged cultivation on blood agar. The pneumococci from pneumonia at first ferment inulin strongly, but also lose it on long cultivation.*" "*Prolonged cultivation on artificial media, a short residence in normal serum, and animal passage, as already pointed out, have a pronounced effect on the morphology and cultural characteristics of these organisms. * * reversion in morphology to the normal type occurs with the return of original pathogenic properties.*"	**Rosenow** 1910. A Study of Pneumococci from Cases of Infectious Endocarditis.
(See Table V on Fermentation Reactions.)	**Beattie and Yates** 1911. Sugar Tests and Pathogenicity in the Differentiation of Streptococci.
"*Der S. conglomeratus A verliert auch bei Vortzüchtung sehr bald das Vermögen, in Bouillon feste Konglomerate zu bilden; in Bouillon wächst er dann in lockeren Flocken ähnlich den Uebergangsformen.*"	**Thalmann** 1911. Weitere Mitteilungen über Streptokokken, inbesondere über pyogene Streptokokken bei Erkrankungen der Atmungsorgane und deren Komplikationen.
"*As regards the streptococci, * * *. If cultures of these organisms are examined at considerable intervals of time their reactions in the test media are by no means constant, but on the contrary are found to undergo remarkable changes. Further, * * continued culture in a particular medium may entirely alter the reactions of these organisms not only to that medium itself, but (it may be) to others also of the carbohydrate media.*" "*The differences observed cannot in any sense be regarded as permanent, but would seem to be due to merely temporary changes in the metabolism of the organisms concerned.*"	**Walker** 1911. On Variation and Adaptation in Bacteria, Illustrated by Observations upon Streptococci, with Special Reference to the Value of Fermentation Tests as Applied to these Organisms.
Employing the microscopic criteria of Mandelbaum the author attempted to repeat the work of Zöppritz in exposing streptococci to the action of saliva, milk, horse serum, and horse blood plasma. He concludes that: "Transmutation of one type of streptococcus into another through cultivation on blood agar, agar, in bouillon, saliva, milk, or horse	**LeBlanc** 1912. Zur Artenfrage der Streptokokken.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
serum does not occur. The transformation of *S. mitior* into *S. pathogenes* can not be admitted on the basis of their similar macroscopic appearance on the blood agar plate. The microscopic study of their colonies is absolutely necessary." (Free translation.)	**LeBlanc**—*Continued.* 1912.
The author tried cultivation of various streptococci for many mos. in horse serum, horse blood plasma, human and animal blood agar plates, human blood plasma, and various standard media of various reactions. He obtained no variations. Having in view the conclusions of Zangemeister (1910) the author made sterile wounds on animals, infected them with pure strains of hemolytic, green-producing, and non-hemolytic streptococci, protected the wounds from accidental infection by glass caps, and always recovered from these wounds only the organism inoculated.	**Rolly** 1912. Experimentelle bakteriologische Untersuchungen von verschiedenen Streptokokkenstämmen.
Retested the fermentation reactions of his strains 2 or 3 times at intervals of several mos. and found no changes except slight quantitative variations in the amount of acid produced. The appearance on blood agar was constant for any given species of blood but was somewhat different for different species.	**Saito** 1912. Versuche zur Abgrenzung des *Streptococcus acidi lactici* von *Streptococcus pyogenes* und *Streptococcus lanceolatus.*
3 strains, originally hemolytic, became like "*viridis*" after prolonged cultivation on blood agar. By cultivation in bouillon the hemolytic character was again restored in full vigor. Variation in the form, size, and consistency of colonies was also noted.	**Jaffé** 1912. Beobachtungen bei blutlösenden und hei gramnegativen Streptokokken.
The author found that by prolonged cultivation of streptococci in sugar bouillon the hemolytic power might be more or less permanently reduced.	**Kuhn** 1912. Einfluss von Zucker auf Hämolyse und Virulenz.
The morphological characters of mastitis streptococci ("*Staketenform*" and paired arrangement within the chain) became greatly modified by artificial cultivation. The characteristics of the growth in bouillon were very variable. The hemolytic characters in blood agar and blood bouillon were found inconstant and therefore not a reliable means of differentiation.	**Gminder** 1912. Untersuchungen über Mastitisstreptokokken und ihre Differenzierung von saprophytischen Streptokokken.
The author points out that he had previously recognized that the peculiar morphological characters of mastitis streptococci were not constant during artificial cultivation and that other, perhaps saprophytic, streptococci might exhibit them under some conditions; *e.g.*, cultivation in raw milk or serum. (See Ernst, 1910, in Table VIII on General Conclusions, etc.)	**Ernst** 1912. Eine Entgegnung zu A. Gminders Arbeit: "Untersuchungen über Mastitisstreptokokken u. s. w."

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
The author has come to the conclusion that *S. conglomeratus* A and B are only degeneration forms of other streptococci (*S. pyogenes* and *S. viridans*), brought about by cultivation or growth under unfavorable conditions—possibly poor oxygen supply. "*Ich fand die Form des Conglomeratus B besonders in der Tiefe der Lakunen exterpierter Mandeln —und zwar hier mitunter in enormen Mengen—und bei chronischer Entzündung des Zahnfleisches. Die kleinen unregelmässigen, höcherigen, in den Nährboden, eingewachsenen Kolonieen zeigen mitunter ausgesprochene Hämolyse, ein Zeichen, wie diese Eigenschaft der pyogenen Streptokokken auch unter ungünstigen Bedingungen festgehalten wird.*"	**Thalmann** 1912. *Streptococcus viridans* im Blut ohne Veränderung der Herzklappen. Zur Einteilung der Streptokokken.
"*Die Keime verlieren ihre Hämolyse nur temporär. Die Stärke der Hämolyse ist abhängig von den Substraten, auf denen die Bakterien zu wachsen gezwungen werden. Aus dem Verhalten des temporären Verschwinden des Hämolyse lässt sich keineswegs der Schluss ziehen, dass durch den Traubenzucker eine Verwandlung der hämolytischen Streptokokken in ahämolytische statthat. Der Verlust der Hämolyse ist als nichts anderes aufzufassen, als die Hemmung einer Eigenschaft eines Streptococcus, der Hämolyse, bedingt durch das Nährsubstrat.*"	**Benthin** 1912. Beiträge zur Hämolysefrage der Streptokokken.
The coagulation of milk, reduction of neutral red, and morphology were excluded from the tests employed because found to give inconstant results.	**Broadhurst** 1912. A Biometrical Study of Milk Streptococci.
The fermentation reactions were usually constant but showed some irregularities. "*On repeating the tests with a particular culture it is not unusual to find irregularity in the results, * *. The characters which an organism manifests when first isolated or when growing in full vigor can usually be brought out repeatedly in subsequent tests, so that the evidence seems to be in favor of the idea that their characters are fixed, and that the irregularities in the results are to be attributed to lack of sufficient growth.*"	**Bergey** 1912. Differentiation of Cultures of Streptococcus.
With regard to the peculiar streptococcus found in the secondary lesions and sometimes in the throats and believed to be responsible for the epidemic, the author says: "*When grown on ordinary mediums the encapsulated coccus—at least some strains—loses its capsule and the growth on slants is less profuse and moist. By animal passage the capsule and the other properties mentioned return.*" With regard to the strain resembling the "*ordinary hemolytic streptococcus,*" isolated from the gargety cow, he says: " * * *after three animal passages the cocci in the peritoneal exudate of the guinea pigs acquired distinct capsules and their growth*	**Davis** 1912. Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
on blood agar slants and plates became more watery and mucoid. These properties were again readily lost on ordinary media. In other words, after animal passage it simulated in every respect the encapsulated streptococci described above in the exudates of the epidemic cases. Before animal passage it simulated the hemolytic streptococci commonly found in the sore throats."	**Davis**—*Continued.* **1912.**
"The conclusion drawn in my earlier papers that the cocci in question are modified and attenuated pneumococci has received additional support because growths from single cocci from strains isolated originally from the blood of cases of endocarditis, as well as from throats, when passed through animals, take on cultural and pathogenic properties indistinguishable from those of typical pneumococci." *"I know that the idea that these strains from endocarditis are modified pneumococci is not generally held, but in view of the facts cited, the conclusion is forced on me that the organisms which are isolated from this type of endocarditis, the organisms designated by Schottmüller as Streptococcus viridans and by Horder as 'saprophytic streptococci,' are in reality pneumococci that have become attenuated and peculiarly modified as the result of environmental conditions."*	**Rosenow** **1912.** Experimental Infectious Endocarditis.
The peculiar streptococci from cases of epidemic sore throat *"sooner or later assume the characteristics of streptococcus pyogenes"* in artificial cultivation. *"By placing Streptococcus pyogenes in unheated sterile milk it becomes modified so as to correspond to the streptococci of epidemic sore throat. The modifications may be accentuated by passage through guinea pigs, and in some cases cultures like those of streptococcus mucosus may result."* Tubes of unheated sterile milk inoculated with ordinary hemolytic streptococci *"placed at 37°C., and in which growth, with acid production, occurred did not appreciably change the streptococci, whereas the streptococci in the milk kept outside the incubator were modified perceptibly."* *" * * subcultures on blood agar plates from the upper and much dried portion of old blood agar tube cultures (72 days) of streptococci from fatal cases for several generations produced dry fine colonies surrounded by a well defined zone of hemolysis, while cultures from the lower and moist part of the same old tubes gave moist large colonies with little hemolysis."*	**Rosenow** **1912.** A Study of Streptococci from Milk and from Epidemic Sore Throat, and the Effect of Milk on Streptococci.
(See Table V on Fermentation Reactions.)	**Stokes and Hachtel** **1912.** Septic Sore Throat. A Milk-Borne Epidemic in Baltimore, Md. Bacteriological Study of the Outbreak.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
The authors believe that the hemolytic characters of the streptococci are more or less variable, being influenced probably by the media on which they are cultivated prior to inoculation onto blood agar plates, and also by growth in and on the animal body.	**Sitzenfrey and Vatnick** 1913. Zur Frage der prognostischen Verwertung bakteriologischer Befunde bei puerperalen Prozessen.
Very few changes in fermentation reactions were observed after cultivation of the strains for several wks.	**Stowell, Hilliard, and Schlesinger** 1913. A Statistical Study of the Streptococci from Milk and from the Human Throat.
Reports the transformation of *S. hemolyticus* into *S. mucosus* by passages through guinea pigs, and the transformation of *S. mucosus* into *S. hemolyticus* by the injection of the former intravenously into a rabbit and recovery of the latter from a joint exudate.	**Davis** 1913. Interrelations in the Streptococcus Group with Special Reference to Anaphylactic Reactions.
The depression of the fermentative activity was not permanent and was evidently due only to the immediate use of the extract bouillon. Growth in extract bouillon effected no change in the fermentative powers of the strains which persisted after they were transferred to more favorable media.	**Broadhurst** 1913. Effect of Meat and Meat Extract Media upon the Fermentative Activity of Streptococci.
"*While most of the organisms have retained their hemolytic power for many weeks, a number of cultures lost this property after several months' cultivation on blood serum.*" "*In no instance did the cultures, which were non-hemolytic, acquire the property of hemolysis while under cultivation.*" The fermentative "*properties persist after long cultivation on ordinary media.*"	**Floyd and Wolbach** 1914. On the Differentiation of Streptococci. (Preliminary Notes.)
By exposing the organisms to various more or less adverse conditions and to repeated animal passages the author reports the following transmutations: (1) change of hemolytic streptococci into *S. viridans* or non-hemolytic streptococci by drying on blood agar slants, growth on blood agar near colonies of *B. subtilis*, growth on ascites dextrose agar in an atmosphere of oxygen, growth in hypotonic media, and passage through rabbits; (2) change of *S. viridans* into pneumococci by passage through guinea pigs; (3) change of pneumococci into *S. mucosus* by passage through guinea pigs; (4) change of *S. mucosus* into *S. viridans* by passage through guinea pigs followed by prolonged cultivation on blood agar; (5) change of pneumococci into *S. viridans* by growth on ascites dextrose agar in oxygen atmosphere and by growth in hypertonic	**Rosenow** 1914. Transmutations within the Streptococcus – Pneumococcus Group.

Results and conclusions.	Reference.
dialyzed beef serum; (6) change of pneumococci into hemolytic streptococci by drying on blood agar slant, sealing infected blood in tube for 5 yrs., growth on ascites dextrose agar in oxygen atmosphere, growth in defibrinated blood in oxygen atmosphere, and growth on blood agar near colonies of *B. coli* (hemolytic); (7) change of *S. viridans* into hemolytic streptococci by growth on ascites dextrose agar in oxygen atmosphere, by growth on blood agar for 1 yr., and by suspension in distilled water for 1 month. By successive steps hemolytic streptococci were changed into *S. mucosus.* The primary criterion of change was the appearance of growth on blood agar plates but these changes were found to be accompanied by appropriate changes in fementation reactions (especially inulin), solubility in bile, agglutination reactions, morphology, pathogenicity, ability to grow in broth culture filtrates (Marmorek's test), etc.	**Rosenow**—*Continued.* 1914.
"*By appropriate means strains of the three varieties* (of streptococci from rheumatism) *have been converted each one into the other.*" A green-producing strain was changed into one producing a narrow zone of hemolysis by "*soaking*" in distilled water for 3 wks.; its morphology and pathogenicity changed correspondingly. "*After prolonged cultivation the strains which produced green at first come to resemble S. viridans in morphology, in cultural, and pathogenic properties. The strains which produced a slight, hazy hemolysis at first now usually produced a wide zone of hemolysis, and when injected produced arthritis but no endocarditis and pericarditis.*" "*By means of animal passage strains of each group have been converted into typical pneumococci.*"	**Rosenow** 1914. The Etiology of Acute Rheumatism, Articular and Muscular.
"*Amongst fifty different strains of streptococci frequently tested* (by the author's special method ?) *as to their hemolytic powers during the period that they were in subculture—a period varying from six months to three and one-half years—I have never met a hemolytic strain which lost the power of producing hemolysis. In one instance a streptococcus which at the outset was non-hemolytic gradually developed the power of hemolysin production, and ultimately was only slightly less hemolytic on an artificial media than some of the strains which were distinctly hemolytic from the outset. This was strain P. M. 8488.*" The author later admits that this result may possibly have been due to mixed culture.	**M'Leod** 1914. Criteria of Virulence amongst Streptococci, with Some Remarks on Streptococcal Leucocidin.
Both strains of streptococci (originally hemolytic) had inconstant hemolytic properties when injected into rabbits. From some rabbits only hemolytic cultures were recovered; from others only non-hemolytic; while from others both hemolytic and non-hemolytic colonies developed at the same or different times.	**Reichstein** 1914. Ueber den Nachweis der Streptokokken im strömenden Blute.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
The fermentation reactions were in general quite constant, the few exceptions being probably due to poor growth at times.	**Hopkins and Lang** 1914. Classification of Pathogenic Streptococci by Fermentation Reactions.
"No change was noted in the cultural or pathogenic properties of the streptococci after growing in the udder of the cow for four weeks."	**Davis and Capps** 1914. Experimental Bovine Mastitis Produced with Hemolytic Streptococci of Human Origin.
(See Table V on Fermentation Reactions.) Though recognizing that the variability in the action of bacteria on various substances may be due to differences in technical procedure the author regards *"the exceptional variability of the group of bacteria"* as also a true cause.	**Thro** 1914. Experiments on the Variability of the Fermentative Reaction of Bacteria, Especially the Streptococci.
"The constancy of carbohydrate reactions is evidenced by the fact that less than five per cent of the strains tested showed any change on subsequent inoculation."	**Lyall** 1914. On the Classification of the Streptococci.
"The growing of hemolytic streptococci with cultures of the Bacillus subtilis for long periods has no effect in altering their hemolytic properties." *"Hemolytic streptococci, growing on blood agar in mixed culture with a green-producing form, show an apparent predominance, * * *."* *"* * * the streptococcus predominates on culture media while the pneumococcus gains the ascendency in the animal body."* *"Isolation from animals of streptococci, differing in important characteristics from those injected, cannot be taken as proof of a change in the characteristics of the injected streptococcus within the animal body, owing to the ease with which streptococci are able to enter the tissues from the intestines or respiratory tract of animals as well as man."*	**Holman** 1914. The Relative Longevity of Different Streptococci and Possible Errors in the Isolation and Differentiation of Streptococci.
In regard to 2 strains of streptococci from the Chicago epidemic (1912), 1 from the blood of a fatal case and the other from the udder of a cow, he says: *"These organisms have maintained their properties and their virulence for animals unimpaired for over two years."* No variations were noted as a result of treating the streptococci with milk or milk products.	**Davis** 1914. The Growth and Viability of Streptococci of Bovine and Human Origin in Milk and Milk Products.
*"Some * * characters were inconstant, notably the sedimentation or clouding of bouillon."* *"The hemolytic activity of the β types has remained fairly constant. In no case has it disappeared. Strain B-15 from a cow forms an apparent exception, but this strain has in the course of our studies split up into a series of forms differing in their laking capacity, some being non-hemolytic at present."*	**Smith and Brown** 1915. A Study of Streptococci Isolated from Certain Presumably Milk-Borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
"When we, however, observe these races over a long period of time we are struck by the persistence of apparently minor cultural and other characters under laboratory conditions. In our hands none of the strains studied have lost or gained any capacity to ferment certain sugars or alcohols." *"In our own repeated tests of the various races of streptococci extending over a period of fourteen months, we have observed some loss of hemolytic activity in a certain group and perhaps some decline of pathogenic power, but no loss of identity when all characters were considered."* *"Spontaneous changes in cultural characters do not proceed rapidly enough if they go on at all, to interfere with current bacteriological methods."*	**Smith and Brown** —*Continued.* 1915.
The virulence of 1 strain was greatly raised by inoculation into the anterior chamber of the eye of a rabbit. Recovered from the eye 3 days later, 5 cc. killed a rabbit in 24 hours (intraperitoneally). 1 cc. of culture from this last rabbit killed a third in 3 wks. *"Although this streptococcus was raised in virulence from nil to the maximum, it showed no changes in its cultural characters."*	**Hartzell and Henrici** 1915. A Study of Streptococci from Pyorrhea Alveolaris and from Apical Abscesses.
Virulence was markedly increased by animal passage. *"Chain formation of the Streptococcus lacticus is favored by animal passage and by cultivation in media containing blood serum, without the addition of carbohydrate."* Both strains originally non-hemolytic acquired to some extent the power to hemolyze human and goat blood after animal passage. *"By animal passage, the amount of acid produced by the original strain progressively decreased, and fermentation of some of the substances was inhibited."* (Notably salicin and mannite.) *"The fermentative ability is not changed materially by repeated transfers through litmus milk."*	**Heinemann** 1915. The Variability of Two Strains of *Streptococcus lacticus.*
With regard to growth on blood agar plates: *"The results were not always constant and in two or three cases it was uncertain in which group the organism belonged."* With regard to fermentation reactions: *"The results of the second series of titrations* (2 weeks after the first and in 2 per cent instead of 1 per cent peptone broth) *were similar to those of the first, except that in a number of instances salicin was fermented in the second series only. This is in all probability due to the more favorable character of the former* (2 per cent peptone) *medium."*	**Kligler** 1915. A Study of the Correlation of the Agglutination and the Fermentation Reactions among the Streptococci.
Reports variations with regard to the fermentation of salicin and raffinose by individual colonies from plates of supposedly pure cultures of streptococci.	**Thro** 1915. Further Experiments on the Variability of the Fermentative Reaction of Bacteria, Especially the Streptococci.

TABLE VII—*Continued.*

Mutations and Variability.

Results and conclusions.	Reference.
Of 134 representative strains retested in carbohydrate media at intervals for 1 yr., 63–77 per cent remained constant. The variation which did occur took place along certain well defined paths from which the author constructs a "*family-tree*" concerning which she says: "*A glance * * will show (1) that these 134 strains included several stable groups of considerable size; (2) that between these groups there are usually well-worn paths; (3) that the variation is more often progressive; and (4) that these combinations fall into three main groups, each probably sharing a different ancestral history, through lactose, through saccharose, or through salicin.*" Variations of temperature and of media were attended hy only temporary variations in the characteristics of the organisms. The exposure of strains to the action of saliva, intestinal extract, and to the products of growth of other strains caused changes in fermentation reactions and minor changes in appearances on blood agar. Exposure to raw or heated milk caused no change. Strains recovered after being fed (within or without celloidin capsules) to puppies showed changes in fermentation reactions and in hemolytic properties.	**Broadhurst** 1915. Environmental Studies of Streptococci with Special Reference to the Fermentative Reactions.
"*The hemolytic properties and fermentative reactions in various sugar media of the streptococci from the tear sac* (of the patient) *were studied from time to time after growth on culture media and after successive passages through animals. The zone of hemolysis on blood agar plates became somewhat smaller after 2 months growth on culture media* (*blood agar*). *After 6 months the 11th subculture showed no further decrease in the zone of hemolysis.*" "*When first isolated, cultures formed acid in media containing dextrose, saccharose, lactose, or salicin, but not in media containing raffinose, mannite, or inulin. In later cultures* (*2 months*), *mannite and raffinose were inconstantly fermented. After passage through a rabbit* (*intraocular injection*), *the streptococcus, being recovered in pure culture, when placed in sugar media again gave the same reactions as when first isolated from the patient.*"	**Irons, Brown, and Nadler** 1916. The Localization of Streptococci in the Eye. A Study of Experimental Iridocyclitis in Rabbits.
The author believes that most reported mutations or variations were due to (1) mixed cultures; (2) unfavorable culture media; (3) insufficient incubation; and (4) the recovery from animals of other strains than the one injected.	**Holman** 1916. The Classification of Streptococci.
"* * * *no noteworthy changes in the morphology or cultural characteristics of the invading organisms were observed in frequent examinations of the milk throughout the course of the infections.*"	**Mathers** 1916. Different Types of Streptococci and Their Relation to Bovine Mastitis.

TABLE VII—*Concluded.*

Mutations and Variability.

Results and conclusions.	Reference.
During 9 mos. "*only slight variations in certain properties, as for example changes in sugar reactions, were occasionally noted. No radical change was observed in the character of hemolysis on human blood agar plates in any of the strains. I have, however, observed slight loss or gain in hemolytic power in some of the strains, in others slight alterations in the character of hemolysis, * * . That virulence of streptococci can be altered by repeated transfer from animal to animal, or by growth on artificial media, is a well known phenomenon. * * Any alterations suggesting the origin of mutants were not observed.*"	**Davis** 1916. Hemolytic Streptococci Found in Milk.
"*My experience has been that under similar conditions, a given organism, if freshly isolated (within from 2 to 3 weeks), will produce a definite and uniform type of hemolysis, and that the diameter of the hemolytic zone will be constant within a moderate latitude.* * These hemolytic characteristics change, however, in some instances after prolonged cultivation on artificial media.*"	**Smillie.** 1917. Studies of the Beta Hemolytic Streptococcus (Smith and Brown).

TABLE VIII

General Conclusions with reference to the Correlation of Source, Pathogenicity, Hemolysis, Fermentation Reactions, etc.

TABLE VIII.

General Conclusions with Reference to the Correlation of Source, Pathogenicity, Hemolysis, Fermentation, etc.

Results and conclusions.	Reference.
The authors regard the prevalent form of mild infectious chronic mastitis in dairy cows as due to the streptococcus described and transmitted from cow to cow by the hands of the milkers. The organisms were streptococci forming long chains and an abundant soft deposit in bouillon without clouding it.	**Nocard and Mollereau** 1887. Sur une mammite contagieuse des vaches laitières.
The authors describe a small diplococcus growing as small chains in fluid media (mixture of equal parts of milk and bouillon). It was Gram-positive. Small transparent granular colonies were formed on blood agar (no other particulars). No growth took place on plain agar or gelatin.	**Poynton and Paine** 1902. Eine kurze Zusammenfassung der Resultate einer Untersuchung betreffend die Pathogenesis des akuten Rheumatismus.
Found that filtrates from bouillon cultures of streptococci of human origin would no longer serve as media for the growth of other strains, while staphylococci and pneumococci grew well. He regards this as a specific reaction indicating that all human streptococci are of the same species. One of the scarlet fever strains grew slightly in a streptococcus filtrate and the "*Streptococcus der Druse*" grew well. The former is regarded as a variant and the latter as a distinct species.	**Marmorek** 1902. L'Unité des streptocoques pathongènes pour l'homme. (Die Artenheit der für Menschen pathogenen Streptokokken.)
The hemolytic activity and virulence of streptococci were found to be generally parallel.	**Schlesinger** 1903. Experimentelle Untersuchungen über das Hämolysin der Streptokokken.
" * * *it has been found that highly virulent pneumococci (such as have been passed through a series of rabbits) possess a greater power of hemolysis than do those of a lower degree of virulence,* * * ."	**Rosenow** 1904. Studies in Pneumonia and Pneumococcus Infections.
In general the strains pathogenic for animals were hemolytic (few exceptions).	**Kerner** 1905. Experimenteller Beitrag zur Hämolyse und zur Agglutination der Streptokokken.

Strains.	Source.	Hemolysis.	Pathogenesis.
4	?	Distinct.	High.
2	Scarlet fever.	Moderate.	Negative.
2	Normal Throats.	None.	"
1	Intestine.	"	"
1	Cholecystitis.	"	"
1	Endocarditis.	"	"

(The pathogenesis indicated is for rabbits.)

From work on agglutination the author concludes that this is not a very reliable or delicate method of determining relationship among streptococci. He could find no quantitative relation between hemolysis and virulence though "it seems that a	**Natvig** 1905. Bakteriologische Verhältnisse in weiblichen Genitalsekreten.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
streptococcus which has the power to produce a considerable amount of hemolysis in alkaline media, is in general virulent for the ordinary laboratory animals." (Translation.) *"Man sieht, dass zwischen den 'longus'-ähnlichen und den 'mitior'-ähnlichen Stämmen keine scharfen Grenzen zu ziehen sind. Nach dem obigen dürfte die betreffende Eintheilung Schottmüller's nur wenig Berechtigung haben."* The morphological, cultural, biochemical, and pathogenic properties of the facultative anaerobic streptococci were considered too variable to afford a safe basis for differentiation.	**Natvig**—*Continued.* **1905.**
A general relation between source, fermentative characters, hemolytic powers, and virulence (with numerous exceptions), is indicated in the authors' tables. The following are the type forms.	**Andrewes and Horder** **1906.** A Study of the Streptococci Pathogenic for Man.

Species.	Milk clotted.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth in gelatin at 20°.	Morphology.	Hemolysis. Pathogenicity for mice.	Source.
S. equinus...	.	.	+	.	.	.	±	+	.	.	*Medius.*	.	Horse dung, air, dust.
S. mitis.....	.	+	+	+	.	.	+	.	.	+	*Brevis.*	.	Human saliva and feces.
S. pyogenes..	.	.	+	+	.	.	+	.	.	+	*Longus.*	++	Pathological conditions.
S. salivarius.	+	±	+	+	±	.	.	.	.	±	*Brevis.*	.	Normal mouth and intestine.
S. anginosus.	+	±	+	+	+	.	.	.	.	±	*Longus.*	++	Pathological conditions; saprophytic.
S. fecalis....	+	+	+	+	.	.	+	+	+	±	*Brevis.*	. .	Pathological conditions; saprophytic.
Pneumococcus.....	±	.	+	+	+	±	.	.	.	.	*Brevis.*	+	Pathological conditions; saprophytic.

"Scarlatina.—All the blood agar plates inoculated with material from scarlatinal throats contained many S. pyogenes colonies and varying numbers of green colonies." *"Normal throats.—All blood agar plates inoculated with material from normal throats contained many green and slightly hemolyzing greenish colonies. S. pyogenes colonies were found in 30 of 51 throats which were studied, but were never present in large numbers and were entirely absent in 21 cases (41.2 per cent)."*	**Ruediger** **1906.** The Streptococci from Scarlatinal and Normal Throats and from Other Sources.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"Measles throats.—The findings in the throats from measles patients corresponded closely to those of normal throats." *"Tonsillitis.—S. pyogenes colonies always predominated in these plates, but green colonies also were present."* *"Pharyngitis."*—In 4 cases no colonies of *S. pyogenes* were found, but many green or brown colonies were found. *"Pneumonia.—Three of the five sets of plates inoculated with material from the tonsils of pneumonia patients contained very few S. pyogenes colonies, and the other two sets did not contain any. * * Typical pneumococci were easily isolated from all plates."* *" * * green colonies were found in all blood agar plates except two sets which were inoculated with material from scarlatinal throats. * * Some of these organisms seem to correspond to Schottmüller's S. viridans, but others are very closely related to the pneumococcus. In addition to the green colonies there are often found slightly hemolyzing colonies which may sometimes have a green tint. These colonies, which are more abundant in plates inoculated with material from normal throats and throats of measles patients than in plates inoculated with material from scarlatinal or tonsillitis throats, must not be confounded with the S. pyogenes colonies."*	**Ruediger**—*Continued.* 1906.
Used human blood agar plates. Found non-hemolytic streptococci in 16 per cent of normal gravida and in a small percentage of normal puerpera, but hemolytic streptococci in those with puerperal fever. No hemolytic streptococci were found in normal gravida or puerpera.	**Gonnet** 1907. Streptocoque pyogène et infection puerpérale.
Could obtain no trustworthy results by agglutination because of spontaneous agglutination of controls. The clouding of bouillon and length of chain hore no apparent relation to the source or other characteristics of the strains studied.	**Nieter** 1907. Zur Streptokokkenfrage.
Did not find the size of the hemolyzed zone to be an index of virulence.	**Mandelbaum** 1907–08. Zur Streptokokkenfrage.
(See Table VII on Mutations and Variability.)	**Fromme** 1908. Klinische und bakteriologische Studien zum Puerperalfieber.
While prepared to admit that decidedly hemolytic streptococci without a high degree of virulence may be found, the author maintains that the finding of hemolytic streptococci in puerperal cases is always a grave indication.	**Heynemann** 1908. Die Bedeutung der hämolytischen Streptokokken für die puerperale Infection.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
The authors could attach no prognostic value to the hemolytic character and could note no relation between hemolysis and virulence as judged either by the condition of the patient or by inoculation of the strains into mice.	**Lüdke and Polano** 1909. Ueber Hämolyse der Streptokokken.
"Es geht daraus mit voller Klarheit hervor, dass sich zwar unter den hämolytischen Streptokokken lier- und voraussichtlich menschenvirulente häufiger finden als unter den nichthämolytischen, dass diese Eigenschaften aber nicht völlig parallel gehen, dass sie nicht voneinander abhängen." *"Ich halte das hämolytische Vermögen bis zum gewissen Grade für einen Energiebeweis, da die nichthämolytischen Streptokokken sich im allgemeinen schwerer fortzüchten lassen, langsamer wachsen, weniger Säure bilden etc."*	**Zangemeister** 1909. Die Hämolyse der Streptokokken.
*" * * Of the 30 scarlet fever throats, 8 cases showed no hemolyzing colonies at all; 4 showed no green colonies, and 18 showed both green and hemolyzing colonies. Of the 24 measles throats, all showed green colonies and 4 showed no hemolyzing colonies."* *"In contrast to the throat cultures, these strains from blood and pus (27 strains), when tested in pure culture in blood plates each gave good hemolyzing colonies with one exception."*	**Anthony** 1909. Some Characteristics of the Streptococci Found in Scarlet Fever.
Concludes that: Hemolysis and virulence do not run parallel since after long cultivation strains may still be hemolytic but may have lost their virulence. On the other hand, hemolysis is a property of strains which under proper conditions may be very pathogenic. Hemolysis and acid production are both dependent upon the ability of the streptococci to multiply. Hemolysis, however, is not a function of the acid produced in cultures. Dextrose bouillon diminishes the hemolytic activity only by checking the growth of the culture because of a development of acid.	**Sachs** 1909. Ueber Streptokokken-Hämolyse.
The author concludes that although hemolysis on blood agar is no index of virulence, yet this does not contradict Schottmüller's conclusion that the streptococci of severe infections are hemolytic.	**Sigwart** 1909. Untersuchungen über die Hämolyse der Streptokokken in der Schwangerschaft und im Wochenbett.
Found streptococci from cases of severe puerperal sepsis always hemolytic, and considers the finding of hemolytic streptococci in such cases always a grave prognostic sign.	**Fromme** 1909. Die Streptokokken in dem Genitalsekret von Schwangeren und Wochnerinnen.
Graduated amounts of 2 per cent lecithin emulsion and 24 hr. bouillon cultures of hemolytic streptococci were mixed and incubated. The growth of strains isolated from the more severe pathological	**Fromme** 1909. Neue Untersuchungen über die Differenzierung der hämolytischen Streptokokken.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
conditions was inhibited less by the lecithin than that of those from mild infections.	**Fromme**—*Continued.* 1909.
Claims that in stained films of milk sediment one can recognize certain streptococci as "parasitic" hy their paired arrangement within the chains and the broad flattened form ("*Staketten-form*") of the elements. "*In bestimmten morphologischen Merkmalen, z. B. Querstellung der Teilglieder, kapselahnlicher Umhüllung und anderem, haben wir ein Mittel, aus dem Euter stammende Streptokokken von nachträglich in die Milch gelangten zu unterscheiden.*" These observations refer to the appearance of the streptococci in the milk only, not in cultures.	**Ernst** 1909. Ueber Milchstreptokokken und Streptokokkenmastitis.
Claims that *S. lacticus* can be distinguished from *S. pyogenes* by the larger amount of acid formed, growth at lower temperature, lanceolate morphology, formation of involution forms, and the appearance of colonies on agar.	**Bahr** 1910. Vorkommen und Bedeutung der Streptokokken in der Milch.
Regards the organisms isolated by him from cases of endocarditis as modified pneumococci, which, however, when isolated come to resemble *S. viridans.* "*(1) The chains are made up of distinct elongated diplococci, often lanceolate in shape,* * * . *(2) They always produce a variable amount of green on blood agar, but usually much less than the typical pneumococci, and never hemolysis. (3) All the strains ferment inulin.*" (See also Table VII on Mutations and Variability.)	**Rosenow** 1910. A Study of Pneumococci from Cases of Infectious Endocarditis.
Thinks that the amount of hemolytic activity may have some relation to virulence but states that differences between *S. vulgaris* and *S. viridans* can not be explained as being due merely to difference in virulence since "*Eine lange fortgesetzte künstliche Züchtung der Stämme, wodurch wohl sicher auch die Virulenz dem Menschen gegen-über beeinträchtigt wird, verändert die Wachstumseigentümlichkeiten nicht.*"	**Schottmüller** 1910. Endocarditis lenta. Zugleich ein Beitrag zur Artunterscheidung der pathogenen Streptokokken.
"*Le streptocoque de la gourme possède les mêmes caractères morphologiques et culturels des streptocoques pyogènes et de l'erysipèle. Dans les cultures en agar-sang et dans le bouillon-sang, il montre de posséder les proprietés hémolytiques du Streptococcus longus seu erysipelatos.*"	**Pricolo** 1910. Recherches expérimentales sur le streptocoque de la gourme.
The streptococci found on the hands of midwives, in normal secretions, and on furniture, walls, and other surroundings (in the hospital ?) were all non-hemolytic, while almost all of those found in wounds and septic processes, especially puerperal sepsis, were hemolytic.	**Zangemeister** 1910. Ueber die Verbreitung der Streptokokken im Hinblick auf ihre Infektiosität und ihre hämolytische Eigenschaft.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
The author concludes that: "*Die hämolytischen Streptokokken sind stets als infektiös anzusehen; denn sie verdanken ja ihre Hämolyse nur die Fähigkeit, kürzlich infiziert zu haben.*" "*Die anhämolytischen Streptokokken sind keineswegs ausschliesslich harmlose Schmarotzer,*" since some of them at least may become virulent. "*Die Nichthämolyse bietet also keine Garantie für eine Avirulenz.*"	**Zangemeister**—*Continued.* 1910.
Conducted experiments with Marmorek's test and concludes that "*Das Verhalten der Streptokokken im Bouillonfiltrat spricht für ihre Einheit,*" but classifies the various varieties as follows: *S. pyogenes*, *S. longissimus*, *S. conglomeratus* A and B, and *S. brevis*—according to their growth on glycerol agar, blood agar, and in bouillon. Regards all but *S. pyogenes* as common inhabitants of normal throats and regards angina as the common means of dissemination of *S. pyogenes*.	**Thalmann** 1910. Streptokokkenerkrankungen in der Armee. Einteilung der Streptokokken und ihre Bekämpfung.
Cited by E. W. A. Walker (1911) as follows: Investigations carried out by Jensen "*and his coworker Holth on a number of microorganisms, including streptococci, by the application of long series of chemical and biological tests. Jensen states that Holth has examined 150 different strains of streptococci in relation to all the carbohydrates and polyvalent alcohols which he could obtain, and to a number of glucosides in addition. The conclusion arrived at is that culture in bouillon containing these substances, followed by titration of the reaction affords a valuable means of identification, differentiation and classification of these organisms. These observers regard lactose, trehalose, cellobiose, and gentobiose as of great value for the purpose of classification, and find that further differentiation is possible by the use of various polyvalent alcohols and glucosides.*" "*Using these methods Holth and Jensen find, even in 'closely similar conditions of disease,' that the strains of streptococci present in different cases are different, * * . Jensen states that Holth's experiments (not yet published) lead to the conclusion that the differences are actually fixed.*" "* * *Jensen next proceeds to discuss the special streptococcal disease of horses, namely, strangles. After pointing out the fact that the course and pathology of this disease varies remarkably in different cases, he states that Holth, * * , has definitely proved them all to be identical* (*i.e.*, the streptococci). *And the constancy of the reactions thus found is not attributable to the residence of the organisms in the horse, since this animal harbours many other streptococci which present quite different*	**Jensen** 1910. Adkillelse og gruppering of naerstaaende bakterieformer ved forhold til kemiske forbindelser.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
series of reactions in the same media." Holth studied about 40 different strains of strangles streptococci from Danish, German, English, and Swedish horses.	**Jensen**—*Continued.* 1910.
The authors point out that although hemolysis on blood agar is not an index of virulence, yet they have found the rapidity with which hemolysis occurs to be significant, the virulent strains producing clear zones in less than 24 hrs. The number of colonies of hemolytic streptococci found on plates inoculated directly with blood (septicemia) or with lochial secretion was also significant. Applying the "*Blutschwamm*" and "*lecithin*" tests of Fromme the authors found virulence indicated for hemolytic streptococci from both severe cases of streptococcus infection and from normal afebrile puerpera. They conclude that there are "*porteurs sains*" of hemolytic streptococci which may be sources of infection for other individuals.	**Fabre and Bourret** 1910. Quelques notions nouvelles sur les streptococcies des suites de couches.
Referring largely to the work of Fromme (1909) the author says: "*Zusammenfassend möchte ich behaupten, dass dem Lecithin auf pathogene Streptokokken besonders wirksame Eigenschaften nicht zukommen. Die wachstumhemmende Kraft des Lecithins ist zum grössten Teil an den Grad seiner Acidität gebunden und ist daher nicht spezifisch. Eine Differenzierung der hämolytischen Streptokokken in verschiedene Arten ist vermittelst Lecithins nicht möglich.*"	**Sachs** 1910. Ueber die Bedeutung des Lecithins für die Unterscheidung verschiedener Arten unter den hämolytischen Streptokokken.
Finding septicemia produced by organisms from all the fermentative groups, the authors could discover no coordination between source, fermentation reactions, and pathogenicity for rabbits.	**Beattie and Yates** 1911. Sugar Tests and Pathogenicity in the Differentiation of Streptococci.
Using Fromme's technique the author found no relation between virulence (as judged by the sources of the strains) and growth in lecithin bouillon.	**Mächtle** 1911. Zur Differenzierung der hämolytischen Streptokokken mittels Lezithinbouillon.
Did not regard hemolysis as an index of relative virulence of strains. (See Table VII on Mutations and Variability.)	**Thalmann** 1911. Weitere Mitteilungen über Streptokokken, inbesondere über pyogene Streptokokken bei Erkrankungen der Atmungsorgane und deren Komplikationen.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"The conclusion, therefore, is apparently unavoidable that, in spite of the very extensive observations of Gordon, Houston, and Andrewes and Horder on the streptococci, there is still no evidence of the existence in the human subject of more than one microorganism, Streptococcus, though this may vary as greatly in its chemical reactions in different cases as it is known to do in virulence. This view agrees with those consistently advanced by Marmorek and others."	**Walker** 1911. On Variation and Adaptation in Bacteria, Illustrated by Observations upon Streptococci, with Special Reference to the Value of Fermentation Tests as Applied to these Organisms.
Found hemolysis on blood agar to be no index of virulence. Found hemolytic streptococci which were non-virulent for animals, and a strain of *S. anhemolyticus vulgaris* (septicemia) which was quite virulent for mice. Isolated *S. viridans* 13 times and *S. anhemolyticus vulgaris* twice from the blood in cases of sepsis.	**Rolly** 1912. Experimentelle bakteriologische Untersuchungen von verschiedenen Streptokokkenstämmen.
S. acidi lactici was found to resemble *S. lanceolatus* (the pneumococcus) culturally and morphologically and these two species are considered by the author to be nearly related.	**Saito** 1912. Versuche zur Abgrenzung des *Streptococcus acidi lactici* von *Streptococcus pyogenes* und *Streptococcus lanceolatus*.
The author does not find that hemolysis and virulence run perfectly parallel but believes them both associated with a parasitic ("carnivorous metabolism") mode of life. The presence of sugar in the medium results in a suppression of the carnivorous metabolism and the adoption of an herbivorous metabolism—saprophytic existence. Experimentally he finds hemolysis in blood bouillon or blood agar much reduced by the presence of sugar in the medium. (See Table VII on Mutations and Variability.)	**Kuhn** 1912. Einfluss von Zucker auf Hämolyse und Virulenz.
Pathogenesis and hemolytic activity were found to bear no relation to each other. "The differentiation of mastitis streptococci among themselves as well as from saprophytic streptococci, is not possible from the standpoint of cultural methods only." (Translation.) Bouillon was somewhat clouded by only 5 of the mastitis strains. Of the 26 mastitis strains 11 formed chains of less than 50 elements and 9 or possibly 11 formed chains of over 400 elements, leaving only 4 of intermediary length. Does not believe that the morphological peculiarity described by Ernst (1909) can be relied upon for the differentiation of mastitis streptococci from others.	**Gminder** 1912. Untersuchungen über Mastitisstreptokokken und ihre Differenzierung von saprophytischen Streptokokken.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
In reply to Gminder the author states that he had not maintained that the differential characters previously described by him (1909) persisted during cultivation on artificial media.	**Ernst** 1912. Eine Entgegnung zu A. Gminders Arbeit: "Untersuchungen über Mastitisstreptokokken u. s. w."
Concludes that bovine milk or mastitis streptococci are harmless as regards summer diarrhea of children, that there is no difference in the streptococcal flora of the intestines of normal children and of those with diarrhea, and that there is no reason to believe that streptococci are the cause of summer diarrhea in infants.	**Puppel** 1912. Ueber Streptokokken in der Milch und im Säuglingsstuhl.
In reply to Puppel the author points out that he maintained only that the finding of "*Staketenform*" streptococci in milk was diagnostic of mastitis in the cows giving the milk, but did not maintain that all bovine mastitis was caused by such streptococci.	**Ernst** 1912. Eine Berichtigung zu R. Puppels Arbeit: "Ueber Streptokokken in der Milch und in Säuglingsstuhl."
Considers Schottmüller's differentiation of *S. hemolyticus* and *S. mitior* fundamental. States that pyogenic streptococci from the mouth are often less hemolytic than those from suppurations: "*Es finden sich alle Abstufungen,*" however. Found comparative uniformity in the cultural characteristics of the *S. pyogenes* (*hemolyticus*) group but regarded the *S. mitior* group as made up of a number of varieties. The streptococci found in human feces and in pathological processes related to the intestine, *e.g.*, appendicitis and peritonitis, produced green colonies on blood agar, were non-pathogenic for mice, and clouded bouillon. The green-producing streptococci from the mouth, on the other hand, were pathogenic for mice. *S. longissimus* formed large moist green colonies on blood agar, produced very long chains of ovoid elements without clouding the bouillon, and was quite pathogenic for mice. The author proposes for *S. longissimus* a special place in the *S. viridans* (*mitior*) group.	**Thalmann** 1912. *Streptococcus viridans* im Blut ohne Veränderung der Herzklappen. Zur Einteilung der Streptokokken.
"*Virulence and hemolytic power are closely associated amongst pathogenic streptococci,*" provided the hemolytic power is measured by the special method used by the author. (See Table IV on The Use of Blood Bouillon and Other Fluid Media.)	**M'Leod** 1912. On the Hemolysin Produced by Pathogenic Streptococci, and on the Existence of Antihemolysin in the Sera of Normal and Immunized Animals.
Did not employ blood agar or fermentation reactions, but from a study of the various strains in bouillon, on agar, in milk, and injected into mice, the author is unable to differentiate virulent from avirulent streptococci or bovine mastitis strains from those of human origin.	**Salus** 1912. Untersuchungen zur Hygiene der Kuhmilch.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"*Streptococcus lacticus* has no sanitary significance, as it is found in nearly all samples of clean, soured or fresh milk, and very often in the healthy milk ducts. *Streptococcus pyogenes*, on the other hand, seems to occur but rarely in milk and is indicative of the existence of an inflamed condition of the udder of the cow furnishing the milk." (Quoted from abstract in *Science*.)	**Ruediger** 1912. A Study of Thirty-Five Strains of Streptococci Isolated from Samples of Milk.
The author states that the differential diagnosis of streptococcus mastitis must depend upon the finding of "*Mastitisstreptokokken*" in the milk sediment. He agrees with Ernst that this streptococcus can be differentiated microscopically. (See Ernst, 1909.)	**Trommsdorff** 1912. Ueber den gegenwärtigen Stand der Mastitisfrage in ihrer Beziehung zur Milchhygiene.
The author divides streptococci into four main groups: *S. longus*, *S. lacticus*, *S. mucosus*, and pneumococcus. Under *S. longus* (*pyogenes*) he includes streptococci forming chains of six or more elements and describes as belonging to this group: (1) *S. pyogenes* (hemolytic), (2) the obligate anaerobic streptococci, (3) *S. conglomeratus*, (4) *S. longissimus*, (5) *S. mitis seu viridans*. " * * *halte ich den S. longissimus, S. conglomeratus (ebenso wie den viridans) nur für Modifikationen des S. longus, die ihre Eigentümlichkeiten in Reaktion auf die Einwirkung der besonderen Oertlichkeit gebildet haben, aber kein 'Arten' darstellen.*" With regard to *S. viridans* found in the blood in cases of endocarditis he says,—"*Ich nehme hieran, dass sich die Hämolyse hier unter der Einwirkung des erkrankten Organismus verloren hat.*" "*Infektionen des Darmes.*"—"The streptococci found in the normal intestine are the same as those found in the mouth, throat, and sputum—the non-hemolytic *S. longus* and especially *S. lacticus* (Kruse)." "*Infektionen im Puerperium.*"—The author found streptococci resembling *S. longus pathogenes* in every respect in most vaginas examined. He states that hemolytic streptococci are common in normal vaginal secretions and lochial discharge, and are of no pathogenic significance.	**von Lingelsheim** 1912. Streptokokken.
In films from throats and exudates the cocci occurred in pairs and short chains, often paired within the chain, were strongly Gram-positive and had definite capsules which were soon lost during artificial cultivation. "*The organism isolated, is an encapsulated hemolytic streptococcus of high virulence. It appears to occupy a position between the ordinary hemolytic streptococcus and the Streptococcus mucosus.*"	**Davis and Rosenow** 1912. An Epidemic of Sore Throat Due to a Peculiar Streptococcus.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"The streptococci of the sore throat and the normal throat show no cultural differentiation in relation to the carbohydrates used. Virulence tests might have separated the two groups." *"The throat streptococci do not readily ferment at 20°C. any of the sugars used, while the milk organisms attack the same sugars and to the same extent at this temperature as at 37°C. This is, perhaps, the most valuable differential feature between chained cocci from these two sources."*	**Stowell and Hilliard** 1912. A Comparison of the Streptococci from Milk and from the Human Throat.
*"The epidemic of sore throat in Chicago in the winter of 1911–12 was caused by an organism belonging to the streptococcus group. In the exudates and in the body it was usually encapsulated, but not infrequently in the throats a capsule was not present. It was highly pathogenic for animals, readily producing arthritis in rabbits, and occasionally endocarditis. * * From a typical case of mastitis in a cow from a farm in this region a streptococcus was obtained pure which * on animal passage agreed in all essential respects to the human epidemic streptococcus. A coccus identical in morphology, in culture, and in pathogenicity was obtained from a human case of tonsillitis and arthritis on the same farm. The relation of these streptococci to the common hemolytic variety, Streptococcus pyogenes, is certainly very close. They may be identical, the differences noted being caused by environmental factors."*	**Davis** 1912. Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat.
(See Table VII on Mutations and Variability.)	**Rosenow** 1912. Experimental Infectious Endocarditis.
"Streptococci, virulent for animals, but which differ from typical Streptococcus pyogenes in a more abundant growth, in being encapsulated and not forming chains, and in causing but little hemolysis, occur in predominating numbers in epidemic sore throat of milk-borne origin."	**Rosenow** 1912. A Study of Streptococci from Milk and from Epidemic Sore Throat and the Effect of Milk on Streptococci.
There was no apparent relation between the hemolytic power of the organisms and the condition of the patients from which they were isolated. The cocci and diphtheroid organisms were found to constitute the chief flora of the vagina, as they do of other mucous membranes (nose, throat, mouth). The author does not regard hemolysis as of great prognostic or pathogenic significance since hemolytic cocci were found in normal cases. Furthermore the hemolytic power of one and the same strain of streptococcus was found variable.	**Sitzenfrey and Vatnick** 1913. Zur Frage der prognostischen und praktischen Verwertung bakteriologischer Befunde bei puerperalen Prozessen.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
No correlation between hemolytic activity and fermentation reactions was observed. *"We believe that the following features are sufficient to separate milk from the throat streptococci: (1) they yield over 2.5 per cent acid in lactose and saccharose at 37°C.; (2) they seldom ferment a substance higher than saccharose in the metabolic series (Dextrose, Lactose, Saccharose, Salicin, Raffinose, Inulin, Mannite); (3) they readily grow in dextrose, lactose, and saccharose at 20°C. Throat streptococci, on the other hand, (1) seldom yield over 3.5 per cent acid in any substance at any temperature; (2) over 40 per cent yield 1.2 per cent acid in either salicin or raffinose or in both at 37°C.; (3) at 20°C. they almost never attack any of the test substances."*	**Stowell, Hilliard, and Schlesinger** 1913. A Statistical Study of the Streptococci from Milk and from the Human Throat.

Relations of Streptococci.

Organisms.	Blood agar. Hemolysis.	Blood agar. Green colonies.	Capsules.	Solubility in bile.	Inulin fermented.	Experimental arthritis.	Experimental endocarditis.
S. hemolyticus	+++	0	0	0	0	+++	±
S. epidemicus	++	0	+	±	0	+++	+
S. mucosus	±	++	+++	+++	++	++	±
S. pneumoniae	0	+++	+	++	++	±	+?
S. viridans	0	+++	0	0	+	±	+++

Davis 1913.
Interrelations in the Streptococcus Group with Special Reference to Anaphylactic Reactions.

In addition to the differences noted in the tables on Fermentation Reactions and on Pathogenicity and Virulence the authors state that in horse serum bouillon *S. equi* usually produces less clouding than *S. longus*. *"Die zwischen beiden Streptokokkenarten von früheren Untersuchern und von uns festgestellten neuen Unterschiede beweisen, dass der Streptococcus longus seu erysipelatos und der Druse-Streptococcus zwar nahe verwandte, aber verschiedene Arten sind."*	**Koch and Pokschischewsky** 1913. Über die Artverschiedenheit des *Streptococcus longus seu erysipelatos* und des *Streptococcus equi* (Druse-streptococcus).
The author believes that the pneumonia following *"la gourme"* in horses is secondary and due to *S. pyogenes*, a different streptococcus from that causing *"la gourme,"* having no capsule and different fermentation reactions.	**Bemelmans** 1913. La spécificité des streptocoques de la gourme.
"On the whole we feel that our work supports the validity of Gordon's tests." The results *"would indicate in general that the more active the fermentative powers, the less is the virulence. * * the more virulent cultures grew less profusely."*	**Floyd and Wolbach** 1914. On the Differentiation of Streptococci. (Preliminary Notes.)

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"While the properties of hemolysis and formation of long chains in general go with virulence, neither of these properties is characteristically associated with virulence, or grouping according to fermentative properties."	**Floyd and Wolbach—** *Continued.* 1914.
"The degree of hemolysis which the streptococci tested produced in vivo was intimately related to their virulence." Regarding hemolysis in blood agar and in heated serum bouillon cultures, " * * *amongst the strains of streptococci tested there is no intimate relationship between the degree of hemolytic power as observed in these artificial media and virulence for the rabbit. Nor is there much relationship between the amount of hemolysis on one medium and that on another; and, lastly, there is no constancy in the amount of hemolysis produced by any strain of streptococcus when tested repeatedly on the same medium."* If, however, the streptococci are grown for 12–18 hrs. in fresh unheated and undiluted serum *"of the species of animal for which it is desired to determine the virulence of the streptococcus"* and the *"hemolytic index"* then determined, *"it is seen at once that the last method affords a means of differentiating very sharply between the virulent and non-virulent streptococci, which under more artificial conditions of culture appear to be equal in hemolytic power."* *"Amongst hemolytic streptococci the power to produce hemolysin in the animal body, or in cultural conditions closely resembling those met with in the animal body, is closely allied to virulence."*	**M'Leod** 1914. Criteria of Virulence amongst Streptococci, with Some Remarks on Streptococcal Leucocidin.
Notwithstanding the lack of hemolysis, the authors think most of their strains agree quite well with Davis' description of *S. epidemicus.* *"Bacteriological examination showed that cultures from the throats of 4 patients contained streptococci apparently identical with strains of streptococci obtained from the milk slime from the 2 cows suffering with garget* (Group D in Table V on Fermentation Reactions). *Cultures from the throats of 8 other patients contained streptococci of this same type, but differing, by slight variations only, in their carbohydrate fermentations."*	**North, White, and Avery** 1914. A Septic Sore Throat Epidemic in Cortland and Homer, N. Y.
*"Some of the strains, notably the mannite fermenters, produced no lysis. Others obtained from septicemias produced a wide zone in less than 24 hrs. * * The property is present to some extent in most streptococci, and while in general it is more pronounced in the more virulent type, the differences are essentially quantitative * * ."*	**Hopkins and Lang** 1914. Classification of Pathogenic Streptococci by Fermentation Reactions.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
Although "*in general, the strains which coagulated milk were of low pathogenicity, and vice versa; yet there were many exceptions* * * ." "*We found that most of the virulent pyogenic strains formed long chains and that most of those found in mild infections of the mucous membranes formed short chains. There were many exceptions, however. We do not think, therefore, that morphology can be used as a basis for classification,* * * ."	**Hopkins and Lang—** *Continued.* 1914.
"*Serum broth is the most favorable medium for the isolation and growth of streptococci. It is so well suited to the growth of streptococci that in mixed cultures, even vigorous organisms such as the bacillus coli are overgrown in 24 hours.*" "*Cultures containing different forms of streptococci should be planted in serum broth for at least 24 hours before plating on blood agar. If this is not done, many of the more pathogenic forms are liable to be overlooked.*"	**Holman** 1914. A Method for Making Carbohydrate Serum Broth of Constant Composition for Use in the Study of Streptococci.
"*Of the six strains isolated from the throats four grow like the streptococcus isolated during the Chicago epidemic two years ago. The other two* * * *resemble the ordinary hemolytic streptococcus.* * * *The organisms from the cows and from the throats agree in practically every detail.*" The above mentioned organisms are tabulated as being hemolytic, having capsules, being pathogenic for rabbits, and fermenting dextrose, lactose, saccharose, and maltose.	**Capps and Davis** 1914. An Epidemic of Streptococcus Sore Throat in Jacksonville, Ill., Which Was Traced to the Milk of Cows Affected with Streptococcus Mastitis.
"*So far as we know at present, any appreciable number of hemolytic streptococci found in cow's milk obtained carefully in sterile tubes directly from the cow's udder always means a diseased condition.* * * *It is, of course, true that udder disease is often caused by non-hemolytic streptococci, but as already stated, so far as we know, these bear no relation to sore throat. I have examined many of these organisms and they have little or no virulence for animals. On the other hand, the hemolytic streptococci, that I have examined from the udder, are always pathogenic to some degree for animals and usually quite highly virulent.*" (See also Table III on The Use of Blood Agar.)	**Davis** 1914. The Growth and Viability of Streptococci of Bovine and Human Origin in Milk and Milk Products.
Bases a system of classification on fermentation of salicin, raffinose, mannite, and inulin, and the hemolytic character as determined by a special method. "*The hemolytic titre does not afford any absolute titre of virulence.*" "*Hemolysin production is inhibited by the addition of sugar. It does not appear to be associated with the amount of acid produced.*" (See Table IV on The Use of Blood Bouillon.)	**Lyall** 1914. On the Classification of the Streptococci.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"Hellmuth describes in detail a series of experiments undertaken to show whether hemolysis does occur with the vaginal bacteria during menstruation, and he finds that it does not. Even in two cases in which metorrhagia had persisted for weeks no hemolytic bacteria could be demonstrated. In the three cases in which hemolytic bacteria were found he thinks that they did not develop during menstruation from non-hemolytic ones but reached the vagina through invasion or inoculation. He does not feel that he has proved that the faculty of hemolysis could not develop under long continued hemorrhage, as for example in cases of myoma, but he thinks his experiments were sufficient to prove that hemolysis is not merely the result of the blood content of the nutrient medium of the bacteria." (Review from *J. Am. Med. Assn.*)	**Hellmuth** 1914. Does Menstruation Influence Hemolysis by Vaginal Bacteria?
All the strains found to he pathogenic for rabbits were of the β hemolytic type and belonged to the following two fermentative groups, (See also Table V on Fermentation Reactions.) All but 2 of the strains in these 2 groups injected into rabbits were pathogenic. *"The streptococci of cow mastitis or garget are different from the streptococci of human tonsillitis."* Epidemic milk-borne tonsillitis is regarded as being probably due to streptococci of human origin inoculated into the udders of cows during milking, multiplying in the milk ducts and being shed into the milk for some time after.	**Smith and Brown** 1915. A Study of Streptococci Isolated from Certain Presumably Milk-Borne Epidemics of Tonsillitis Occurring in Massachusetts in 1913 and 1914.
(See Table III on The Use of Blood Agar.)	**Rosenow** 1915. The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli.
*"To summarize, we have shown that streptococci of the viridans group are constantly present in peridental suppurations. * * These streptococci are normally of low virulence but are at times able to produce lesions of the heart, aorta, kidneys, and joints, * * . We can not claim from the evidence so far obtained, that these streptococci bear an etiologic relationship to dental abscesses and pyorrhea, but from the point of view of metastatic infections it is important to know that such organisms are constantly*	**Hartzell and Henriel** 1915. A Study of Streptococci from Pyorrhea Alveolaris and from Apical Abscesses.

	Dextrose.	Maltose.	Saccharose.	Lactose.	Raffinose.	Mannite.	Inulin.	Salicin.
1.	+	+	+	+	−	−	−	+
2.	+	+	+	−	−	+	−	+

TABLE VIII—*Continued.*

General Conclusions.

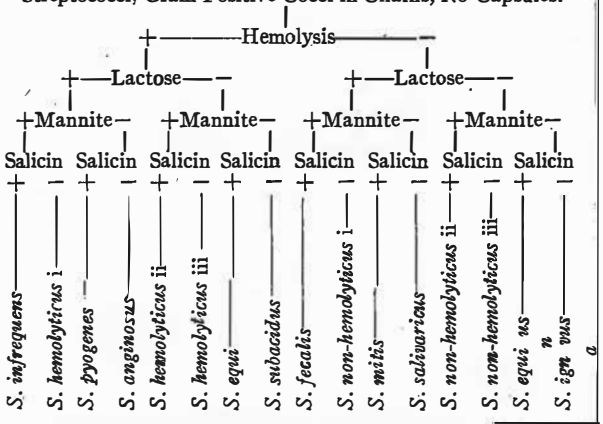
Results and conclusions.	Reference.
present in lesions presenting a large ulcerated surface, through which they may, and probably do, frequently pass into the deeper tissues and the blood stream."	**Hartzell and Henriel**—*Continued.* 1915.
"The agglutination reaction was not found to separate the streptococci into large groups. However, by its correlation with the fermentation reactions, the probable relationship of these types is indicated." *"The agglutination tests tend to show that a division of the streptococci on the basis of hemolysis is not warranted, whereas a separation according to the fermentation reactions appears to coincide more closely with their natural relationship."* The author suggests the following classification: *"S. pyogenes.—Salicin fermenters, which do not ferment raffinose or mannite, are generally hemolytic, and strongly pathogenic."* *"S. salivarius.—Raffinose fermenters, usually ferment salicin, but do not ferment mannite, generally produce a green colony on blood agar, and usually cause subacute and chronic infections.* *"S. fecalis.—Mannite fermenters, generally ferment salicin, rarely ferment raffinose, and are variable in their reaction to blood and in their pathogenicity."* (See also Table III on The Use of Blood Agar.)	**Kligler** 1915. A Study of the Correlation of the Agglutination and the Fermentation Reactions among the Streptococci.
*"Pathologic samples yield the greatest proportion of hemolyzing strains. * * Hemolysis does not seem to be correlated with the results in litmus milk, gelatin, or with any of the Gordon reactions or complexes."* *"Strains from pathologic conditions and from throats often fail to develop in gelatin; strains from other sources usually grow readily at room temperature."* *"Saprophytic strains are usually high fermenters."* *"Morphologic characteristics are not, independently or in connection with other characteristics, definitely differential."*	**Broadhurst** 1915. Environmental Studies of Streptococci.
The authors endorse the conclusions of Smith and Brown (1915) regarding the spread of tonsillitis by the infection of cows with streptococci of human origin and the necessity of studying the strains isolated for *"cultural identity in every detail or immunological identity."* The strains isolated from throat and peritoneal cavity of patients, from persons in the suspected dairy, and from the cow *"were the same in their cultural characteristics."*	**Krumwiede and Valentine** 1915. A Bacteriological Study of an Epidemic of Septic Sore Throat.
"It would appear, then, that these epidemics of appendicitis and parotitis were due to streptococci contained in dairy products." *"The cultures from the dairy products showed a preponderance of non-hemolyzing, short-chain-pro-*	**Rosenow and Dunlap** 1916. An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
ducing streptococci, often in almost pure form and in enormous numbers. Slightly hemolyzing streptococci were found occasionally. All were of a relatively low grade of virulence, but those producing parotitis caused death (in rabbits) *more frequently than did those producing appendicitis."*	**Rosenow and Dunlap**—*Continued.* 1916.
"It would appear, then, that herpes zoster is due to a streptococcus having elective affinity for the ganglia and the posterior roots. The possibility, however, that the disease in some instances may be due to other bacteria having a similar affinity must be admitted." *"Since the streptococci lose the characteristic affinity after cultivation on artificial media, after animal passage, and apparently in the focus of infection after recovery, the conclusion seems warranted that the atrium of infection is not only the place of entrance, but the place where the streptococci, by growth in symbiosis with other bacteria and under varying grades of oxygen pressure, may acquire the peculiar properties necessary to infect in this particular manner."*	**Rosenow and Oftedal** 1916. The Etiology and Experimental Production of Herpes Zoster.
"The classification which has proved of practical value during several years of routine use, and which I wish here to report, is based on the reactions on blood agar and the fermentation or non-fermentation of the carbohydrates, lactose, mannite, salicin, and inulin in serum broth."	**Holman** 1916. The Classification of Streptococci.

Streptococci, Gram-Positive Cocci in Chains, No Capsules.



The above scheme does not include inulin; in the text, however, inulin-fermenting and non-fermenting varieties of nearly all the non-hemolytic organisms are mentioned, but no hemolytic inulin fermenters are mentioned.

TABLE VIII—*Continued.*

General Conclusions.

Results and conclusions.	Reference.
"Hemolytic streptococci having a wide clear zone occur commonly in both pasteurized and unpasteurized (certified) milk. These strains vary among themselves. They are more resistant to heat than human strains of hemolytic streptococci, and possess little or no virulence for rabbits, therefore in all probability none for man. They rapidly acidify and coagulate milk and grow well at 20°C. They form short or long chains, but as seen in milk often appear in pairs or a chain of few elements. While they are definitely hemolytic (Type β, Smith and Brown), the characteristics of the hemolytic zone on plates may vary in certain respects." *"The milk strains are different from certain strains of hemolytic streptococci found at times in diseased udders of cows. These latter resemble the strains of hemolytic streptococci from human sources, and are virulent for rabbits."* *"Between hemolytic area and pathogenicity there exists no constant parallelism."*	**Davis** 1916. Hemolytic Streptococci Found in Milk.
In 2 epidemics of septic sore throat streptococci isolated from the throats of patients were identical in cultural characteristics and agglutination reactions with streptococci isolated from the milk supplies and in 1 case from the udder of a cow in the dairy. *"The types of streptococci isolated"* from the above sources *"are identical in their cultural characteristics and virulence for animals with the type of streptococci described by Smith and Brown in their studies of the milk epidemics of Massachusetts of 1914–1915. This type of streptococcus is a human strain, with a group of definite characteristics**."* *"The streptococcus of this type is seldom found in normal throats (1% of the cases studied.)"* *"The throats of individuals who have had tonsillitis may harbor the beta hemolytic streptococcus for 3 months or more after acute symptoms of the disease have subsided."* *"This streptococcus is found in large numbers in the throats of a certain proportion of cases of sporadic tonsillitis (5 of 20 studied)."* *"The beta streptococcus is frequently found in the throats of acute cases of scarlet fever, and may remain or months in the purulent discharges of the cases complicated by otitis media, etc."* *"These facts explain how the discharges from a case of scarlet fever may contaminate a milk supply and produce a milk-borne epidemic not of scarlet fever, but of septic sore throat."*	**Smillie** 1917. Studies of the Beta Hemolytic Streptococcus (Smith and Brown).

TABLE VIII—*Concluded.*

General Conclusions.

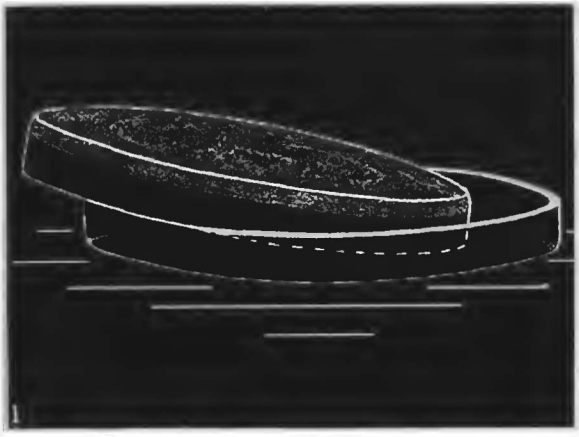
Results and Conclusions.	Reference.
The following scheme of classification "*for clinical purposes*" is advocated: *Streptococci.* Gram-positive, bile insoluble cocci in chains. Hemolysis—Blood agar—Methemoglobin and Indifferent *S. viridans* *S. hemolysans* Lactose+ Mannite− *S. buccalis* Lactose+ Mannite+ *S. fecalis* Lactose− Mannite− *S. equinus* "*While it is fully recognized that more extensive study of the growth-characteristics, pathogenicity, and metabolic activities of various strains of streptococci are of much value in experimental and epidemiological work, such investigations are too cumbersome and time-consuming, and of insufficient value for purposes of routine identification. It is believed that adoption of the proposed method of classification will serve to obviate the confusion that has hitherto existed and to provide an adequate means of identification of streptococci for clinical purposes until such time as an immunological basis for classification shall be developed.*" "*A series of agglutination experiments by the author though somewhat limited in number, lends weight to the value of differentiation by the blood agar plate method.*"	**Blake** 1917. The Classification of Streptococci.

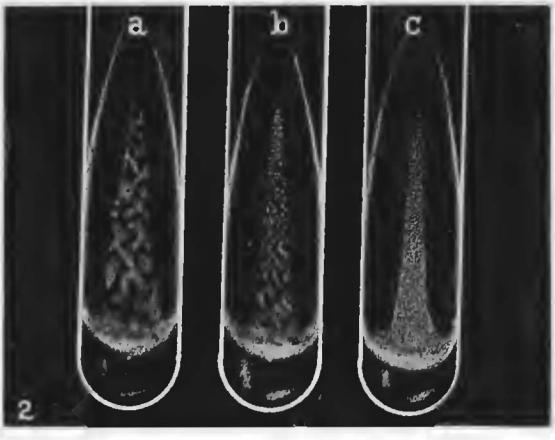
PLATE 1.

FIG. 1. (PAGE 7.) The method of allowing an agar plate to "dry" or "harden" before incubation.

FIG. 2. (PAGE 28.) (*a*) The typical growth of *Streptococcus epidemicus* or of *Streptococcus mucosus* on an agar slant. (*b*) The growth of *Streptococcus epidemicus* or of *Streptococcus mucosus* on an agar slant the upper part of which has become somewhat "dried out." (*c*) The growth of *Streptococcus pyogenes* and most other streptococci and pneumococci on an agar slant inoculated as described.

FIG. 3. (PAGE 20.) Below the line: the appearance of three deep colonies as viewed from the surface of an agar plate. Above the line: the same three colonies as they would appear if viewed laterally.





(Brown: Blood agar for study of streptococci.)

PLATE 2.

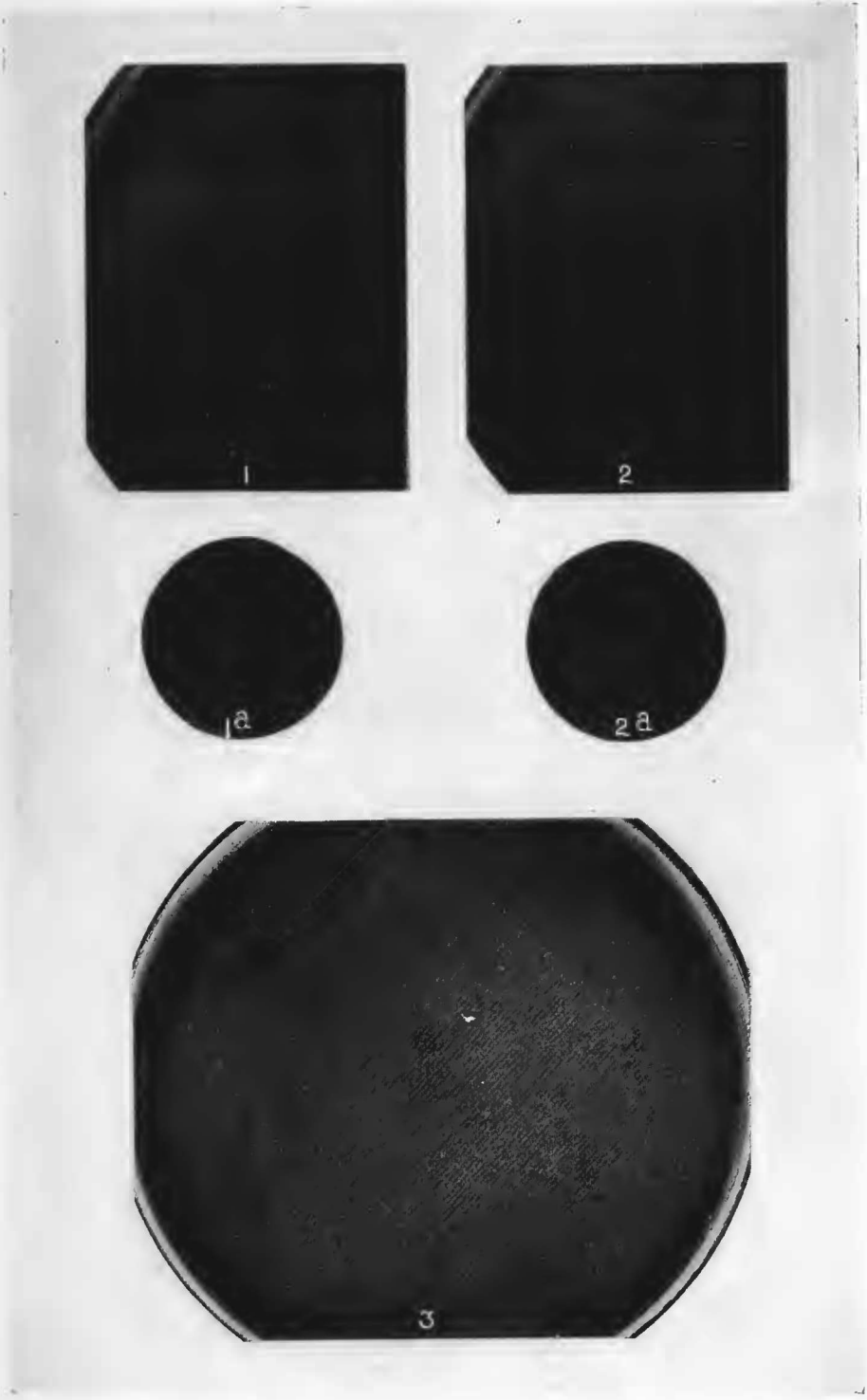
Streptococcus D-1γ.

FIG. 1. March, 1915. (PAGES 17, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 17.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a and 2a. Photomicrographs of a typical deep colony indicated by arrows in Figs. 1 and 2.

FIG. 3. March, 1916. (PAGE 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.



(Brown: Blood agar for study of streptococci.)

PLATE 3.

Streptococcus D-1γ.

FIG. 1. April, 1915. (PAGE 50.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

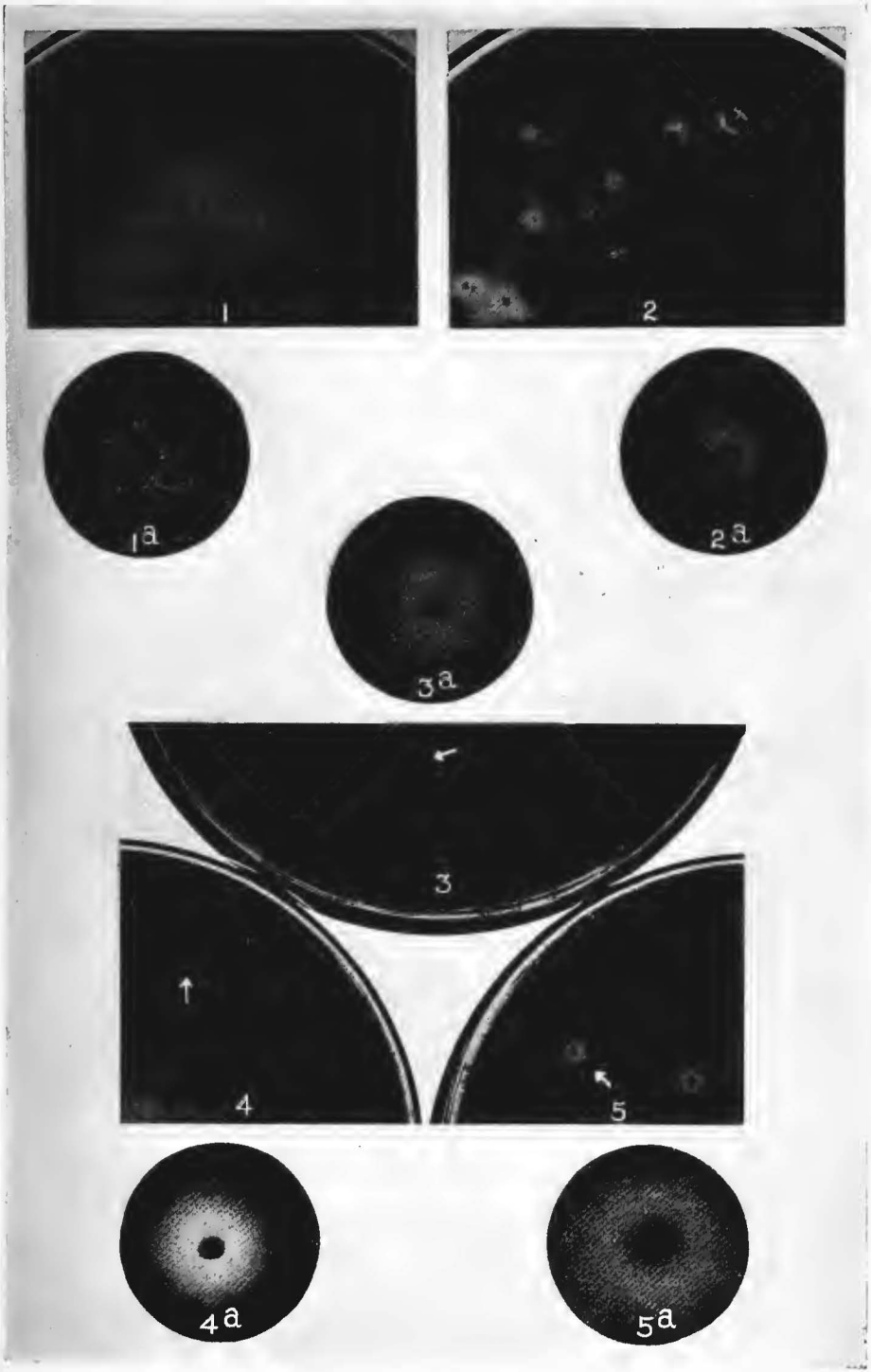
FIG. 2. April, 1915. (PAGE 50.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIG. 3. (PAGE 50.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 50.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 0.1 per cent. Incubated 48 hours.

FIG. 5. (PAGE 50.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 1a, 2a, 3a, 4a, and 5a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, and 5.



(Brown: Blood agar for study of streptococci.)

PLATE 4.

Streptococcus A-18 (Cow)β.

FIG. 1. March 16, 1915. (PAGE 30.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

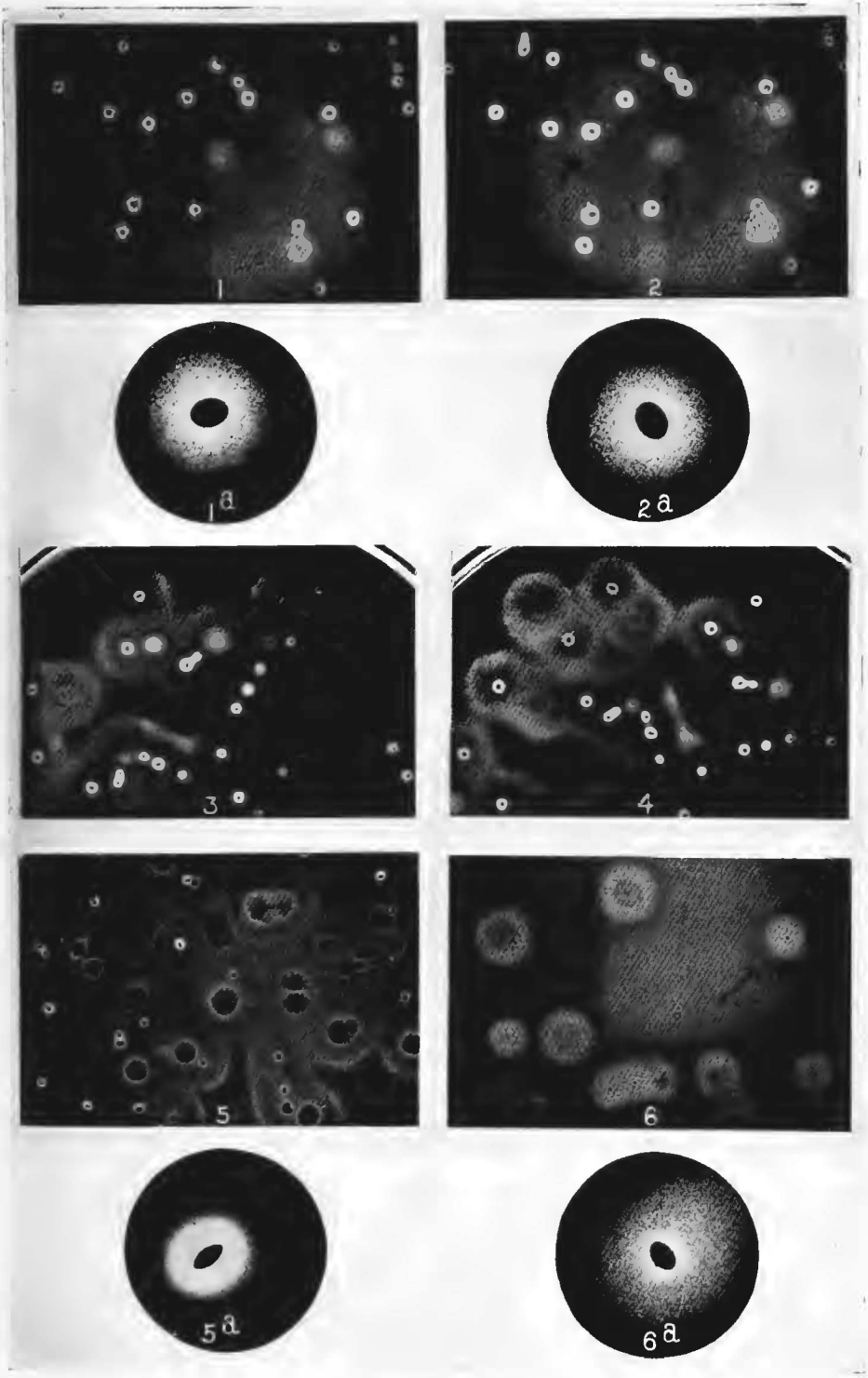
FIG. 3. March 22, 1915. (PAGES 29, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

Fig. 4. The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. March, 1916. (PAGE 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 30.) Washed corpuscles from ⅔ cc. of horse blood; fermented veal infusion Witte peptone agar, 12 cc. Incubated 48 hours.

Figs. 1a, 2a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 5.

Streptococcus B-18 (Cow)β.

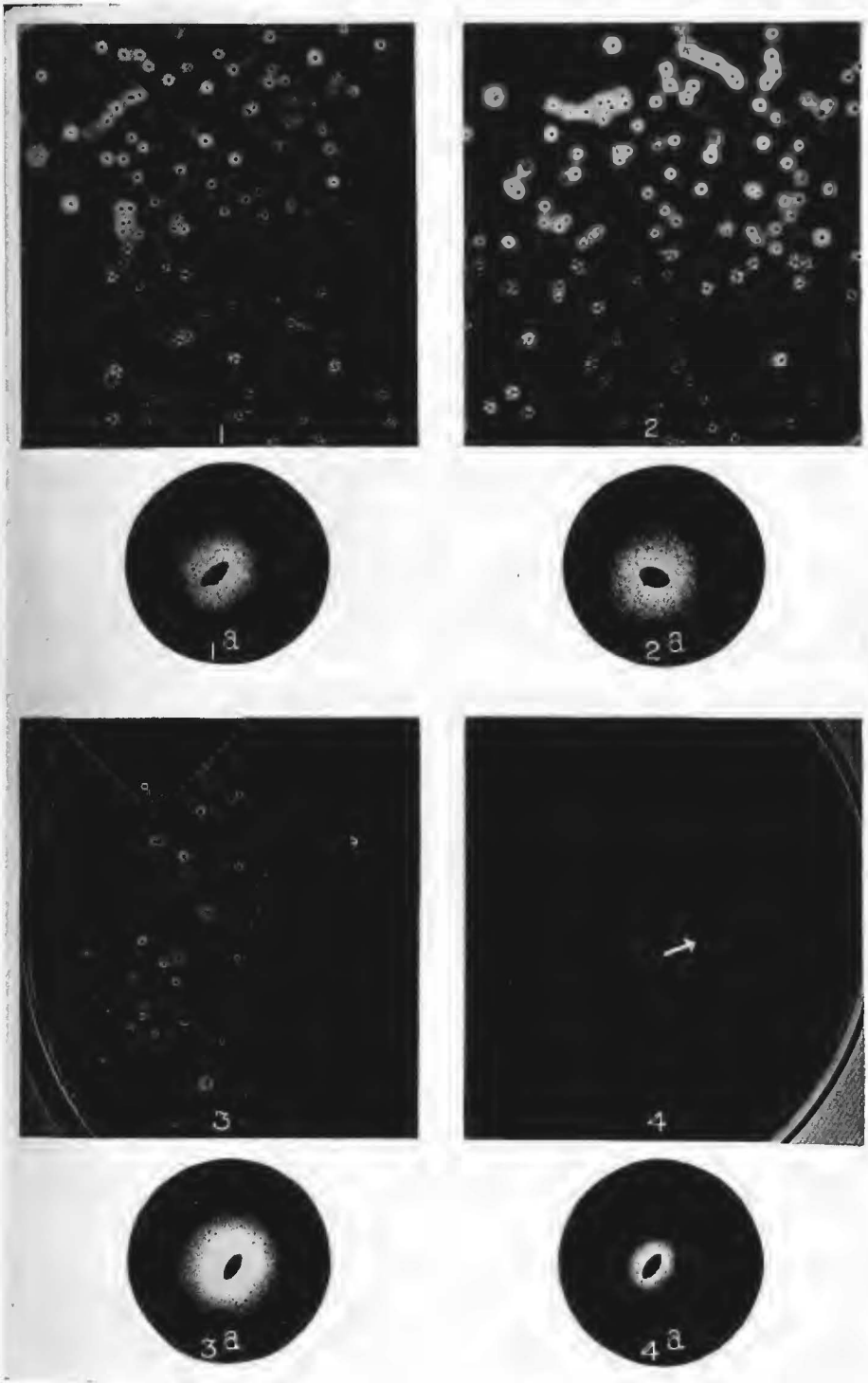
FIG. 1. March, 1915. (PAGES 29, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. March, 1916. (PAGE 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 30.) Washed corpuscles from ⅔ cc. of horse blood; fermented veal infusion Witte peptone agar, 12 cc. Incubated 48 hours.

FIGS. 1a, 2a, 3a, and 4a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, and 4.



(Brown: Blood agar for study of streptococci.)

PLATE 6.

Streptococcus A-STβ.

FIG. 1. March, 1915. (PAGES 14, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 15.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. March, 1916. (PAGES 32, 44, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 44.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 5. (PAGE 32.) Horse blood, 4 cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 32.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 24 cc. Incubated 48 hours.

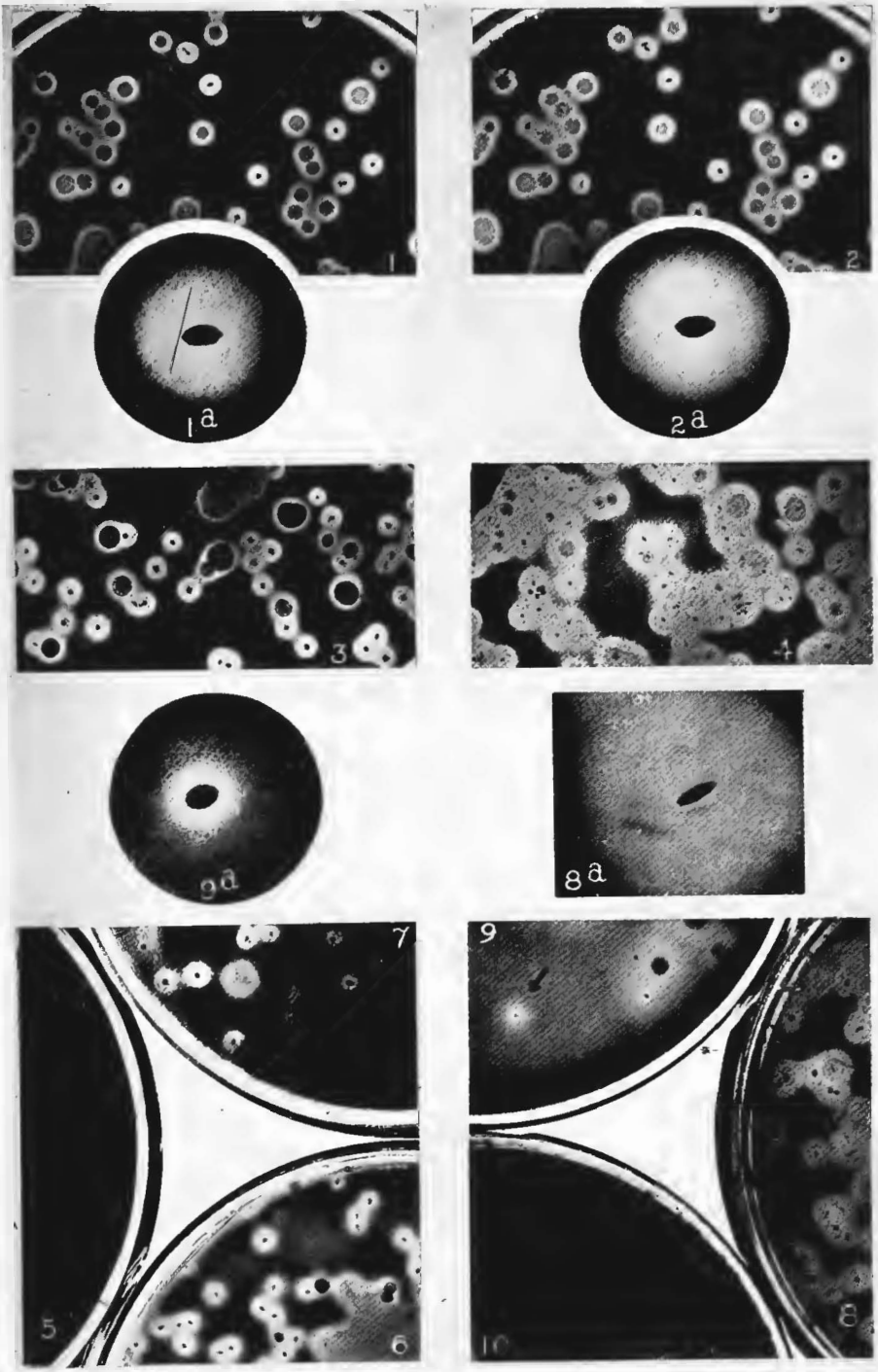
FIG. 7. (PAGE 32.) Horse blood, 1⅓ cc.; standard veal Witte peptone agar, 24 cc. Incubated 48 hours.

FIG. 8. (PAGE 48.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc. Incubated, 48 hours.

FIG. 9. (PAGE 49.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 0.1 per cent. Incubated 48 hours.

FIG. 10. (PAGE 49.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 1a, 2a, 8a, and 9a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 8. and 9.



(Brown: Blood agar for study of streptococci.)

PLATE 7.

Streptococcus D-AD4 β.

FIG. 1. March, 1915. (PAGES 26, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 26.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

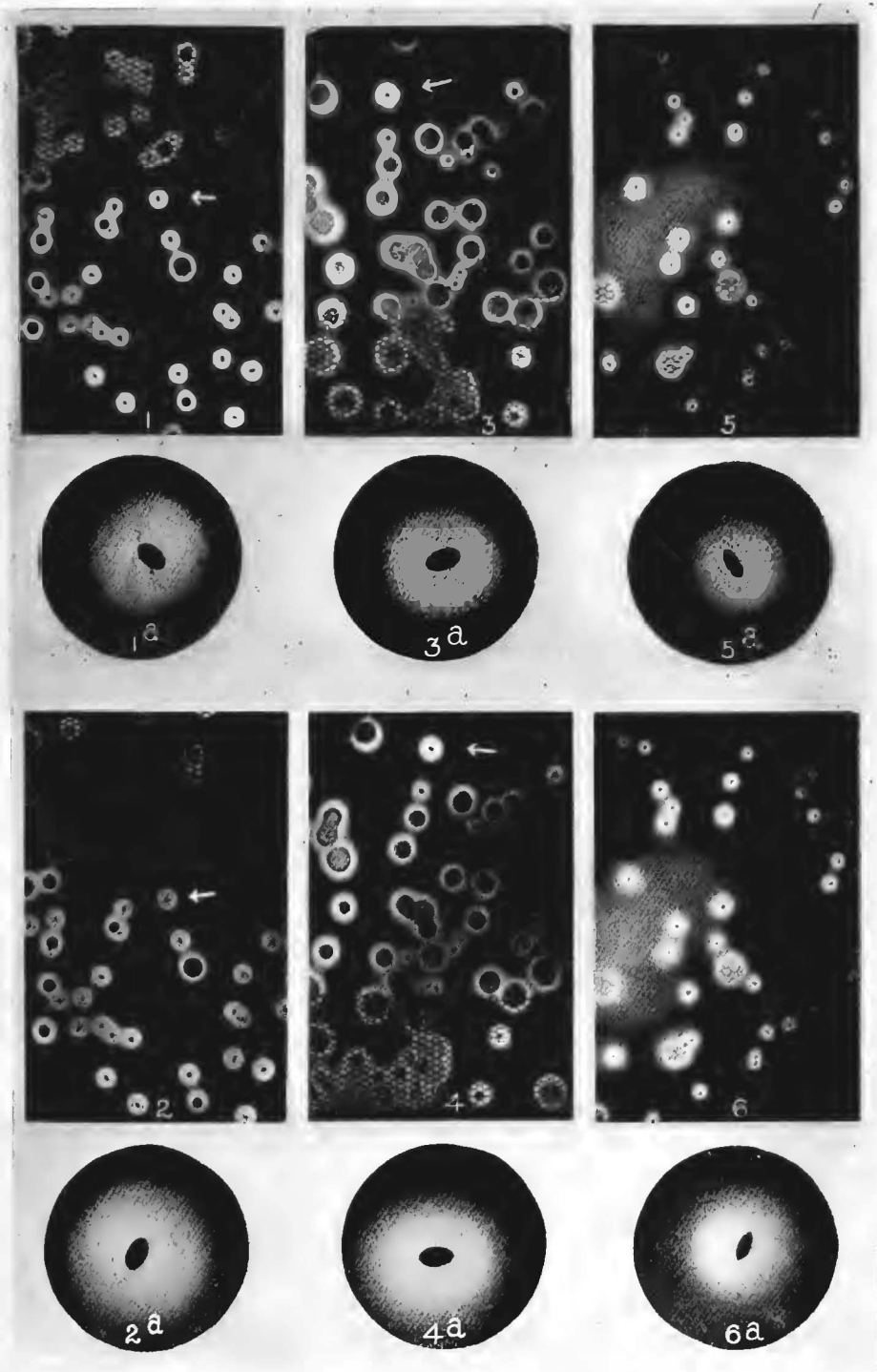
FIG. 3. (PAGE 38.) Rabbit blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 38.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 38.) Human blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 38.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 8.

Streptococcus D-AD4 β.

FIG. 1. March, 1916. (PAGES 48, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

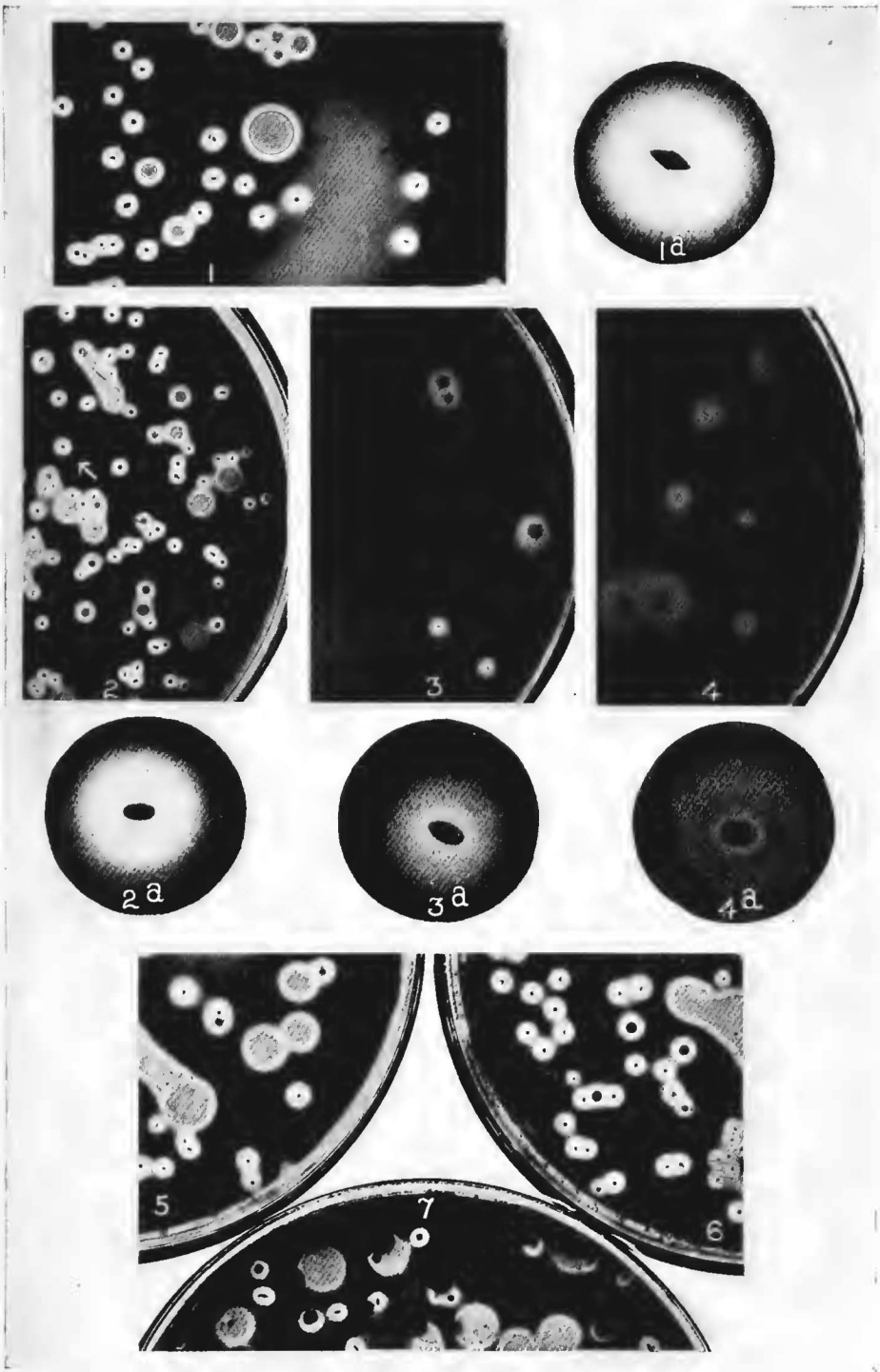
FIG. 2. (PAGE 48.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc. Incubated 48 hours.

FIG. 3. (PAGE 48.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 0.1 per cent. Incubated 48 hours.

FIG. 4. (PAGE 48.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 5, 6, and 7. (PAGE 45.) Streptococci D-AD4 β, C-64β, and X-38 β respectively. Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIGS. 1a, 2a, 3a, and 4a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, and 4.



(Brown: Blood agar for study of streptococci.)

PLATE 9.

Streptococcus B-2b (Cow) β.

FIG. 1. March, 1915. (PAGES 27, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 27.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. March, 1916. (PAGES 44, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

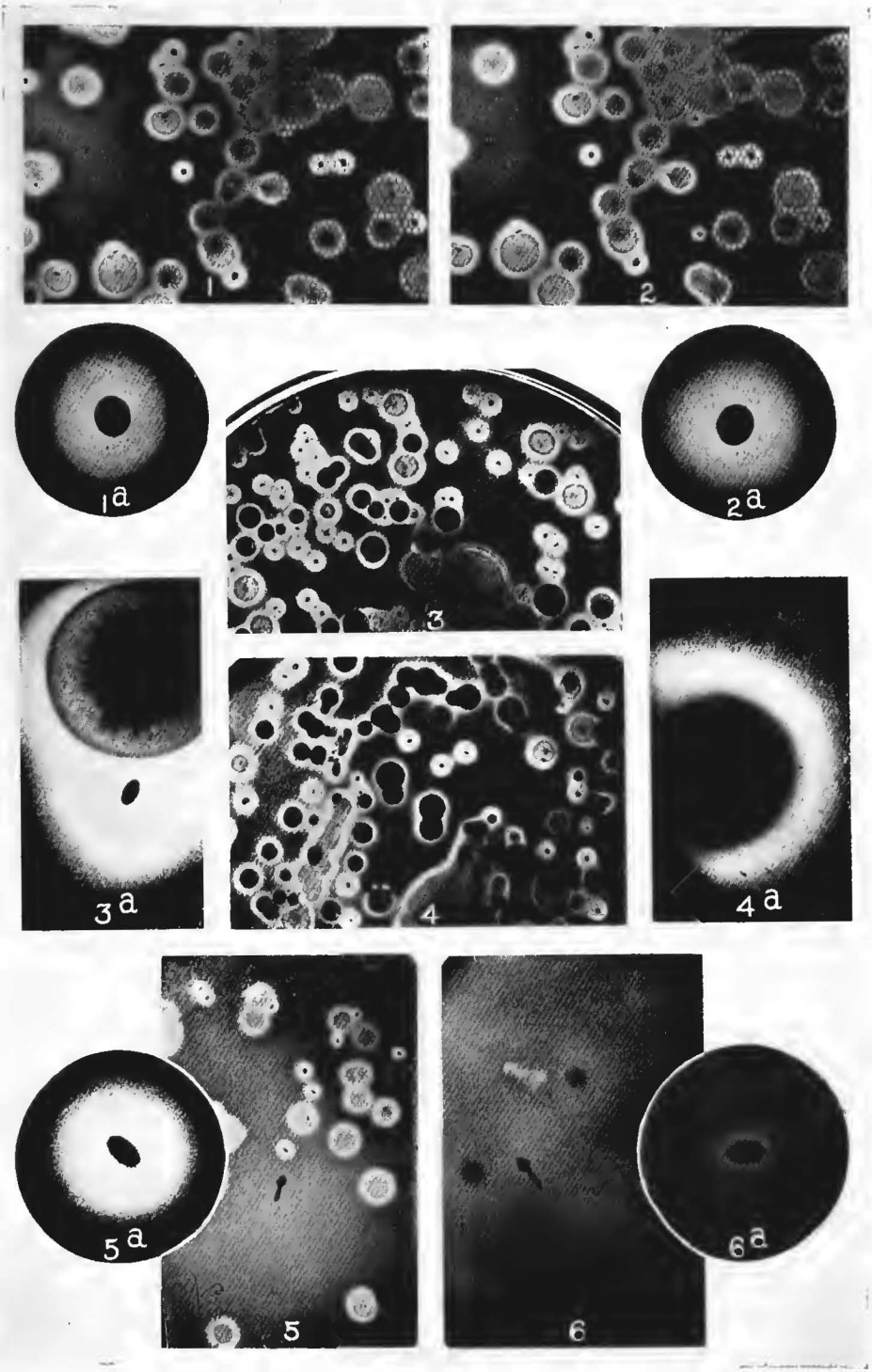
FIG. 4. March, 1916. (PAGE 44.) Horse blood ⅔ cc.; standard veal Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIG. 5. (PAGE 49.) Rabbit blood, 1 cc.; Ruediger sugar-free agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 49.) Rabbit blood, 1 cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 1a, 2a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 5, and 6.

FIGS. 3a and 4a. Photomicrographs of top surface colonies indicated by arrows in Figs. 3 and 4.



(Brown: Blood agar for study of streptococci.)

PLATE 10.

Streptococcus B-6α′.

FIG. 1. March, 1915. (PAGE 16.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 17.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGES 17, 34.) Rabbit blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

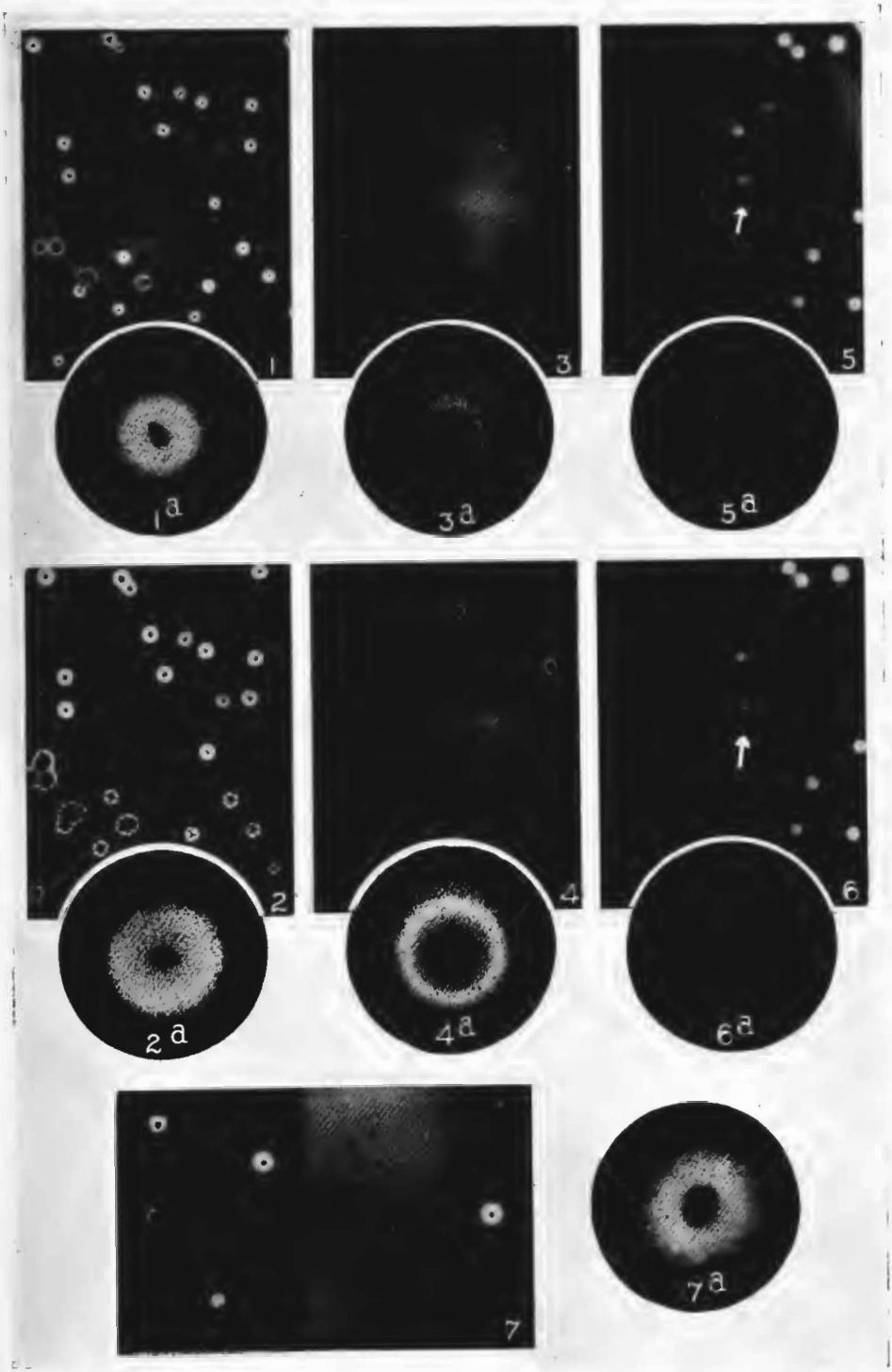
FIG. 4. (PAGES 17, 34.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 35.) Human blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 35.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIG. 7. (PAGE 49.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, 6a, and 7a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, and 7.



(Brown: Blood agar for study of streptococci.)

PLATE 11.

Streptococcus F-2α'.

FIG. 1. March, 1915. (PAGE 26.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 26.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

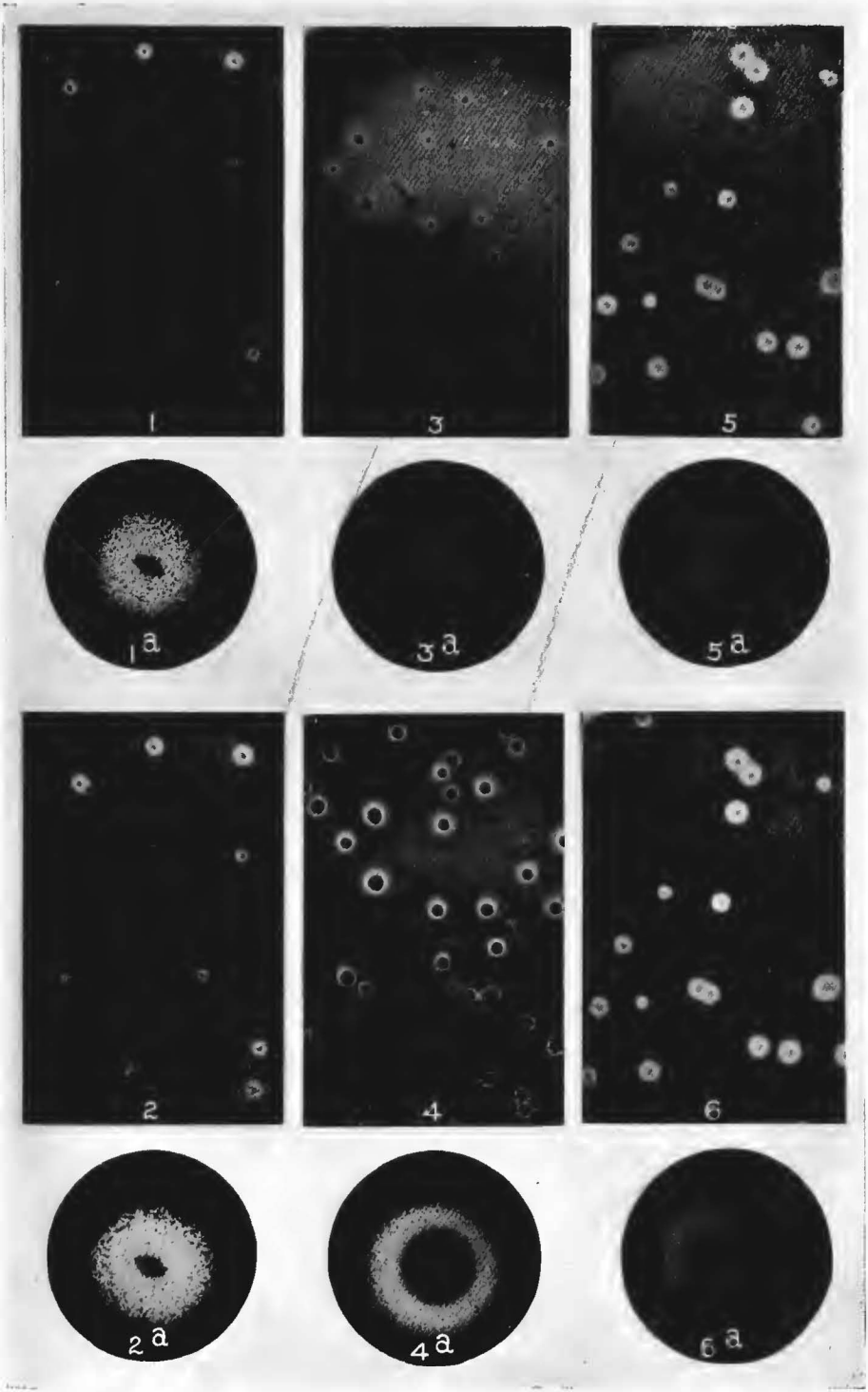
FIG. 3. (PAGE 34.) Rabbit blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 34.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 35.) Human blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 35.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 12.

Streptococcus A-BLα.

FIG. 1. March, 1915. (PAGES 19, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGES 19, 80.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 19.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

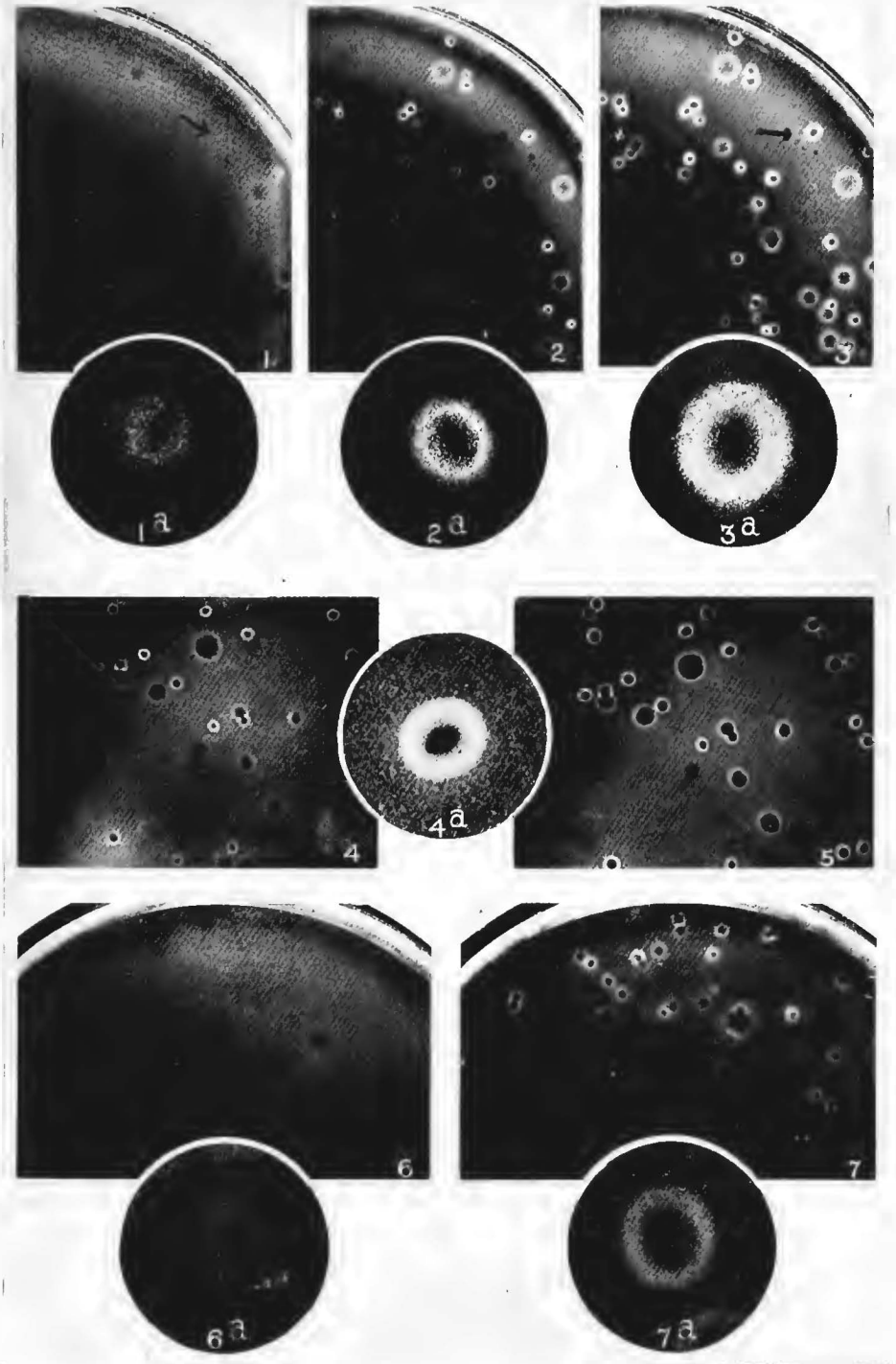
FIG. 4. March, 1916. (PAGES 32, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 5. (PAGES 32, 80.) The same plate as shown in Fig. 4. Incubated 48 hours; refrigerated 48 hours.

FIG. 6. March, 1916. (PAGE 32.) Horse blood, 1⅓ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 7. (PAGE 32.) The same plate as shown in Fig. 6. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 6a, and 7a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 6, and 7.



(Brown: Blood agar for study of streptococci.)

PLATE 13.

Streptococcus A-BLα.

FIG. 1. (PAGE 56.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically.

FIG. 2. (PAGE 56.) The same plate as shown in Fig. 1. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically.

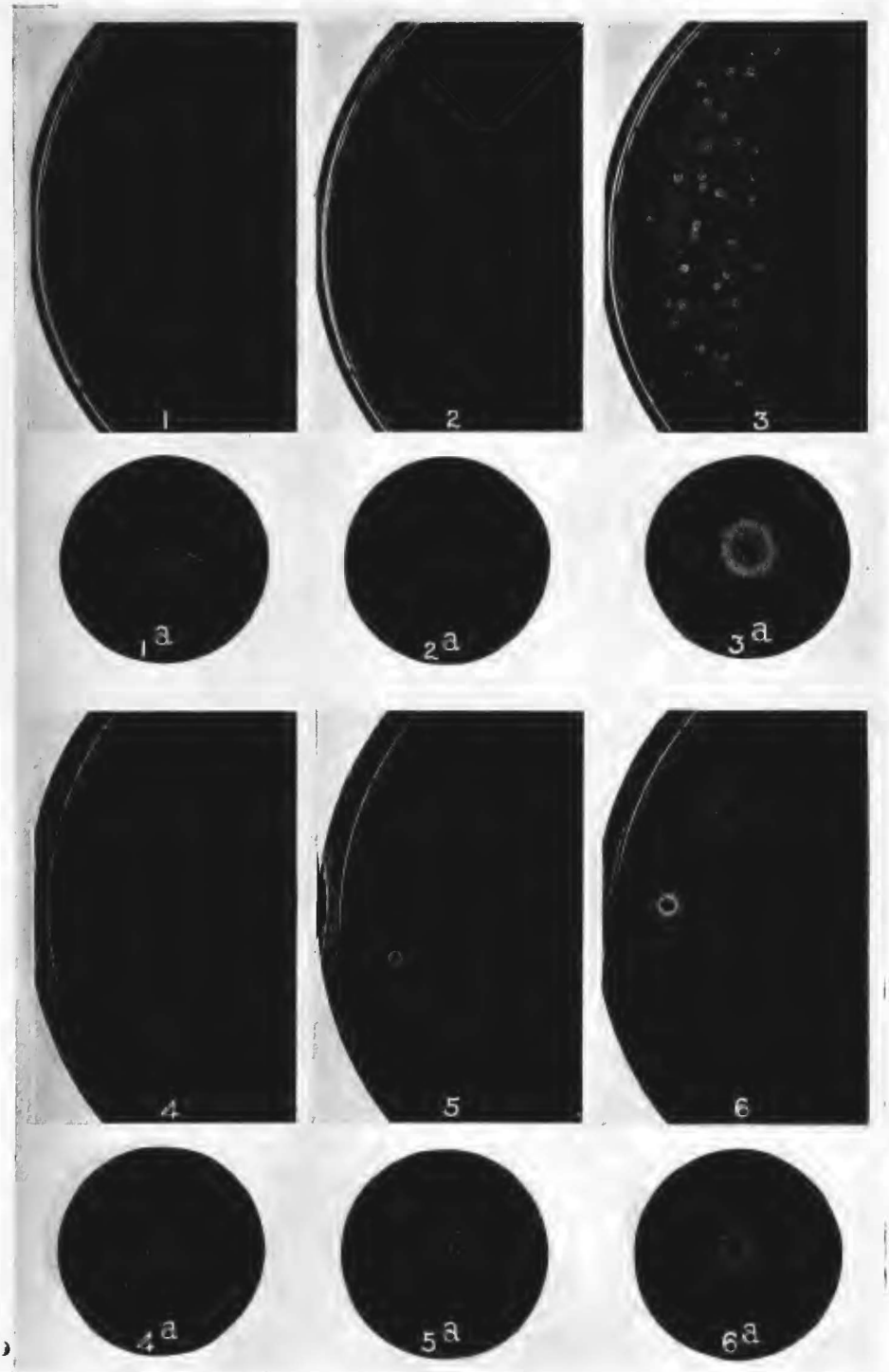
FIG. 3. (PAGE 56.) The same plate as shown in Fig. 1. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically; refrigerated 24 hours aerobically.

FIG. 4. (PAGE 56.) Rabbit blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically.

FIG. 5. (PAGE 56.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically.

FIG. 6. (PAGE 56.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically; refrigerated 24 hours aerobically.

Figs. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 14.

Streptococcus D-4α.

FIG. 1. March, 1915. (PAGE 19.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 19.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 19.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

FIG. 4. (PAGE 36.) Rabbit blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 5. (PAGE 36.) The same plate as shown in Fig. 4. Incubated 48 hours; refrigerated 48 hours.

FIG. 6. (PAGE 36.) The same plate as shown in Fig. 4. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours.

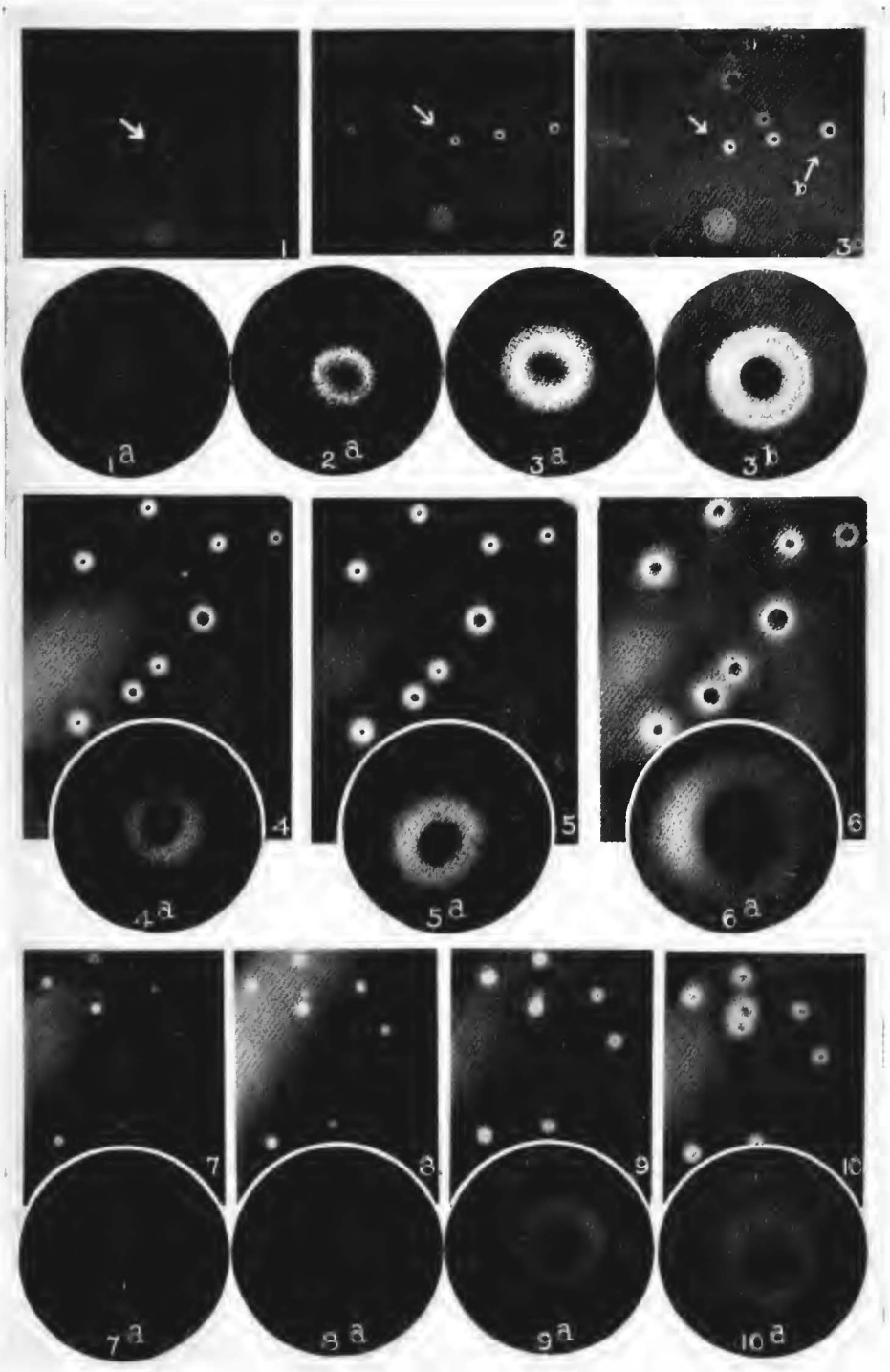
FIG. 7. (PAGE 36.) Human blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 8. (PAGE 36.) The same plate as shown in Fig. 7. Incubated 48 hours; refrigerated 48 hours.

FIG. 9. (PAGE 36.) The same plate as shown in Fig. 7. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours.

FIG. 10. (PAGE 36.) The same plate as shown in Fig. 7. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 3b, 4a, 5a, 6a, 7a, 8a, 9a, and 10a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.



(Brown: Blood agar for study of streptococci.)

PLATE 15.

Streptococcus B-7α.

FIG. 1. March, 1915. (PAGES 8, 35, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGES 10, 35, 80.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 11.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours.

FIG. 4. (PAGE 11.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 35.) Rabbit blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 35.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIG. 7. (PAGE 36.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours.

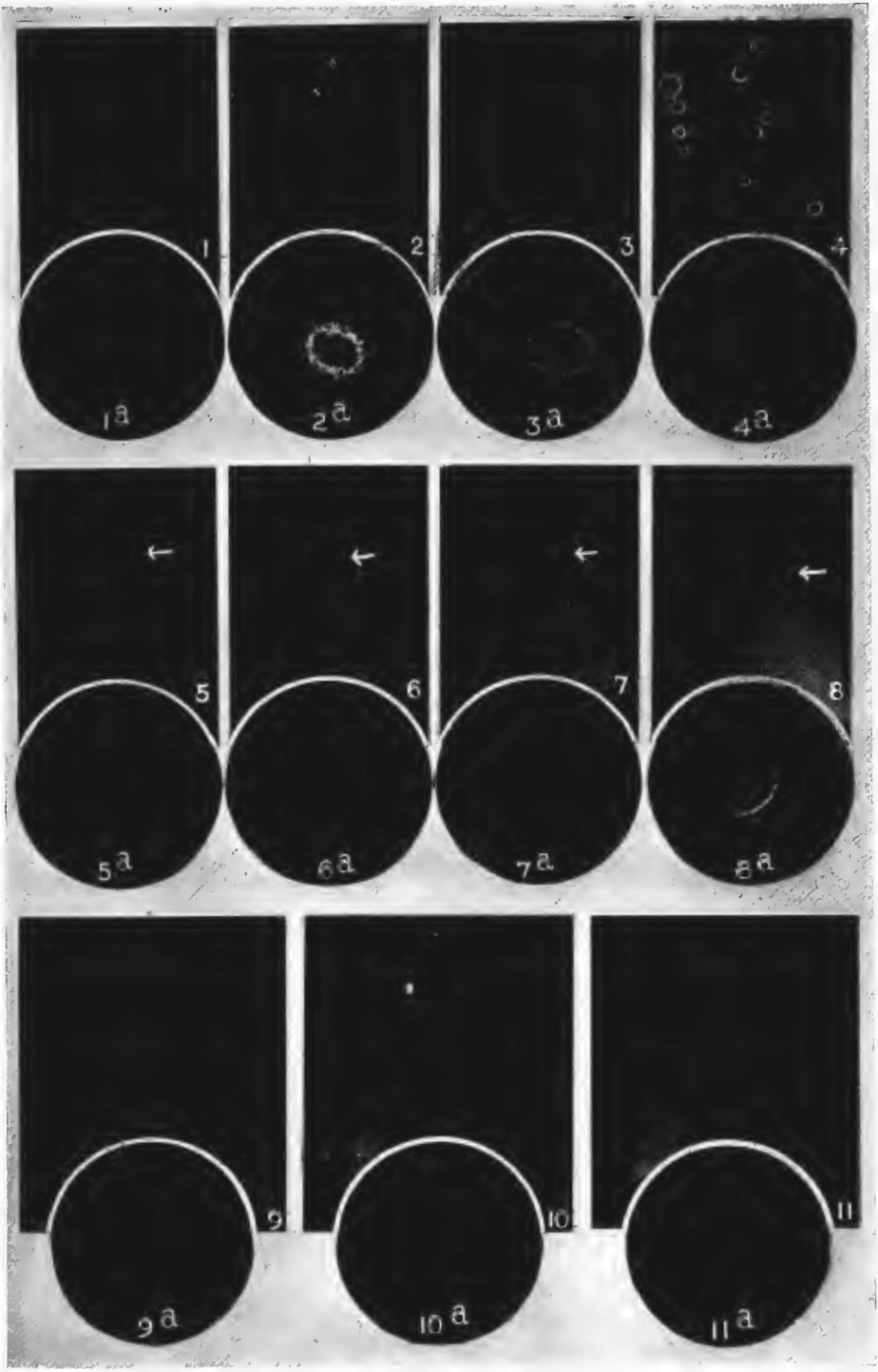
FIG. 8. (PAGE 36.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

FIG. 9. (PAGE 35.) Human blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 10. (PAGE 35.) The same plate as shown in Fig. 9. Incubated 48 hours; refrigerated 48 hours.

FIG. 11. (PAGE 35.) The same plate as shown in Fig. 9. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, 6a, 7a, 8a, 9a, 10a, and 11a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11.



(Brown: Blood agar for study of streptococci.)

PLATE 16.

Streptococcus B-7α.

FIG. 1. March, 1916. (PAGES 42, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 80.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 42.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc. Incubated 48 hours.

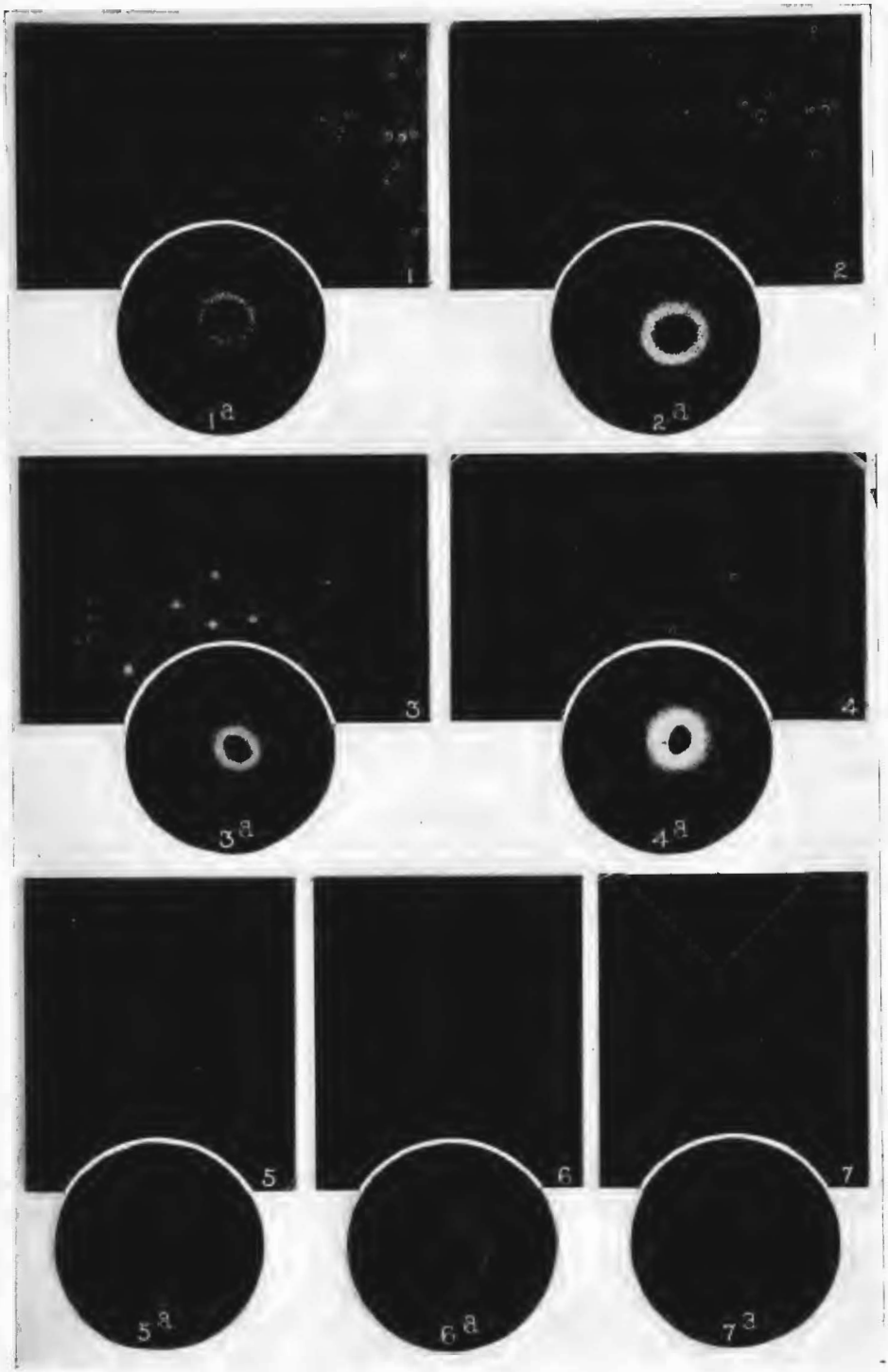
FIG. 4. (PAGE 42.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 53.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 53.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 0.1 per cent. Incubated 48 hours.

FIG. 7. (PAGE 53.) Rabbit blood, ¾ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, 6a, and 7a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, and 7.



(Brown: Blood agar for study of streptococci.)

PLATE 17.

Streptococcus E-4 α.

FIG. 1. March, 1915. (PAGES 19, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGES 19, 80.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 19.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours.

FIG. 4 (PAGE 19.) The same plate as shown in Fig 1. Incubated 48 hours; refrigerated 48 hours; incubated 48 hours; refrigerated 48 hours.

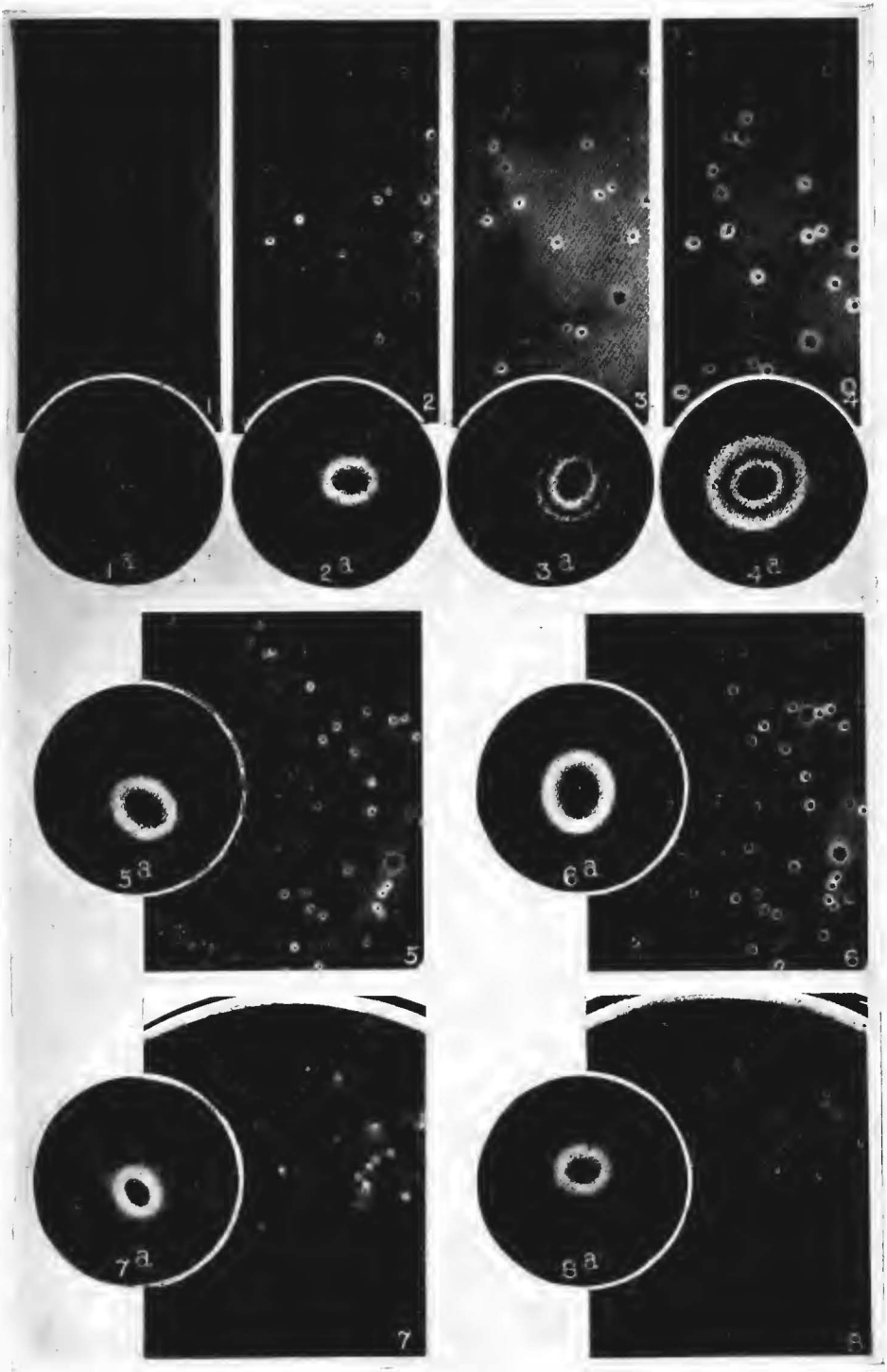
FIG. 5. March, 1916. (PAGES 42, 80.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 80.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIG. 7. (PAGE 42.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 8. (PAGE 42.) The same plate as shown in Fig. 7. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, 6a, 7a, and 8a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, 7, and 8.



(Brown: Blood agar for study of streptococci.)

PLATE 18.

Streptococcus D-AD8$_2$ α.

FIG. 1. (PAGE 22.) Horse blood, $\frac{2}{3}$ cc.; standard beef Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 22.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 37.) Rabbit blood, $\frac{2}{3}$ cc.; standard beef Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 37.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGES 8, 37.) Human blood, $\frac{2}{3}$ cc; standard beef Witte peptone agar, 12 cc. Incubated 48 hours.

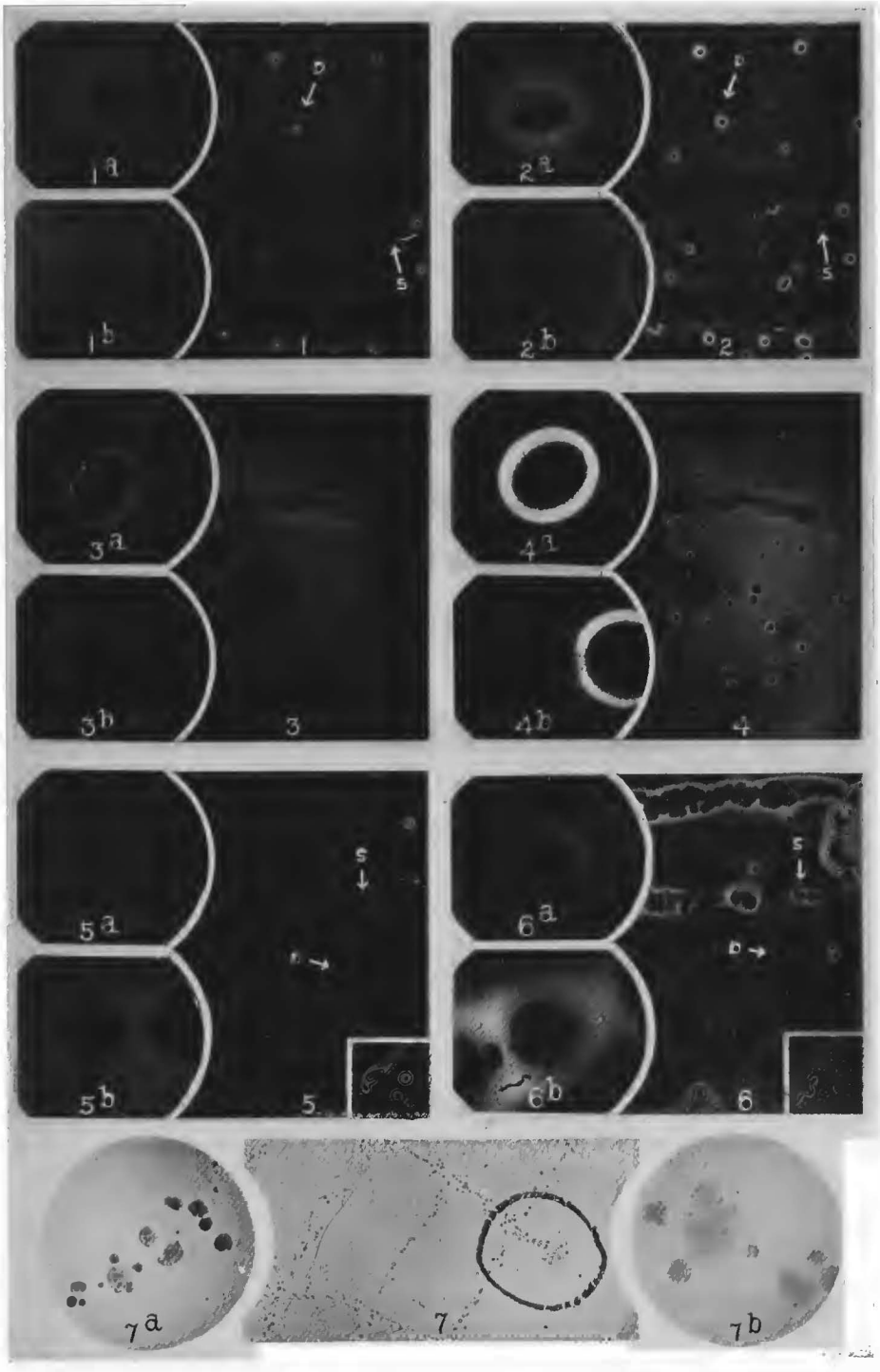
FIG. 6. (PAGES 8, 37.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows marked (D) in Figs. 1, 2, 3, 4, 5, and 6.

FIGS. 1b, 2b, 3b, 4b, 5b, and 6b. Photomicrographs of typical surface colonies indicated by arrows marked (S) in Figs. 1, 2, 3, 4, 5, and 6.

FIG. 7. (PAGE 22.) Standard beef Witte peptone agar, 12 cc. Incubated 48 hours.

FIGS. 7a and 7b. Photomicrographs of colonies within the encircled area of Fig. 7.



(Brown: Blood agar for study of streptococci.)

PLATE 19.

Streptococcus F-2 α.

FIG. 1. (PAGE 21.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

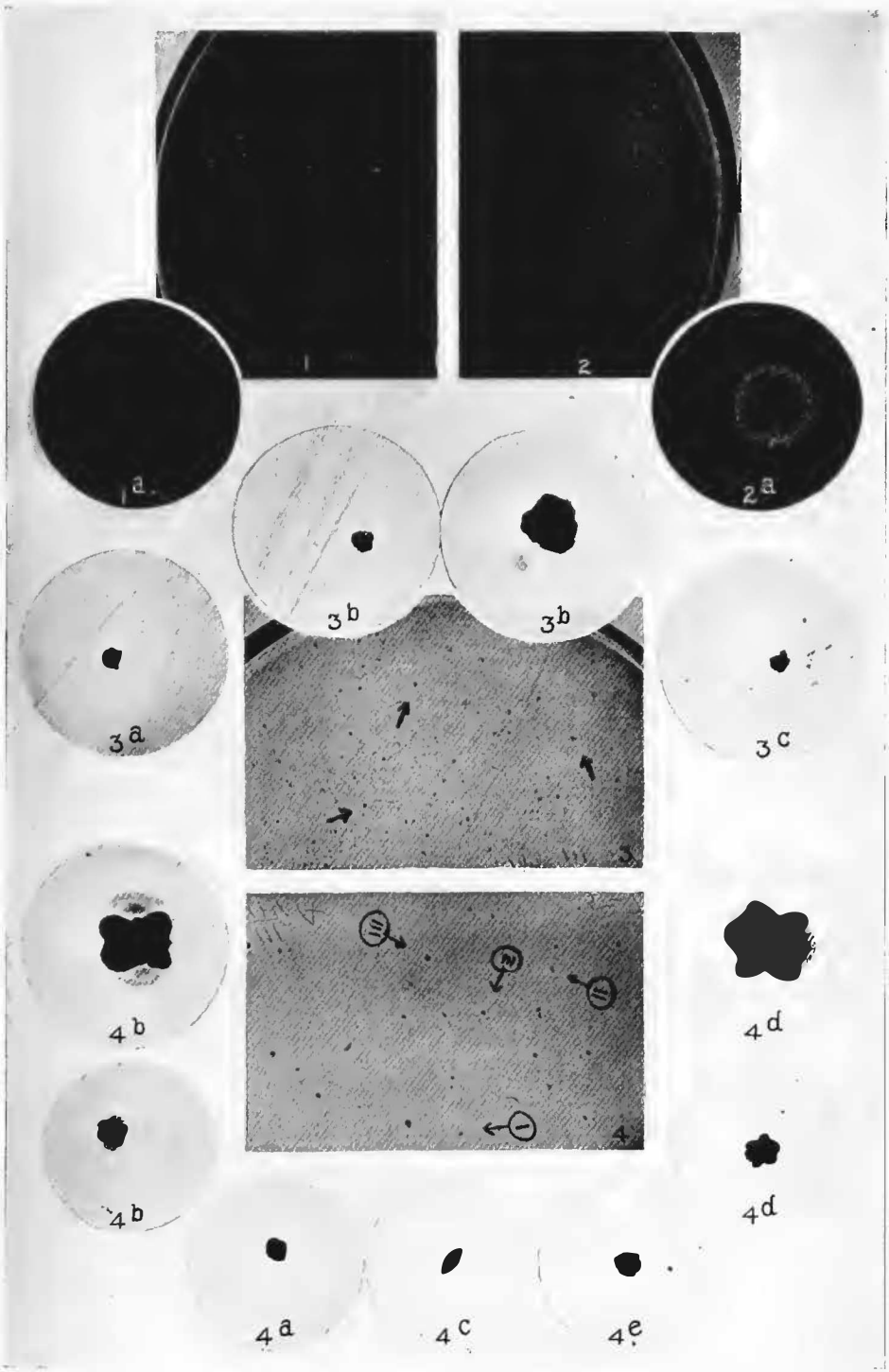
FIG. 3. (PAGE 21.) Standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

Streptococcus D-1 α.

FIG. 2. (PAGE 21.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 21.) Standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIGS. 1a, 2a, 3a, 3b, 3c, 4a, 4b, 4c, 4d, and 4e. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, and 4.



(Brown: Blood agar for study of streptococci.)

PLATE 20.

Streptococcus Brig.-IIaα.

FIG. 1. (PAGE 24.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours aerobically.

FIG. 2. (PAGE 24.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 48 hours aerobically.

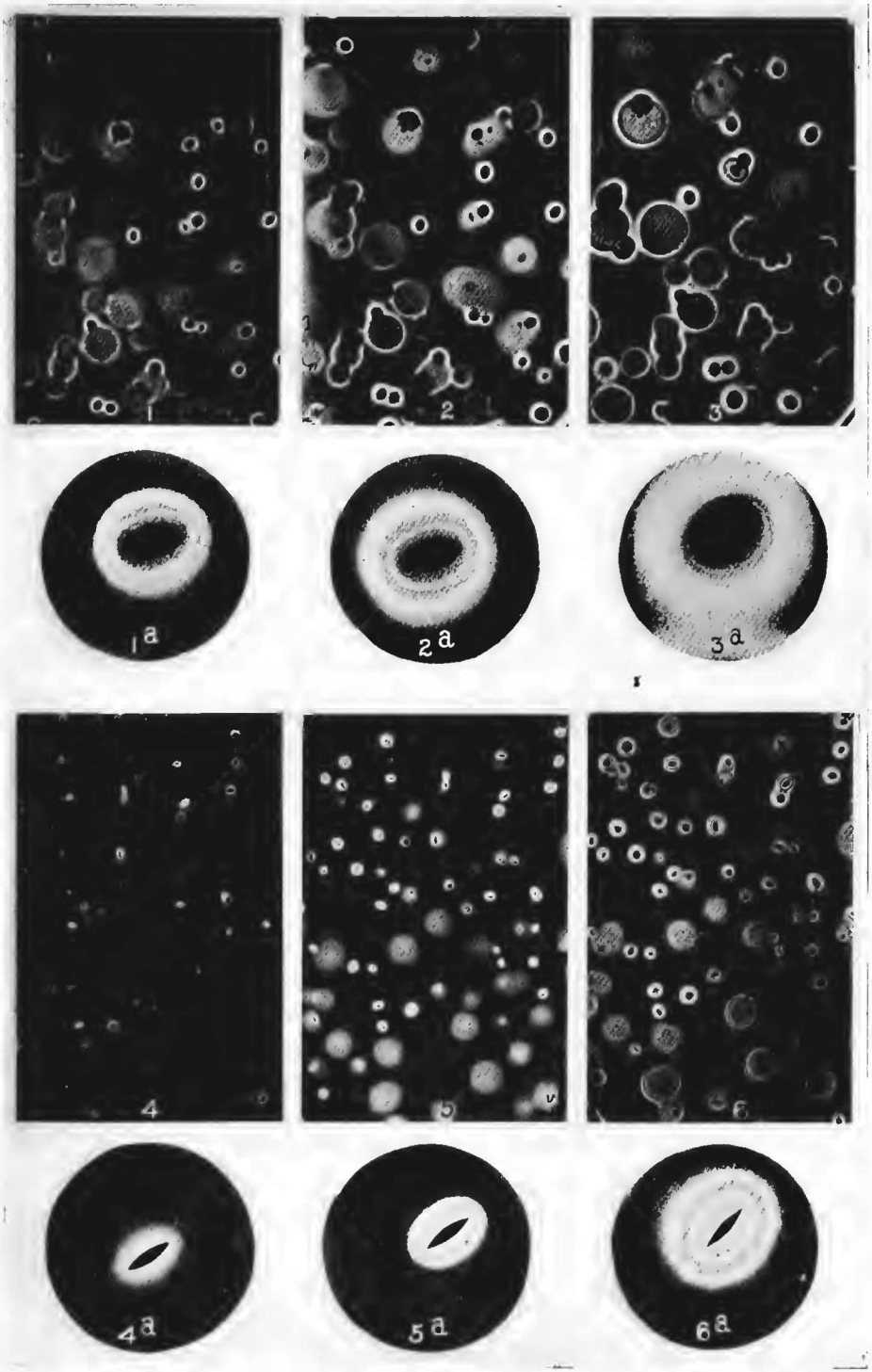
FIG. 3. (PAGE 24.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 48 hours aerobically; incubated 24 hours aerobically; refrigerated 48 hours aerobically.

FIG. 4. (PAGE 57.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically.

FIG. 5. (PAGE 57.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 48 hours aerobically.

FIG. 6. (PAGE 57.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 48 hours aerobically; incubated 24 hours aerobically; refrigerated 48 hours aerobically.

FIGS. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 21.

Pneumococcus Cole I 109.

FIG. 1. (PAGES 25, 43.) Horse blood, $\frac{2}{3}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours aerobically.

FIG. 2. (PAGES 25, 43.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 72 hours aerobically.

FIG. 3. (PAGE 25.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically.

FIG. 4. (PAGE 25.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically; refrigerated 24 hours aerobically.

FIG. 5. (PAGE 57.) Horse blood, $\frac{2}{3}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically.

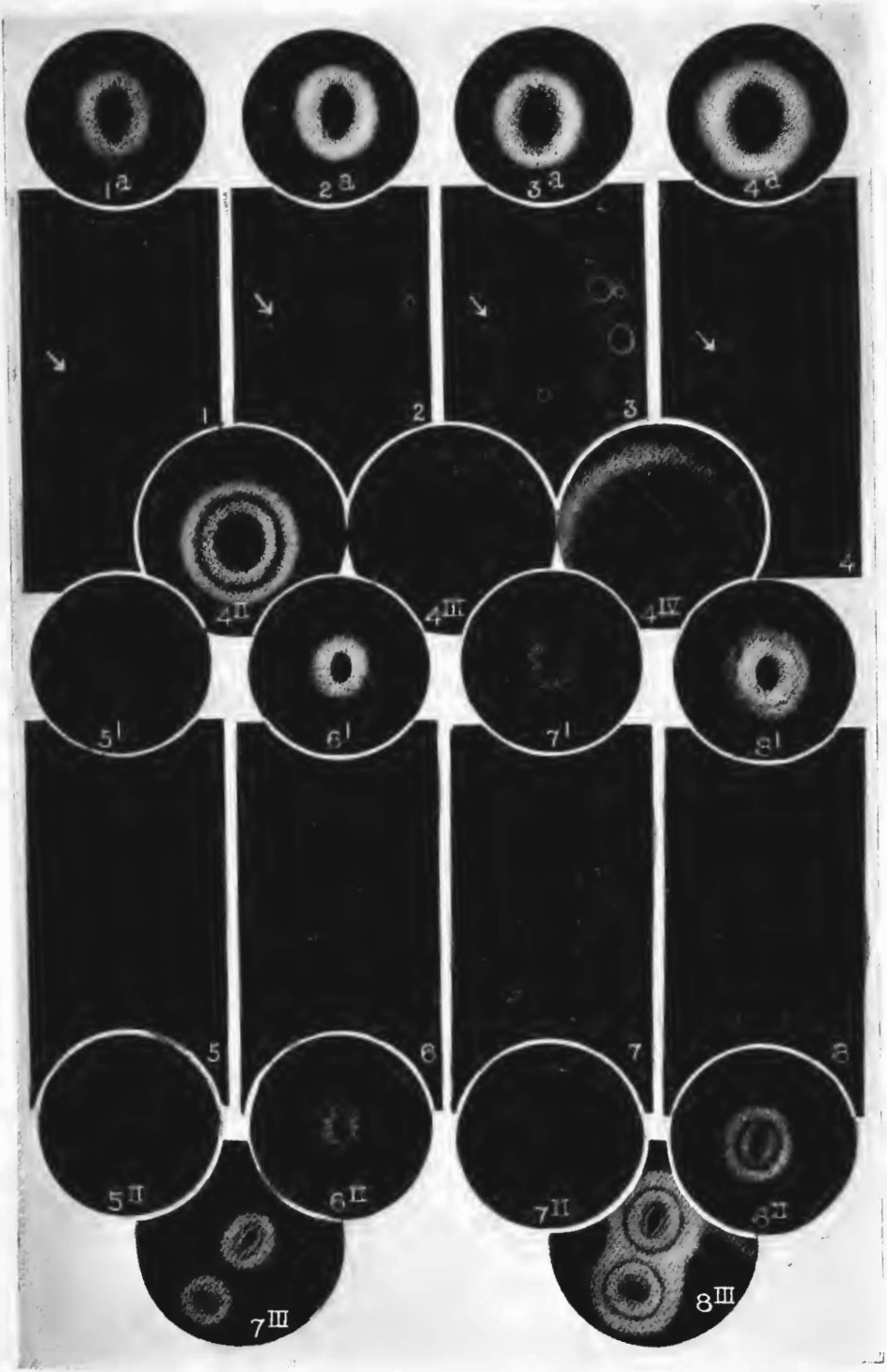
FIG. 6. (PAGE 58.) The same plate as shown in Fig. 5. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically.

FIG. 7. (PAGE 58.) The same plate as shown in Fig. 5. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically.

FIG. 8. (PAGE 58.) The same plate as shown in Fig. 5. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically; refrigerated 24 hours aerobically.

FIGS. 1a, 2a, 3a, 4a, 4^{II}, 5^{1}, 5^{II}, 6^{1}, 6^{II}, 7^{1}, 7^{II}, 7^{III}, 8^{1}, 8^{II}, and 8^{III}. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, 7, and 8.

FIGS. 4^{III} and 4^{IV}. Photomicrographs of bottom and top surface colonies indicated by arrows (III) and (IV) in Fig. 4.



(Brown: Blood agar for study of streptococci.)

PLATE 22.

Pneumococcus Cole I 109.

FIG. 1. (PAGE 37.) Rabbit blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours aerobically.

FIG. 2. (PAGE 37.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 72 hours aerobically.

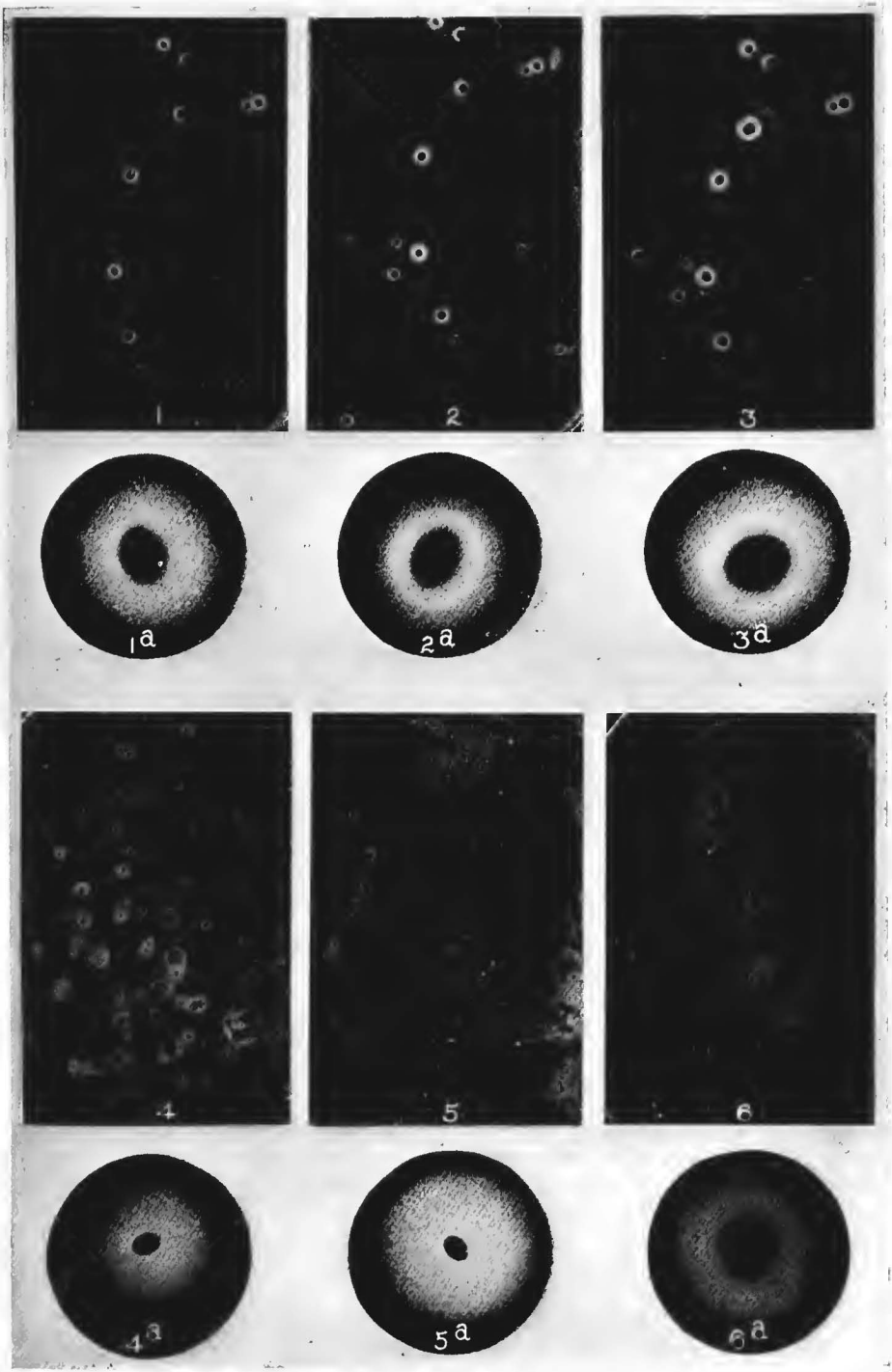
FIG. 3. (PAGE 37.) The same plate as shown in Fig. 1. Incubated 48 hours aerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically.

FIG. 4. (PAGE 57.) Rabbit blood, ⅔ cc.; standard beef Digestive Ferments Co. peptoné agar, 12 cc. Incubated 48 hours anaerobically.

FIG. 5. (PAGE 58.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically.

FIG. 6. (PAGE 58.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 72 hours aerobically; incubated 24 hours aerobically.

FIGS. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 23.

Pneumococcus Cole I 109.

FIG. 1. (PAGE 43.) Horse blood, $\frac{2}{3}$ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 43.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

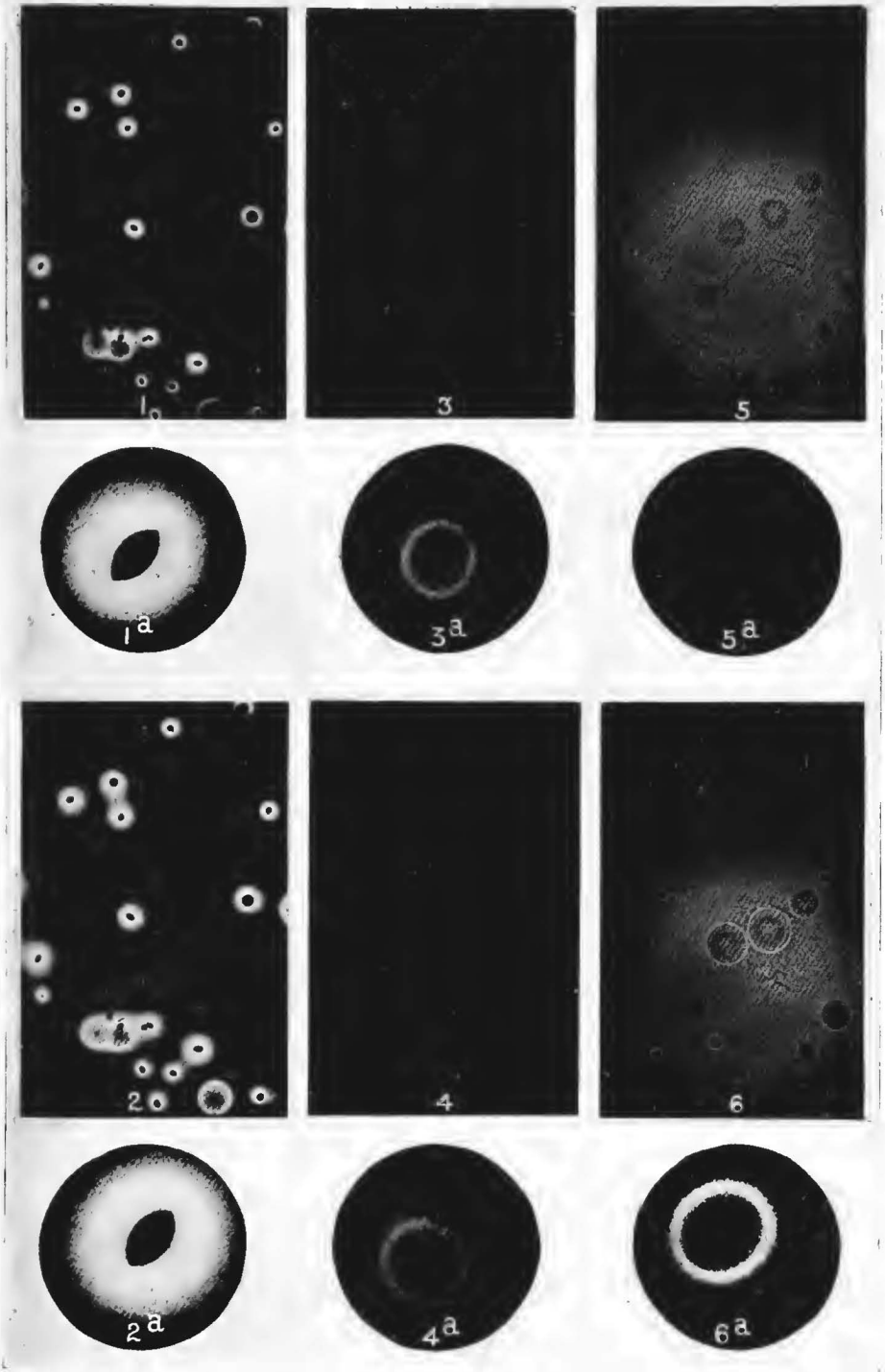
FIG. 3. (PAGE 43.) Horse blood, $\frac{2}{3}$ cc.; standard Liebig extract Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 43.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 38.) Human blood, $\frac{2}{3}$ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 38.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, and 6a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, and 6.



(Brown: Blood agar for study of streptococci.)

PLATE 24.

Pneumococcus Cole II 45.

FIG. 1. (PAGES 25, 44.) Horse blood, $\frac{2}{3}$ cc.; standard veal Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 2. (PAGE 25.) The same plate as shown in Fig. 1. Incubated 48 hours; refrigerated 48 hours.

FIG. 3. (PAGE 44.) Horse blood, $\frac{2}{3}$ cc.; standard veal Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIG. 4. (PAGE 44.) The same plate as shown in Fig. 3. Incubated 48 hours; refrigerated 48 hours.

FIG. 5. (PAGE 43.) Horse blood, $\frac{2}{3}$ cc.; standard Liebig extract Witte peptone agar, 12 cc. Incubated 48 hours.

FIG. 6. (PAGE 43.) The same plate as shown in Fig. 5. Incubated 48 hours; refrigerated 48 hours.

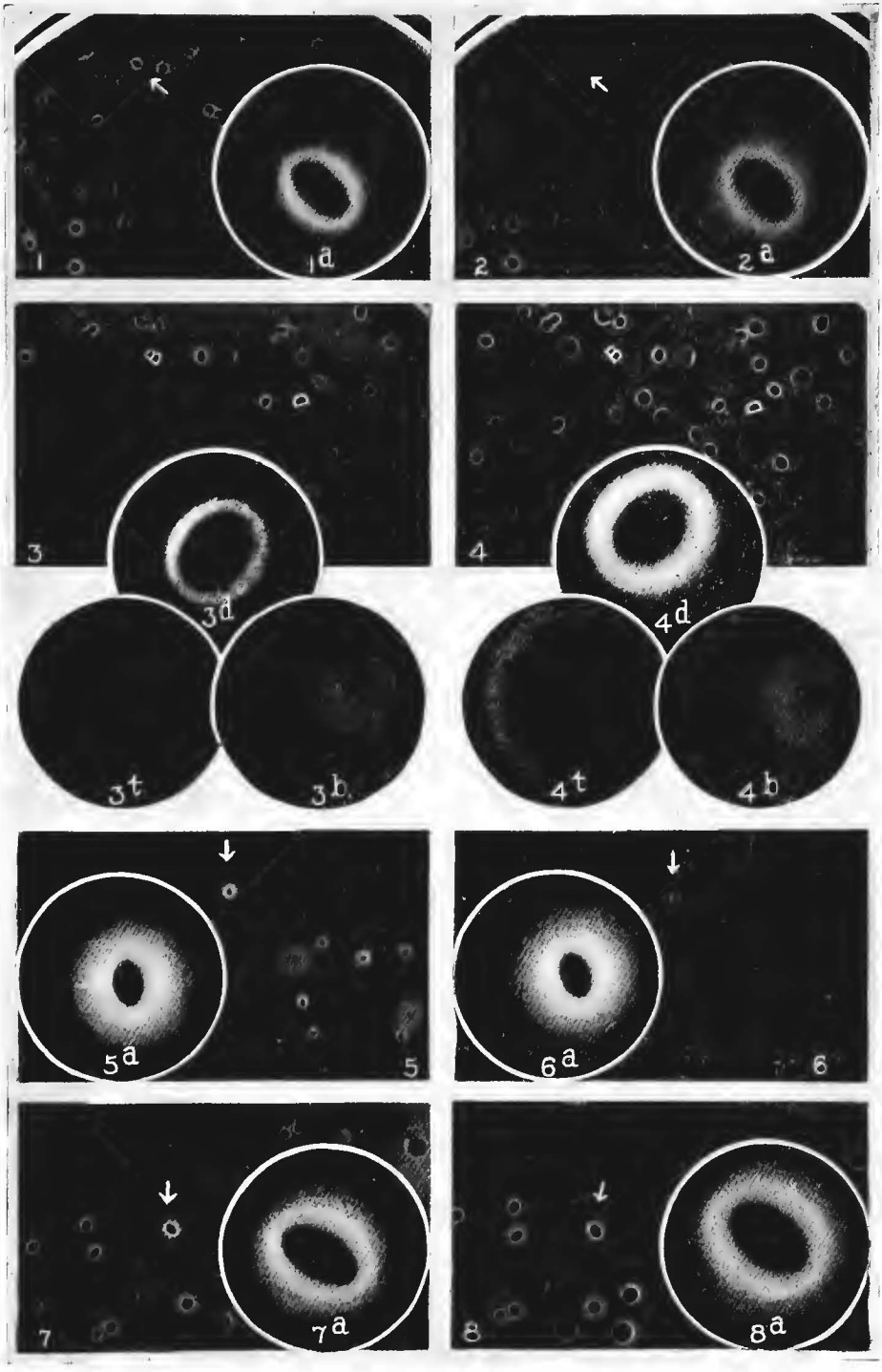
FIG. 7. (PAGE 43.) Horse blood, $\frac{2}{3}$ cc.; standard Liebig extract Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours.

FIG. 8. (PAGE 43.) The same plate as shown in Fig. 7. Incubated 48 hours; refrigerated 48 hours.

FIGS. 1a, 2a, 3d, 4d, 5a, 6a, 7a, and 8a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, 7, and 8.

FIGS. 3t and 4t. Photomicrographs of a typical top surface colony indicated by arrows (T) in Figs. 3 and 4.

FIGS. 3b and 4b. Photomicrographs of a typical bottom surface colony indicated by arrows (B) in Figs. 3 and 4.



(Brown: Blood agar for study of streptococci.)

PLATE 25.

Pneumococcus Cole II 45.

FIG. 1. (PAGE 58.) Horse blood, $\frac{2}{3}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically.

FIG. 2. (PAGE 58.) The same plate as shown in Fig. 1. Incubated 48 hours anaerobically; refrigerated 48 hours aerobically.

FIG. 3. (PAGE 58.) The same plate as shown in Fig. 1. Incubated 48 hours anaerobically; refrigerated 48 hours aerobically; incubated 24 hours aerobically.

FIG. 4. (PAGE 58.) Rabbit blood, $\frac{2}{3}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically.

FIG. 5. (PAGE 58.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 48 hours aerobically.

FIG. 6. (PAGE 58.) The same plate as shown in Fig. 4. Incubated 48 hours anaerobically; refrigerated 48 hours aerobically; incubated 24 hours aerobically.

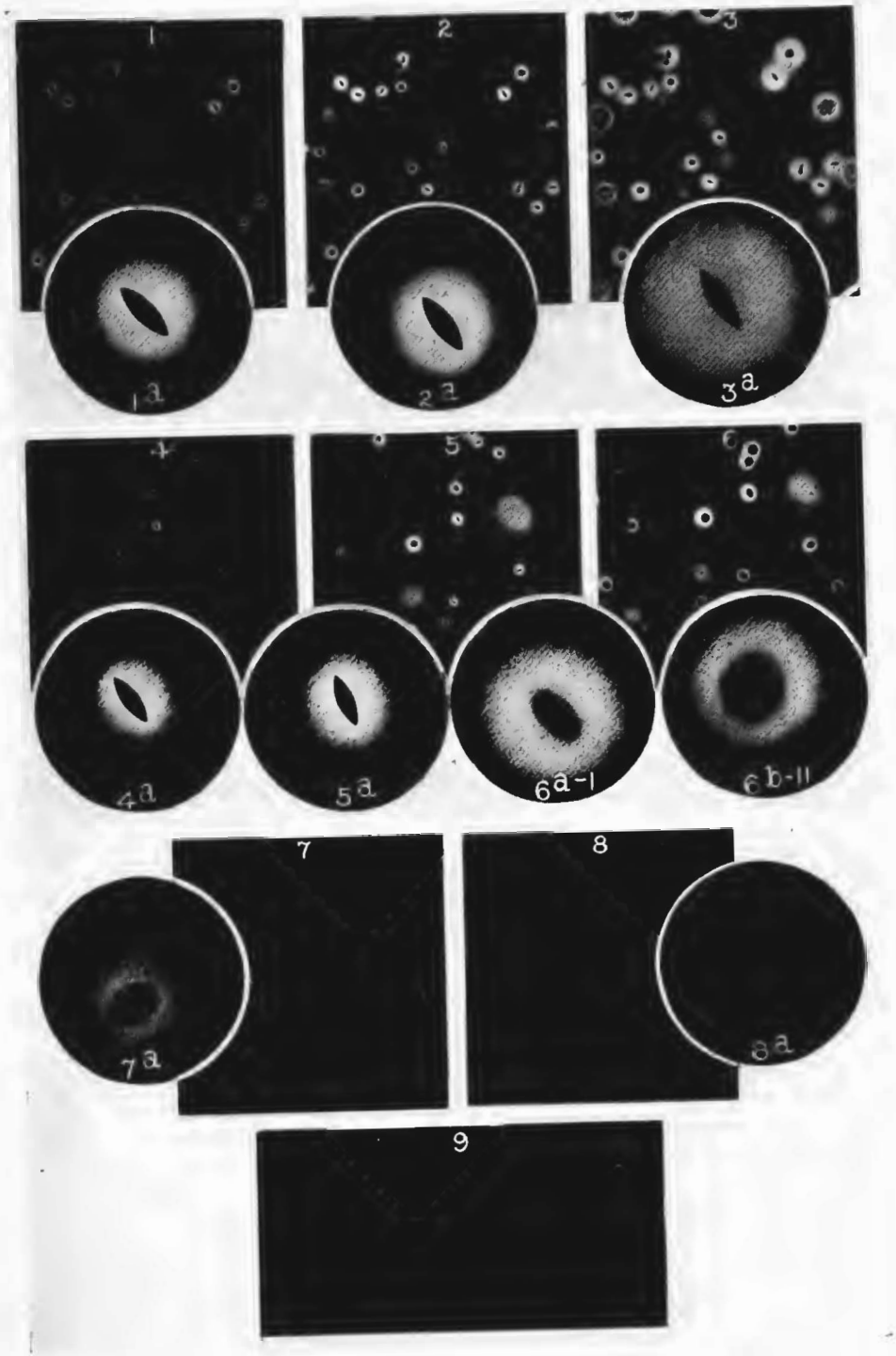
FIG. 7. (PAGE 53.) Rabbit blood, $\frac{2}{4}$ cc.; Ruediger sugar-free agar, 12 cc. Incubated 48 hours.

FIG. 8. (PAGE 53.) Rabbit blood, $\frac{2}{4}$ cc.; Ruediger sugar-free agar, 12 cc.; dextrose, 1 per cent. Incubated 48 hours.

FIGS. 1a, 2a, 3a, 4a, 5a, 6^{a-1}, 6^{b-11}, 7a, and 8a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 2, 3, 4, 5, 6, 7, and 8.

Pneumococcus Cole II 37.

FIG. 9. (PAGE 59.) Horse blood, $\frac{2}{3}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 48 hours anaerobically.



(Brown: Blood agar for study of streptococci.)

PLATE 26.

Streptococcus X-32 β.

FIG. 1. (PAGE 47.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 24 hours.

FIG. 2. (PAGE 47.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 24 hours.

FIG. 3. (PAGE 46.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc. Incubated 24 hours.

Streptococcus C-64 β.

FIG. 4. (PAGE 47.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 24 hours.

FIG. 5. (PAGE 47.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 24 hours.

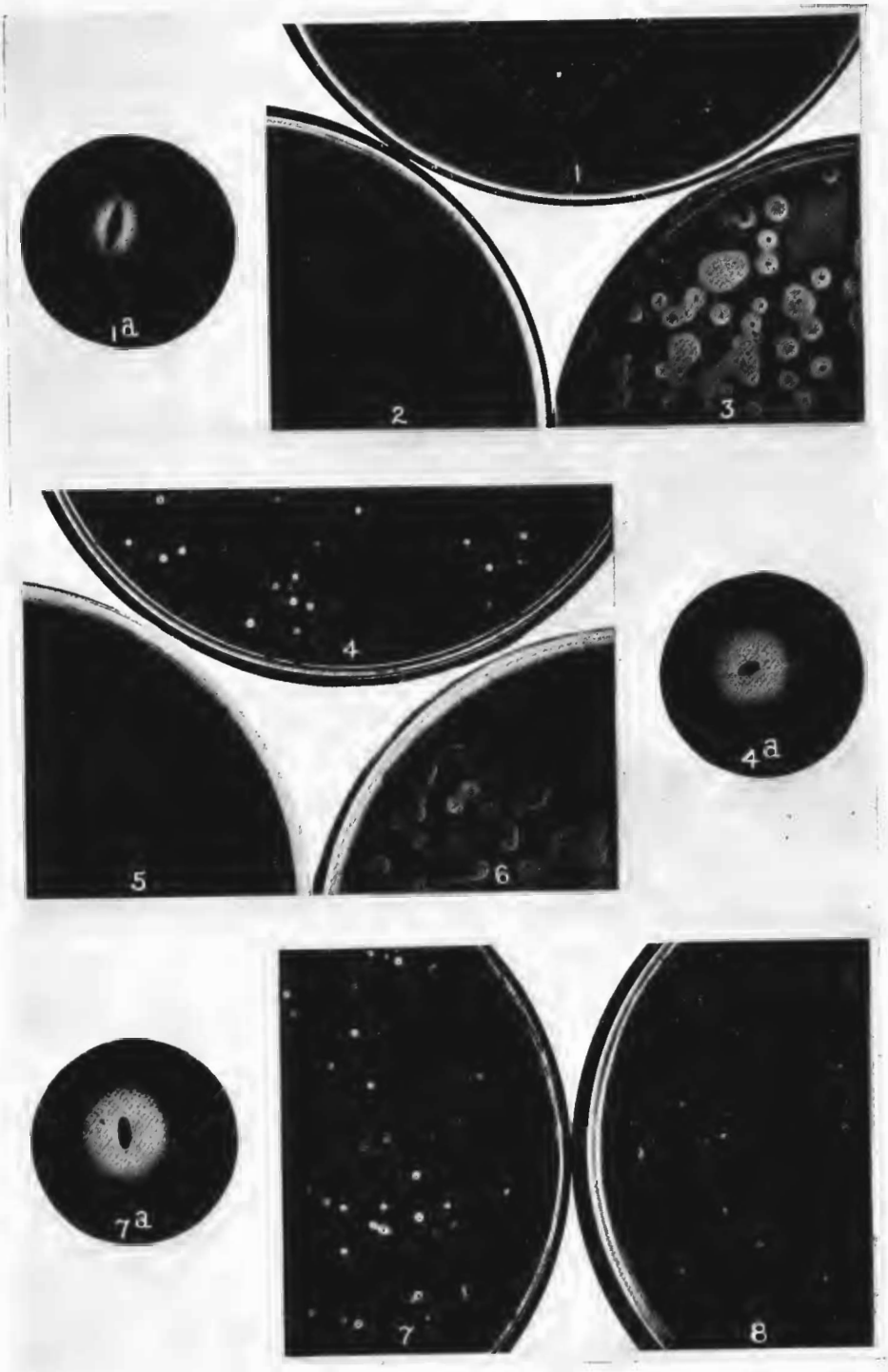
FIG. 6. (PAGE 46.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc. Incubated 24 hours.

Streptococcus A-ST β.

FIG. 7. (PAGE 47.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 24 hours.

FIG. 8. (PAGE 47.) Horse blood, ⅔ cc.; standard Liebig extract Witte peptone agar, 12 cc.; dextrose, 1 per cent. Incubated 24 hours.

FIGS. 1a, 4a, and 7a. Photomicrographs of typical deep colonies indicated by arrows in Figs. 1, 4, and 7.



(Brown: Blood agar for study of streptococci.)

PLATE 27.

Streptococcus B-7α.

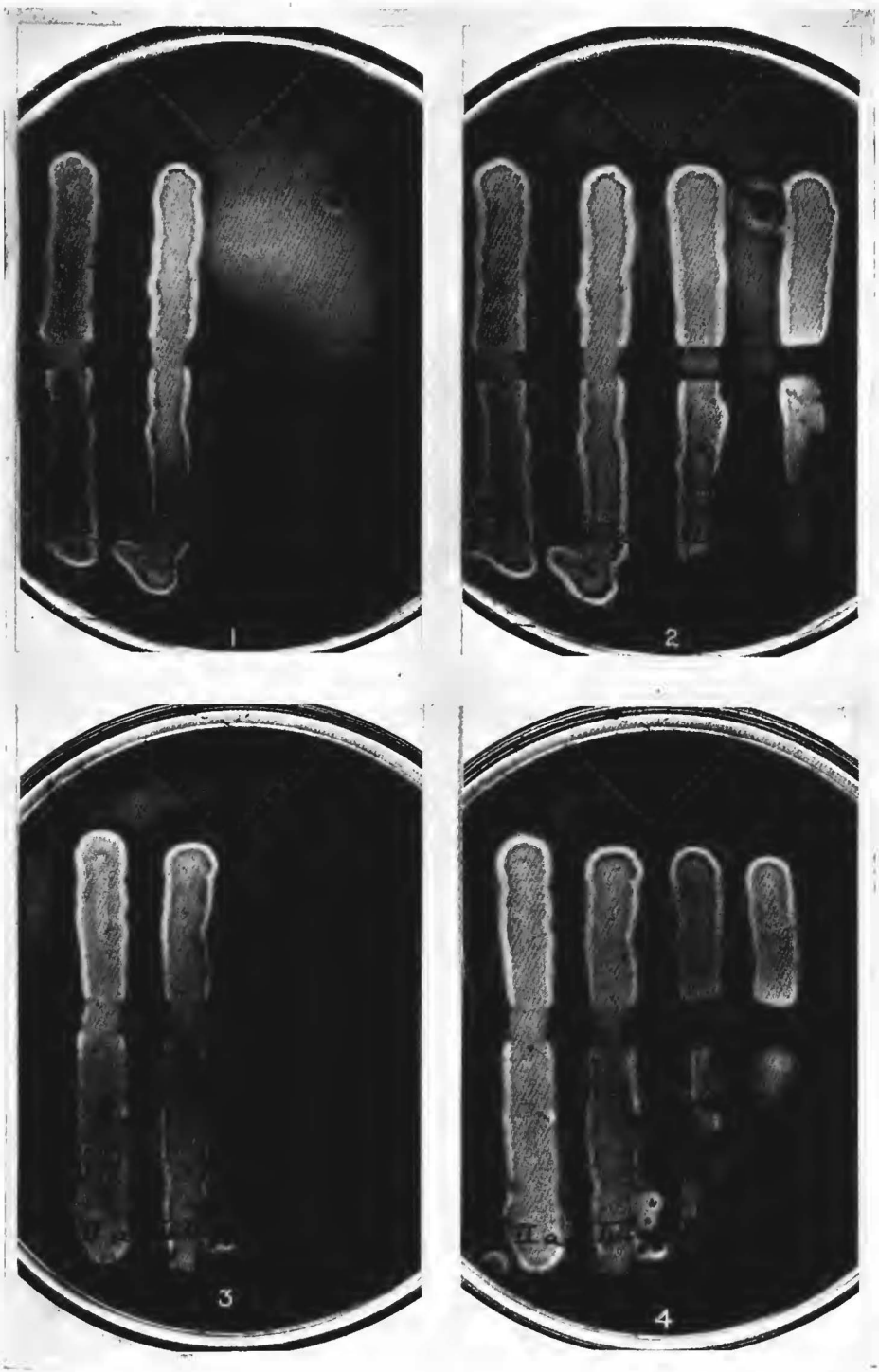
FIG. 1. (PAGE 73.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. STREAK I = streptococcus B-7α. STREAK IIa = streptococcus D-AD4β. STREAK IIIa = streptococcus A-STβ. Incubated 24 hours.

FIG. 2. (PAGE 73.) The same plate as shown in Fig. 1. Streaked as follows after the photograph shown in Fig. 1 had been taken. STREAK IIb = streptococcus D-AD4β. STREAK IIIb = streptococcus A-STβ. Incubated 24 hours longer.

Pneumococcus Cole II 45.

FIG. 3. (PAGE 74.) Horse blood, ⅔ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. STREAK I = pneumococcus Cole II 45. STREAK IIa = streptococcus D-AD4β. STREAK IIIa = streptococcus A-STβ. Incubated 24 hours.

FIG. 4. (PAGE 74.) The same plate as shown in Fig. 3. Streaked as follows after the photograph shown in Fig. 3 had been taken. STREAK IIb = streptococcus D-AD4β. STREAK IIIb = streptococcus A-STβ. Incubated 24 hours longer.



(Brown: Blood agar for study of streptococci.)

PLATE 28.

(PAGES 61 TO 63.)

The medium in all plates is standard beef Digestive Ferments Co. peptone agar, 12 cc.; streaked as follows: STREAK 1 = pneumococcus Cole II 109. STREAK 2 = streptococcus A-BLα. STREAK 3 = streptococcus B-2b (cow)β. STREAK 4 = streptococcus D-AD4β. STREAK 5 = streptococcus A-18 (cow)β.

FIG. 1. Metabolized 48 hours by pneumococcus Cole II 109, then streaked and incubated 48 hours.

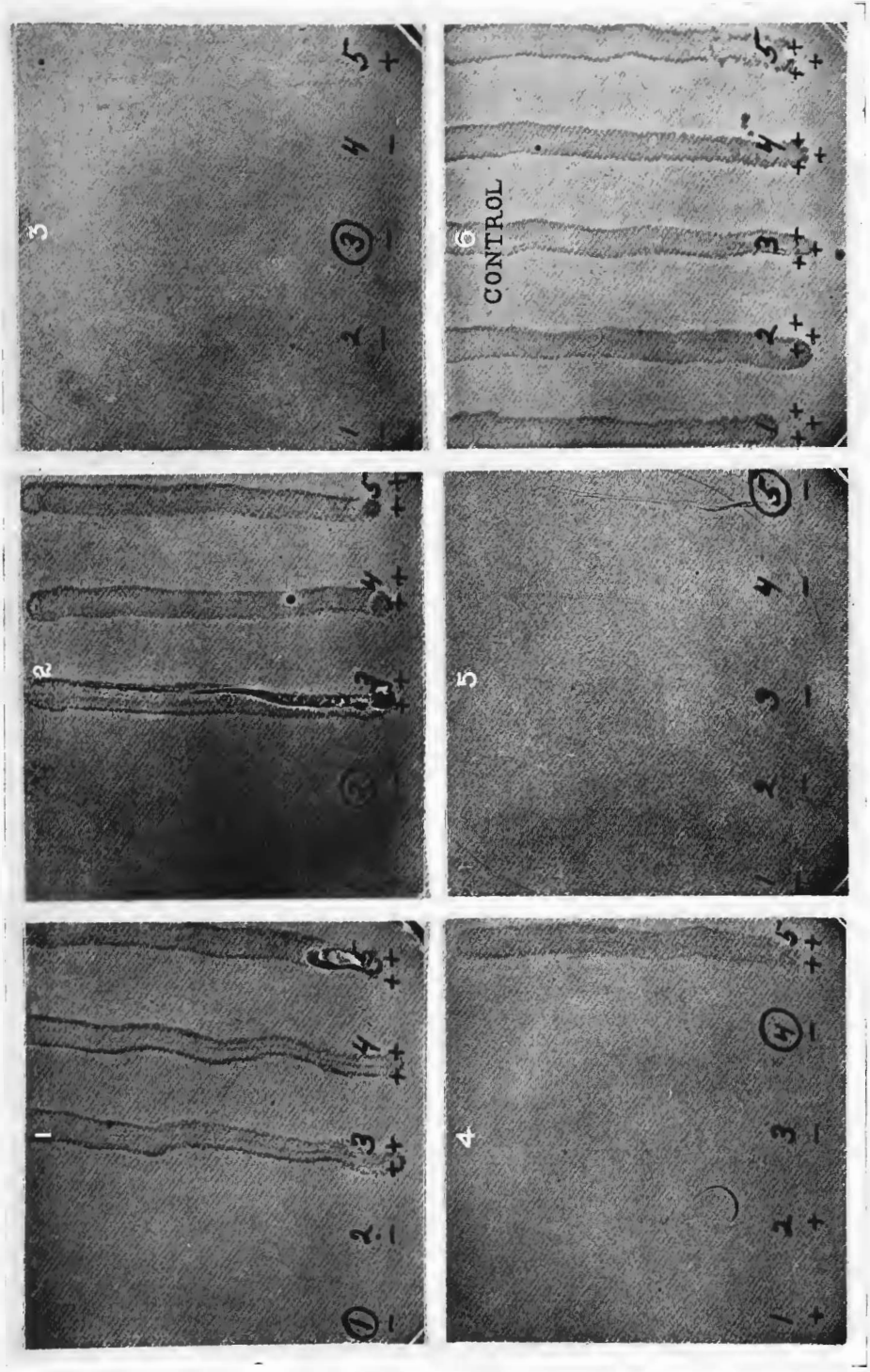
FIG. 2. Metabolized 48 hours by streptococcus A-BLα, then streaked and incubated 48 hours.

FIG. 3. Metabolized 48 hours by streptococcus B-2b (cow)β, then streaked and incubated 48 hours.

FIG. 4. Metabolized 48 hours by streptococcus D-AD4β, then streaked and incubated 48 hours.

FIG. 5. Metabolized 48 hours by streptococcus A-18 (cow)β, then streaked and incubated 48 hours

FIG. 6. Control plate. Incubated 48 hours sterile, then streaked and incubated 48 hours.



(Brown: Blood agar for study of streptococci.)

PLATE 29.

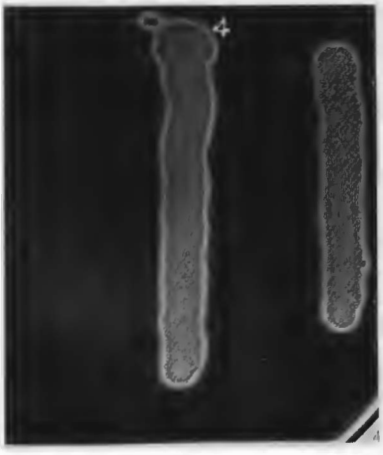
FIG. 1. (PAGE 63.) Horse blood, $\frac{1}{2}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated sterile for 48 hours as control for plates shown in Figs. 2 and 3, then streaked and reincubated 48 hours. STREAK I = streptococcus A-18 (cow). STREAK II = streptococcus B-2b (cow)β. STREAK III = streptococcus D-AD4β. STREAK IV = streptococcus A-STβ.

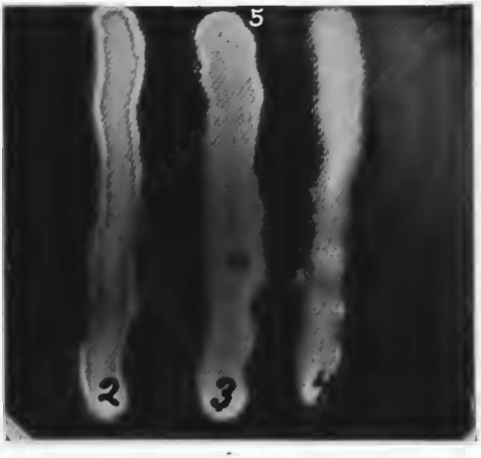
FIG. 2. (PAGES 63, 73.) Horse blood, $\frac{1}{2}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Metabolized 48 hours by streptococcus B-7α, then streaked and reincubated 48 hours. STREAKS = the same as on the plate shown in Fig. 1.

FIG. 3. (PAGES 63, 73.) Horse blood, $\frac{1}{2}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. Metabolized 48 hours by pneumococcus Cole II 45, then streaked and reincubated 48 hours. STREAKS = the same as on the plate shown in Fig. 1.

FIG. 4. (PAGE 64.) Horse blood, $\frac{2}{3}$ cc.; standard veal Witte peptone agar, 12 cc. streaked as follows: STREAK II = streptococcus D-1γ. STREAK III = streptococci D-1γ and D-AD4β mixed. STREAK IV = streptococcus D-AD4β. Incubated 24 hours.

FIG. 5. (PAGE 64.) Horse blood, $\frac{2}{3}$ cc.; standard beef Digestive Ferments Co. peptone agar, 12 cc. streaked as follows: STREAK 1 = streptococcus A-BLα. STREAK 2 = streptococci A-BLα and D-AD4β mixed. STREAK 3 = streptococcus D-AD4β. STREAK 4 = streptococcus D-AD4β and pneumococcus Cole II 45 mixed. STREAK 5 = pneumococcus Cole II 45. Incubated 24 hours.





(Brown: Blood agar for study of streptococci.

PLATE 30.

FIG. 1. (PAGE 66.) Standard beef Digestive Ferments Co. peptone agar, 12 cc. Sterile.

FIG. 2. (PAGE 66.) Horse blood, ½ cc., laked by 2 volumes of distilled water; standard beef Digestive Ferments Co. peptone agar, 12 cc. Heavily inoculated with *Streptococcus pyogenes* G-33β. Incubated 24 hours.

FIG. 3. (PAGE 66.) A sterile plate similar to that shown in Fig. 2. Incubated 24 hours.

FIG. 4. (PAGE 66.) Streptococcus B-2b (cow)β. Horse blood, ⅔ cc., laked by 2 volumes of distilled water and centrifuged 30 minutes at 3,000 revolutions per minute; standard beef Digestive Ferments Co. peptone agar, 12 cc. Incubated 24 hours.

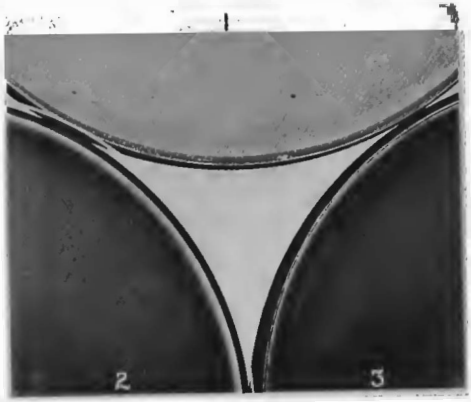
FIG. 5. (PAGE 66.) Streptococcus D-AD4β. The same medium as used in the plate shown in Fig. 4. Incubated 24 hours.

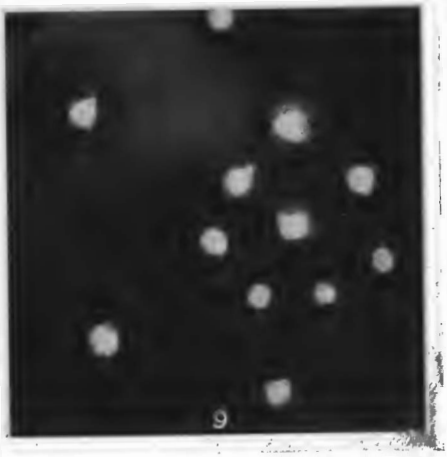
FIG. 6. (PAGE 68.) Streptococcus D-AD4β. Medium similar to that used in the plate shown in Fig. 5 except that the corpuscle shadows were incompletely sedimented by centrifugation. Incubated 24 hours.

FIG. 7. (PAGE 66.) Streptococcus A-STβ. The same medium as used in the plate shown in Fig. 4. Incubated 24 hours.

FIG. 8. (PAGE 69.) Horse blood, ⅔ cc.; standard veal Witte peptone agar, 12 cc. Holes sucked in the medium by means of a glass tube and then filled with sterile laked blood agar. Photographed before incubation.

FIG. 9. (PAGE 69.) The same plate as shown in Fig. 8. Photographed after incubation for 24 hours.





(Brown: Blood agar for study of streptococci.)

PLATE 31.

(PAGE 84.)

Medium used throughout = horse blood, $\frac{3}{4}$ cc.; standard veal Witte peptone agar, 12 cc.

Photomicrographs of typical deep colonies, taken after incubation for 48 hours. (Figs. 1, 3, 5, 7, 9, 11, and 13) and after refrigeration for 48 hours longer (Figs. 2, 4, 6, 8, 10, 12, and 14).

FIG. 1. Streptococcus D-AD4β.

FIG. 2. The same colony as shown in Fig. 1.

FIG. 3. Streptococcus F-2α'.

FIG. 4. The same colony as shown in Fig. 3.

FIG. 5. Streptococcus B-6α'.

FIG. 6. The same colony as shown in Fig. 5.

FIG. 7. Streptococcus A-BLα.

FIG. 8. The same colony as shown in Fig. 7.

FIG. 9. Streptococcus B-7α.

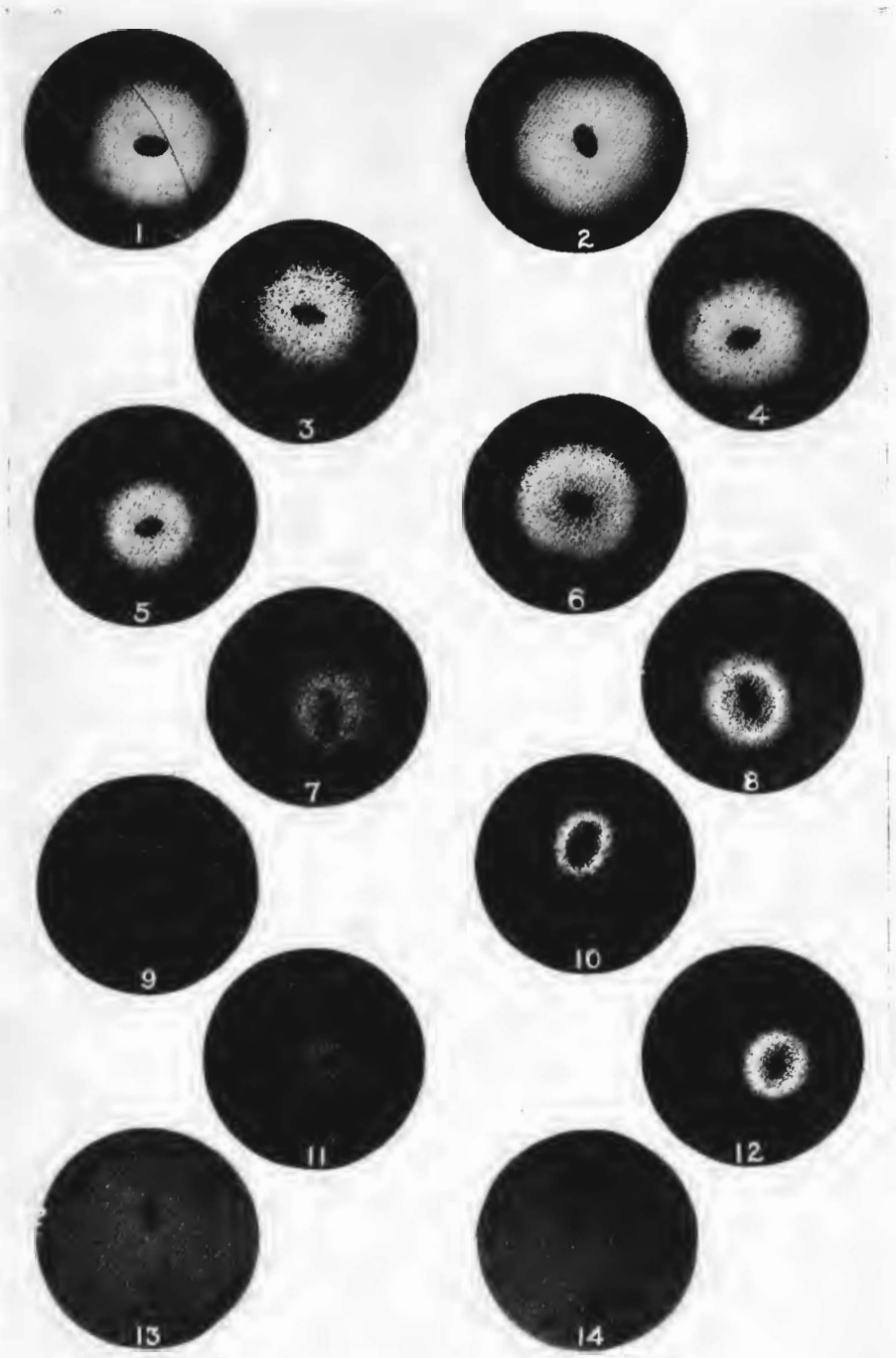
FIG. 10. The same colony as shown in Fig. 9.

FIG. 11. Streptococcus D-4α.

FIG. 12. The same colony as shown in Fig. 11.

FIG. 13. Streptococcus D-1γ.

FIG. 14. The same colony as shown in Fig. 13.



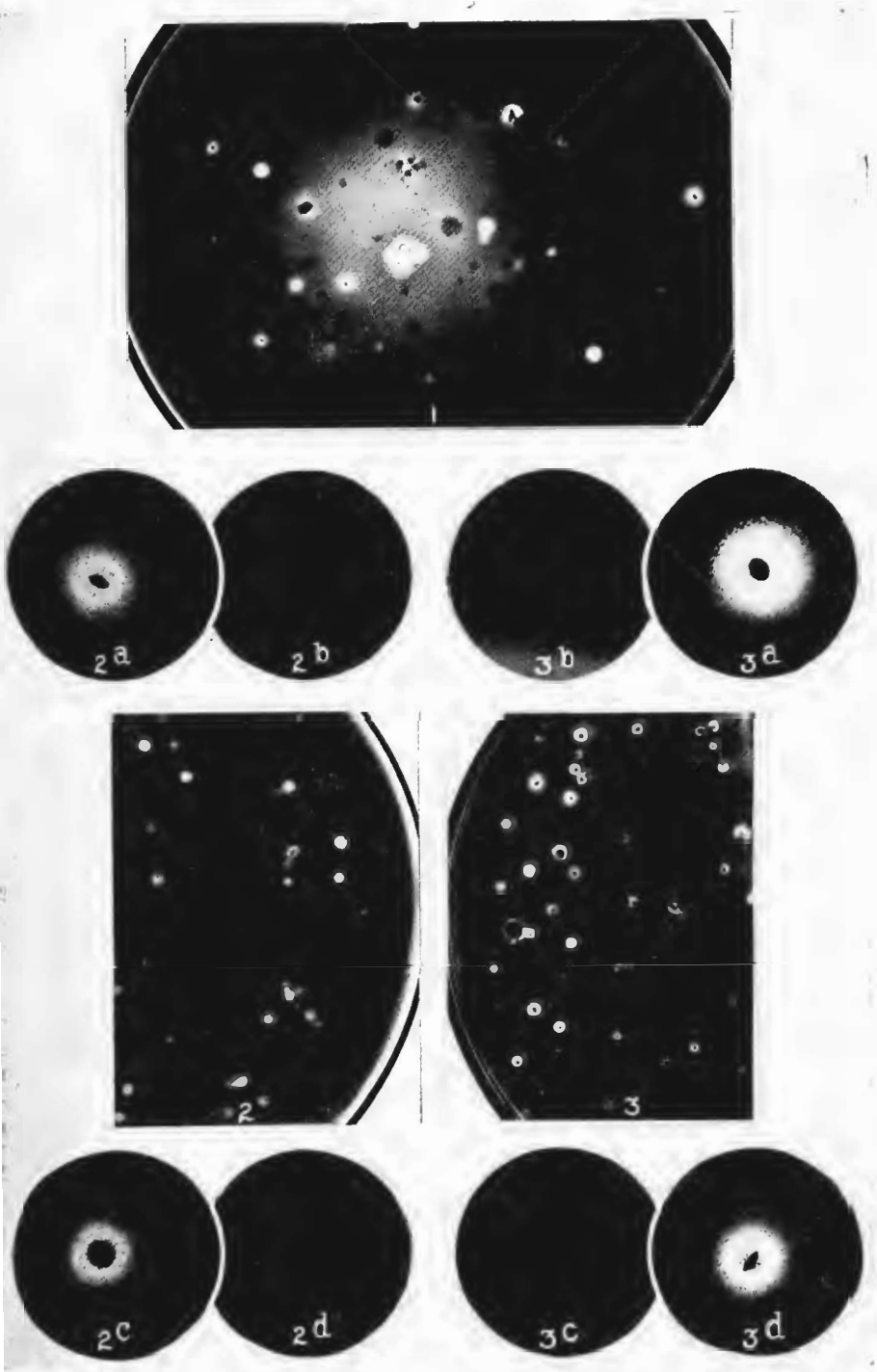
(Brown: Blood agar for study of streptococci.)

PLATE 32.

(PAGE 83.)

FIGS. 1, 2, and 3. Horse blood, $\frac{2}{3}$ cc.; standard veal Digestive Ferments Co. peptone agar, 12 cc. Incubated 24 hours. (Three characteristic plates from convalescent or chronic sore throats.)

FIGS. 2a, 2b, 2c, 2d, 3a, 3b, 3c, and 3d. Photomicrographs of deep colonies indicated by arrows in Figs. 2 and 3.

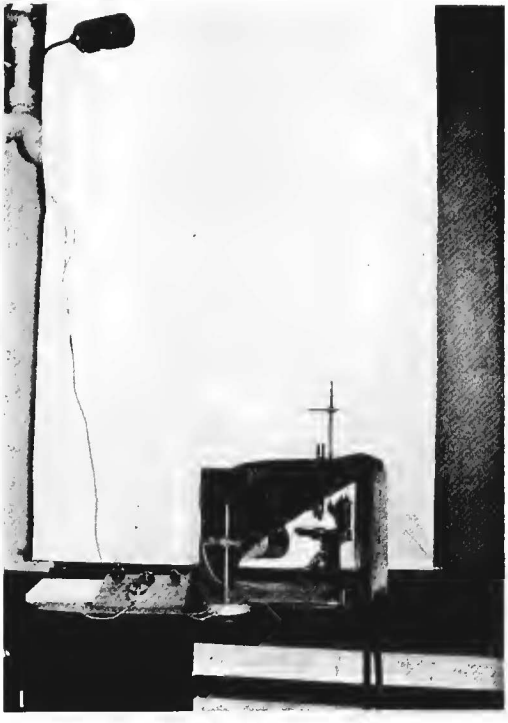


(Brown: Blood agar for study of streptococci.)

PLATE 33.

(PAGE 103.)

The apparatus used for photographing the agar plates and colonies shown in the preceding plates.

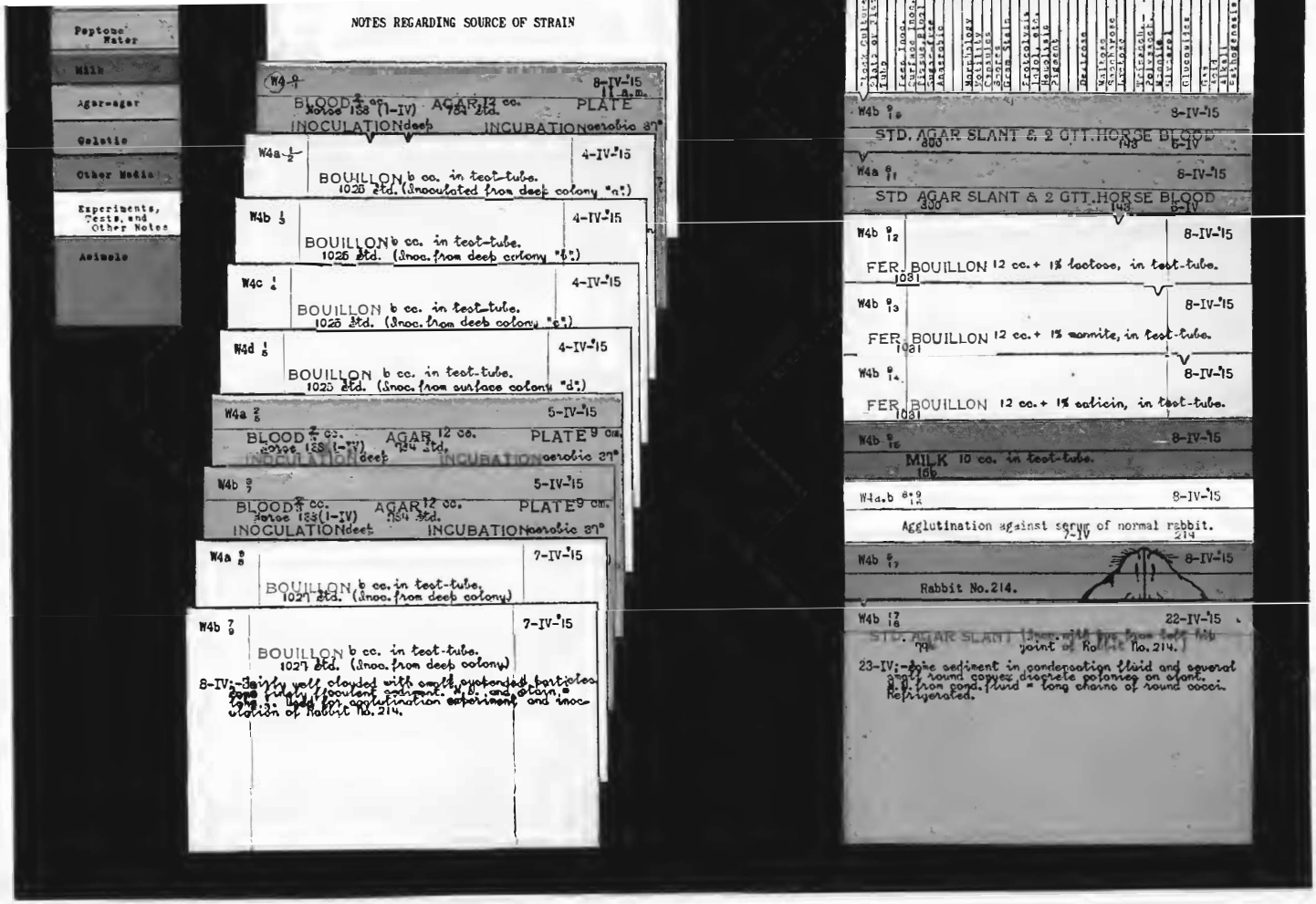


(Brown: Blood agar for study of streptococci.)

PLATE 34.

(PAGE 98.)

A system of notes for preserving the genealogy and other details in the study of bacterial cultures.



(Brown: Blood agar for study of streptococci.)

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