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TYPHOID CARRIERS AND TYPHOID IMMUNITY.*

OMNIS TYPHUS EX TYPHO.

BY ABRAHAM L. GARBAT, M.D.

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I. INTRODUCTION AND GENERAL CONSIDERATIONS.

Importance of the Carrier Problem in Civil Life and in the Army.

In spite of our increased knowledge of typhoid prophylaxis, typhoid fever is still a disease to be reckoned with. *In the army* the generalized use of antityphoid inoculations has undoubtedly prevented the occurrence of large and serious epidemics; but isolated instances and small outbreaks (1) are still frequent. While official reports with the accurate numbers of cases of typhoid infection among the 2,000,000 men in the American Expeditionary Forces are not as yet available, the total will be below 2,000 cases. It is important for patients to realize that antityphoid inoculation *per se* is not an absolute safeguard against typhoid infection and that the observation of the other prophylactic measures is therefore necessary. Failures of vaccination to protect against the disease are in some instances explained by "mass infection;" *i.e.*, the patients imbibe so large a number of bacteria at one time that the antibodies obtained by previous prophylactic injections are not sufficient to overcome the bacilli. In other patients, the disease may arise because the prophylactic inoculations do not stimulate the production of immune bodies. This failure of the tissue cells in certain individuals to react, is well known, and can usually be proven by the absence of agglutinating antibodies in the blood. A Widal test should therefore be made on every person receiving antityphoid inoculations. Whereas if antibodies have been stimulated it does not necessarily follow that a patient is sufficiently immune, it is highly significant of lack of immunity if the Widal test remains negative.

Other factors, such as impotent vaccine, improper technique of inoculation, or infection by a special strain of organism, may also account for failure of adequate protection.

In civil life, typhoid fever continues to merit even greater consideration.

According to Gay (2), this disease remains the ninth contributing cause to the mortality statistics in this country and ranks fifth among the infectious diseases, being exceeded as a cause of death only by

tuberculosis, pneumonia, infantile diarrhea, and diphtheria. Even as recently as in 1919, the death rate from typhoid in certain localities was as high as 58.4 per 100,000 of the population (3). Although the average mortality of typhoid is usually given as 8 to 10 per cent, this percentage is much higher in some cities than in others; in one town, out of 281 cases there were 49 deaths, and in another, out of 35 cases there were 7 deaths. About 150,000 typhoid cases still occur each year in the United States, and this causes an annual production of approximately 7,500 carriers. When one realizes that according to some statistics (4) as many as 44 per cent of all typhoid cases are due to carriers, the economic importance of the carrier problem is readily estimated. It is therefore the duty of every physician not to discharge a typhoid patient until he is certain that the patient no longer harbors any typhoid bacteria in the excretions. It is with this object in mind that the study of duodenal cultures was undertaken.

Origin of the Epidemic.

During the recent war, the internment camp for German civilian prisoners was situated at Hot Springs, N. C., on the west bank of the French Broad River, about 38 miles from Asheville. The internment camp was divided into two areas known as Camp A and Camp B. The water supply proved to be inadequate for both camps so that a separate supply was developed for Camp B by digging a number of shallow wells along the river bank. When these wells also proved inadequate, direct connection was made with the river water (French Broad) and this water was intended for flushing latrines. River water was usually applied once or twice a day. When river water was pumped, chlorination was applied. Instructions were in force not to drink the river water. Examinations of the original well water showed it to be perfect. The water from the French Broad River, however, is polluted and always carries a great deal of suspended matter. The first case of typhoid fever occurred at Camp B about July 20, 1918. On Aug. 5 there were about 50 cases with several suspects. On Aug. 12, 13, and 25 all cases of typhoid and typhoid suspects, a total of 183, were transferred to U. S. A. General Hospital No. 12, at Biltmore, N. C., where the writer was stationed. All these cases came from Camp B, apparently due to

the polluted French Broad River water. Of these 183 cases, there were 16 deaths; only 5 cases were definitely proved not to be typhoid fever.

The study of these cases[1] did not terminate as is usual with the cessation of the acute manifestations. While abundant reports are found in the literature referring to symptoms and laboratory findings during the active stage of typhoid fever, comparatively meager and inaccurate statistics exist pertaining to convalescent typhoid patients. Hardly any large epidemics have been studied in detail, as the work entailed in the care of very many, for example 200, patients under one roof at the same time, has in the past made detailed research studies almost impossible. Conclusions were therefore formerly drawn from collected series of cases. This must lead to somewhat erroneous statistics, as the findings are obtained by different workers, at different times, and with different techniques. It is therefore felt that the report to be given here merits consideration, since all the results were obtained by the same worker under the same circumstances. Through the cooperation of Colonel F. F. Russell the writer was given all the necessary laboratory assistance.

As there are several questions which will be constantly referred to throughout this monograph, it is wise to discuss them here, in order to avoid repetition in the various chapters.

Medium Used for Typhoid-Colon Differentiation.

The Endo medium was chosen for all the differential work, as we found it very accurate and very simple to make up. In its preparation, several details were considered absolutely essential for its proper action. Sterile stock 3 per cent agar (made in the usual way from chopped beef) was kept on hand in quarter liter and liter flasks. The required quantity for the day's work was titrated just before use and the reaction adjusted to 0.2 per cent acid to phenolphthalein. While hot, the agar was treated with chemically pure lactose, 10 gm. per liter. The powdered lactose itself was added, or this quantity was preserved in a solution of sterile water in individual tubes and thus used. Next 1.8 cc. of fuchsin solution per liter were added to the hot agar. A

[1] A clinical study will be published by the writer in conjunction with Dr. E. Henes.

filtered saturated solution of basic fuchsin in 95 per cent alcohol was employed. The agar became deep red. As the last step, the sodium sulfite solution was gradually added until the hot agar was entirely decolorized. It usually required about 25 cc. to the liter. A 10 per cent solution of pure dry sodium sulfite crystals in sterile water was made up fresh every day. Since various fuchsin solutions and also various preparations of sodium sulfite may differ, the absolute quantities given above may not exactly hit the proper balance in separate lots. These were approximate, however, and the proper balance can easily be obtained by a little preliminary testing in which the sodium sulfite solution is added to definite quantities of the fuchsin-colored agar in a test-tube. The finished product was poured into large sterile Petri dishes. The covers were left off until the agar was hard. While hot, a pale rose color was present in the medium which faded to a very faint pink or became almost colorless on cooling. Under no circumstances were the plates exposed to sunlight or daylight, as this colored the medium red. The Petri dishes were covered with a dark towel or kept in the ice box until used. If all these details are strictly observed, the author feels that Endo's medium will be found to give as reliable results as those obtained by the other differential media proposed. (Krystallviolett of von Drigalski and Conradi, malachite green of Loeffler, brilliant green of Krumwiede.)

Recognition of Typhoid Bacilli.—After incubation for 18 to 24 hours the typhoid colonies stood out on the plates as the smaller colorless colonies amidst the deeply fuchsin-stained colon colonies. The suspicious (typhoid) colonies were transplanted upon Russell's double sugar agar slants and further identified by specific serum agglutination tests. In several instances even then the result was doubtful, so that recourse was taken to injection of the doubtful organisms into rabbits and to examination of the obtained specific sera for agglutinins against known typhoid strains.

Division of Typhoid Illness into Two Stages.

For the elucidation of certain statistics, the author divided the *typhoid illness* into *two stages:* the *acute stage* and the *stage of convalescence.* The first day when the temperature reached normal and remained normal was taken as the dividing point between these two

periods. The writer has always considered this a much more logical plan than the usual method of dividing the typhoid illness into 4 weeks. Those who have seen a great deal of typhoid fever realize how almost impossible it is to tell during the course of the disease what week of the illness the patient is in at a particular time. It seems much more rational to wait until the fever has subsided and then use the first day of normal temperature as the landmark. If it is desired to know at what stage of the disease a certain phenomenon occurred, it can very readily be told by calculating backward from the date of normal temperature and be designated in terms of days or weeks either before normal temperature or after the patient went to bed with fever. Similarly in the study of typhoid convalescence, it is more practical to state that a certain test was positive or negative 10 or 15 days after "normal temperature" than to use the designation in terms of "day of disease," as "the 28th day of the disease." "Convalescence" is no longer "disease" nor "illness." The course of typhoid fever is such a variable one that simply stating the "28th day of the disease," for example, associates with it no fixed idea of symptoms. In one patient, this day may coincide with the height of the fever stage, while in another it may coincide with subnormal temperature.

The "duration of the disease" was estimated as the interval between the first and last days of fever. Usually, clinicians include in the "length of the illness" the time of incubation, but this period also is such a varying one that it seems wiser to go by the much more definite symptom of "fever" rather than the usual general guides of malaise or indisposition. The latter may be present a long time before the onset of typhoid fever and still not be dependent upon the infection.

II. DUODENAL CULTURES VERSUS FECES CULTURES AS A MEANS OF DETECTING CARRIERS.

History of the "Typhoid Carrier Problem."

The part played by human carriers in the spread of typhoid fever is a problem the solution of which was directly evolved from the views set forth by Robert Koch in his famous address of November, 1902, "Die Bekämpfung des Typhus" (5). Even at that time, he considered the typhoid patient or convalescent as the most fruitful source of further infection. This view was soon abundantly confirmed by Frosch in 1903 (6), who had assumed the directorship of an experimental typhoid station at Trier, also by von Drigalski (7) at Saarbrucken, and Dönitz (8) in Berlin. To Frosch belongs the credit for suggesting the hypothesis that the typhoid bacillus may lead a saprophytic existence in the intestinal tract, and it was von Drigalski who established on a bacteriological basis the hypothesis put forward by Frosch. Von Drigalski also recorded the first chronic carrier traced from convalescence onwards. In addition, he discovered the first female chronic carrier who gave no history of having passed through an attack of typhoid fever. With the recognition of the carrier state as a definite entity, various statistics appeared proving the validity of Koch's original dictum, and some authors have claimed that as high as 44 per cent of typhoid cases could be traced to typhoid carriers (4). It became of great importance to be able to detect the carrier by means of proper urine and stool examinations. For this purpose all types of special nutritive media were introduced, but, as will be seen presently, radical improvements upon these methods were necessary in order to obtain accurate results.

In an article published by the author in 1916 (9) entitled, "Duodenal Cultures in Typhoid Fever as a Means of Determining Complete Convalescence," 12 cases of typhoid fever were studied, in which comparative examinations made from the duodenal contents (bile) and the feces, demonstrated in 2 cases typhoid bacteria in the bile and not in the feces. In the present publication, the study of the bile as the source of typhoid bacteria in the stools of typhoid

convalescents was resumed on a much larger and more detailed scale and the solution of a number of problems dealing with typhoid carriers has been attempted. This paper deals with duodenal (bile) cultures made on 136 cases of typhoid fever.

Technique for Obtaining Bile.

The Einhorn duodenal tube was sterilized by boiling and given to the patient in the evening at 9 or 10, about 3 hours after the last meal. Various other times of the morning and afternoon were tried, but were given up, because the patient would thus miss a meal, and this often met with great objection. Only in rare instances was there any difficulty in having the patient swallow the tube because of gagging. Usually the tube would go down easily and by the following morning it had passed into the duodenum and bile could be aspirated with a sterile 10 or 20 cc. Luer syringe. It was necessary for the piston of the syringe to be a well fitted one, because at times suction had to be quite strong. The bile was collected in four or five separate sterile test-tubes; the first specimen usually contained mucus or other secretions that had collected in the tube during the night. This technique was found to be perfectly satisfactory and is somewhat simpler than the one described by the author in 1916. In about 5 per cent of the cases, the tube remained in the stomach. This was probably due to gastric atony and the tests had to be repeated.

When there was difficulty in obtaining sufficient bile, it was found that if the patient sat up in bed and bent his head forward between his knees, at the same time pressing upward on his abdomen with the palms of his hands, a flow of bile would set in. Or, if the patient was given a cold drink, the bile would appear, probably due to the reflex relaxation of the sphincter around the opening of the common bile duct. Occasionally, part of the fluid which the patient had drunk would be expelled through the pylorus into the duodenum so rapidly that on aspiration bile mixed with the fluid swallowed would be obtained. Thus, it is wise to give the patient sterile water to drink when this means of stimulation is employed. If secretion is obtained and one is doubtful whether it is bile or not, one can, at the bedside, give the patient some colored liquid to drink, grapejuice for example, and immediately thereafter aspirate. If the tube is in the stomach, the undiluted red-stained fluid is obtained on aspiration. In rare instances, if none of these methods yielded any bile, the author would inject 5 or 10 cc. of sterile fluid through the tube and immediately thereafter aspirate again. This fluid would return bile-stained from its contact with the duodenal wall. In the vast majority of instances, none of these extra manipulations were necessary, and sufficient bile was obtained without any trouble if only patience was practiced.

Characteristics of Bile Specimens.

(*a*) *Quantity.*—It was surprising to note the large quantities of bile that could be aspirated at the first examination. In only 16 cases was less than 10 cc. obtained. The small quantity does not, however, interfere with the finding of the typhoid bacilli, because a positive duodenal culture is recorded in one patient from whom only 2 cc. of bile and in another from whom only 6 cc. had been collected. In Table I the cases are tabulated according to the various quantities obtained.

TABLE I.

No. of cases.	Per cent of cases.	Quantity of bile obtained.
		cc.
16	14	Less than 10.
43	39	10–20
19	17	21–30
21	19	31–40
7	6	41–50
2	1	51–60
1	1	62
1	1	80
26*		

* Not estimated.

(*b*) The *consistency* varied from thin, almost watery secretions, to thick mucous fluids which came through the tube with difficulty. The former were usually absolutely clear, while the latter were turbid or entirely opaque with a varying amount of a dirty granular sediment.

(*c*) A variety of *colors* was noted; very light yellow, canary yellow, golden yellow, light green, dark green, light brown, dark brown, etc.

(*d*) On *microscopical examination*, the clear fluids showed only a moderate number of bacteria, or none at all. The clear fluids were usually sterile. The turbid fluids contained a great deal of mucus mixed with a moderate or great number of leucocytes and numerous bacteria, usually bacilli. Frequently myelin drops were noted. A Gram stain showed mostly Gram-negative bacilli mixed with some Gram-positive cocci.

No absolutely characteristic picture can be ascribed to those bile specimens which contained typhoid bacteria. While almost all of these fluids were thick, very turbid, and contained a good deal of mucus, the same findings were also present in those specimens which on culture showed colon bacilli. As a rule, however, the latter contained very many more leucocytes. In only one instance out of this entire series were actively motile typhoid bacilli (as proven later by culture) noted in hanging drops of the fresh bile specimens.

Technique for Bacteriologic Examination.

Endo's medium was selected for typhoid-colon differentiation as it was found least complicated and very satisfactory. The details for its preparation are given under the general discussion.

(*a*) The *bile* collected in each test-tube was examined separately. A broth culture and an Endo plate from each were prepared, thus making 4 broth tubes and 4 Endo plates from each patient. About 1to 2 cc. of bile were added to the broth tube, and about 0.25 to 0.5 cc. was spread over a large Endo plate. No special dilutions were found more advantageous than others. The broth tube was plated after 24 hours incubation. At the Walter Reed Hospital where duodenal cultures were undertaken, the original bile was first incubated for 24 hours and then plated.

(*b*) The *stool* examination was made by the usual technique; namely, a piece of feces, about the size of a split pea, was rubbed up in about 10 cc. of broth, allowed to stand for about 15 minutes until the heavy parts had settled, and then a large loopful from the upper layer of the fluid was spread carefully over the entire surface of a hardened plate of Endo's medium. In the routine stool examination, two such plates were made from each specimen of feces.

The problems which this study attempted to solve are so varied that possibly the best way of expressing the results is by stating the particular question under investigation followed by the answer as interpreted from the findings.

Is the Repeated Examination of the Feces a Fair Index as to the Absence or Presence of Typhoid Bacilli in the Intestines?

Almost all clinicians and bacteriologists agree that when a stool is reported as "no typhoid bacilli found," this statement in many instances may mean nothing more than that the laboratory methods were such that the typhoid bacilli were not detected. As evidence that bacteriologic examination of the stool for typhoid bacteria is attended with uncertain results, one has but to note the different methods and the new media constantly devised for such examination, in the hope of improving the results.

In our series of cases, we adhered strictly to the army rule of not considering a patient free of typhoid fever until 3 consecutive stools and urines examined at intervals of 6 days proved to be negative. The first specimens were sent to the laboratory when the patient's temperature was nearing normal, or had become normal.

In 136 patients, bile cultures were made *after* 3 consecutive stool examinations had been found negative; *i.e.*, apparently free of typhoid

bacteria. Out of these 136, 20, or 15 per cent, still showed typhoid in the bile. Of these 20, 15 had never shown typhoid colonies in the feces, while in 5, typhoid bacteria had been demonstrated in the stool cultures at some time previously but had apparently cleared up so far as could be judged by the 3 consecutive negative feces cultures. Even more striking is the fact that 15 of these 20 patients, or 11 per cent of the cases examined, had not only 3 negative stools, but also 3 negative urines to their credit when the positive growth in the bile was obtained. In other words, 11 per cent of the typhoid patients who are usually considered ready to leave the hospital and mingle with the community at large, may still be discharging typhoid bacilli undetected by the general method of stool examination.

TABLE II.

Name.	Duration of original infection.	1st day of constant normal temperature.	Date of 3rd negative urine and stool cultures.	Date of positive duodenal culture.	Date of onset of relapse.	Date of positive blood culture during relapse.	Date of normal temperature after relapse.	Date of negative duodenal culture after relapse.
	days							
Poelman.....	49	Sept. 21	Oct. 14	Oct. 14	Oct. 16	Oct. 22	Nov. 5	Nov. 8
Bergenthal.....	44	" 23	Sept. 30	" 3	" 5	" 8	Oct. 31	Jan. 6

The remaining 4 per cent of the cases (5 patients) with positive bile cultures and negative feces cultures had other evidences of the persistence of typhoid besides the positive duodenal culture. Two still showed typhoid bacteria in the urine. Another had recurrent rises in temperature with tenderness over the gall bladder but a negative blood culture, probably recurrent attacks of cholecystitis. The other two developed definite relapses with positive blood cultures, after normal temperature for 25 days and 12 days respectively. In spite of 3 negative urines and stools these 2 patients began to run temperatures several days following the time of the positive bile culture (Table II).

It is interesting to consider the hypothesis that some typhoid relapses may be due to the persistence of bacteria in the gall bladder as the source of a vicious circle. There is, first, the primary bacteremia; next, the settling of the bacteria in the gall bladder; then a reabsorption of the bacteria either from the gall bladder or from the intestines,

giving a renewed bacteremia and relapse in those patients in whom a sufficient degree of immunity has not taken place from the original infection. Thus a positive duodenal culture during the early convalescence may be compared to a strongly positive Wassermann test in a lues asymptomatica—a danger sign of possible trouble. All relapses cannot, however, be explained thus, because there were instances where relapses occurred after a negative bile culture. Further investigation along this line is necessary.

The bile cultures were not always made on the same day as the third negative feces. The different periods of time which elapsed between the last negative feces and the positive duodenal culture are shown in Table III.

TABLE III.

No. of cases.	Interval between last negative feces and positive bile cultures.	No. of cases.	Interval between last negative feces and positive bile cultures.
	days		*days*
5	0	3	7
1	1	1	9
2	2	1	11
5	3	1	12
1	6		

From this table it has been estimated that had bile cultures not been made and were the patients not required to observe the usual typhoid precautions after they had 3 negative urines and 3 negative stools, 15 patients would have been excreting typhoid bacteria in their feces over a total period of 68 days. These figures are comparatively mild when one considers the length of time that these bile cultures continued positive after the third negative stool.

The Army rule of keeping a patient at the hospital until 3 consecutive negative urines and stools at intervals of 6 days are reported, is a comparatively strict precaution. Many health boards and hospitals in this country do not adhere to such a wise requirement. A few years ago, systematic inquiries disclosed that out of 23 leading hospitals in this country, only 9 required an examination of both the urine and stools of their typhoid patients before discharging them from the hospital. One institution examined these excretions only when the patient's occupation brought him into contact with foods. Another exam-

ined the urine and not the feces; one even discharged patients with bacteria in the urine if these persistently remained there. 11 institutions disregarded such examinations altogether; 5 of these definitely stated that the technical phase associated with the inaccuracies of the findings in stool examinations did not warrant the time spent. The author has often felt the same way about the results of stool examinations and it is that sense of inaccuracy which led to the study of bile cultures. It is very discouraging to note that the 1920 New York City sanitary code regulations require that, in typhoid fever, quarantine should be continued until 10 days after the patient's temperature reaches normal and further until 2 specimens of feces collected at an interval of at least 24 hours are found to be free of typhoid bacilli (10). Let us hope that such a dangerous state of affairs, as evidenced by the cited statistics, will be remedied.

Is It Possible that Typhoid Bacilli Are Destroyed on Their Way Down from the Gall Bladder, Thus Actually Giving a Negative Stool and a Positive Bile Culture?

This question was answered in 9 cases. In 2, after an interval of 6 days, the fourth specimen of feces came to the laboratory as part of the regular routine. Typhoid colonies were found. No special dilutions of the feces nor an increased number of Endo plates were necessary for their discovery. In 4 other patients with positive duodenal cultures, subsequent stools were especially reexamined, and, when 10 to 15 Endo plates from various dilutions of the feces were made, typhoid bacilli were recovered.

Furthermore, it was found that in some of these patients with positive duodenal cultures and negative stools, the longer the typhoid bacteria persisted in the bile, the more likely was one to detect the typhoid colonies in the stools. Thus in 3 patients in whom the typhoid bacilli persisted in the bile for over 3 months after normal temperature, isolated typhoid colonies could be detected in the stool plates with little trouble at the end of this time; whereas, in the early days of convalescence, typhoid colonies were either not detected at all in the regular routine stool examinations or only after repeated and numerous cultures. At the same time, we meet with the difficulties of stool examinations even in chronic carriers. In one instance

of a permanent typhoid carrier, a heavy growth of pure typhoid bacteria was isolated from the duodenum on two successive occasions. During the same time it required 60 Endo plates (10 made on each of 6 successive days) to demonstrate the typhoid bacilli in the feces. The first 4 days (40 Endo plates) revealed no typhoid bacteria. On the 5th day 4 out of the 10 plates showed 3 or 4 typhoid colonies per plate. The 6th day revealed no typhoid bacteria. While it is shown that there is a great discrepancy between the findings of duodenal cultures and feces cultures, the typhoid bacilli are not destroyed in the intestines but they are either absent in the particular drop or drops of feces that are examined or through the use of the usual technique they are overgrown by the colon bacillus. In the bile, the typhoid bacilli are more concentrated. Most authorities have been of this opinion, although definite proof *in vivo* was evidenced only by this method of duodenal cultures. At post-mortem, von Drigalski (11) and Jürgens (12) showed by cultural methods that in the intestinal tract from the duodenum down to the rectum, the number of typhoid bacilli decrease. In the duodenum and the upper portion of the jejunum one frequently meets with enormous numbers of typhoid bacilli in nearly or actually pure culture.

On What Day of the Disease May One Expect the Duodenal Cultures to Become Negative?

As was said in the general discussion, it is wise to designate the first day of normal temperature as the dividing point between acute disease and convalescence, so that it is more accurate to put the question: How soon after normal temperature may one expect the bile cultures to be *negative?* In order to have deduced any such fixed periods of time, it would have been necessary to do repeated cultures from the bile on the same patients at different intervals, beginning before the temperatures had reached normal. Since the main object of the present study was a determination of the comparative value of duodenal (bile) and stool cultures, the above plan was not adopted. The duodenal cultures were done at various intervals after the onset of normal temperature and it can be said that no definite conclusion was reached as to when the bile becomes free of typhoid bacteria. Table IV gives the number of cases and the

time at which the duodenal examinations proved negative for typhoid bacilli.

About 40 per cent of typhoid cases had negative duodenal cultures within the first month after the temperature reached normal. Had it been possible to take bile cultures on the other patients at a date previous to the times stated above, this percentage would undoubtedly have been higher. In some cases, duodenal examinations were made even before the temperatures had become normal, and in others after only 1 or 2 negative urines and stools; and at these times, the bile was already free of typhoid bacteria in some instances. It can, therefore, be stated that the period of time after normal temperature when the bile becomes free of bacteria is a varied one. Patients may be dis-

TABLE IV.

No. of cases.	Per cent of cases.	Interval after normal temperature when duodenal culture was negative.
		days
7	6	10
13	11	11–20
34	22	21–30
42	37	31–40
17	13	41–50
2		51–60
1		61–70

charged before 3 stools have been examined and found negative as long as the duodenal cultures show no typhoid bacilli. Such direct examination is much more accurate than stool examinations.

It is much more important to analyze the time relationship of the *positive* duodenal cultures. Only 20 of our cases (15 per cent) showed positive bile cultures during convalescence, but it must be remembered that bile cultures were only started after 3 stools from a given patient had already been examined at intervals of 6 days and reported as negative. Had duodenal cultures been instituted at an earlier date, it is more than probable that a greater number of positive cultures would have been obtained. The presence of typhoid bacteria in the bile during the *acute stage* of typhoid fever is a different problem. Table V shows the number of days between the onset of normal

temperature and the *first positive bile culture.* Here we see that in 10 cases (7.5 per cent) the duodenal cultures were positive later than 1 month after the beginning of normal temperature.

TABLE V.

No. of cases.	Interval after normal temperature when duodenal culture was positive.	No. of cases.	Interval after normal temperature when duodenal culture was positive.
	days		*days*
4	1–10	8	31–40
3	11–20	1	51–60
3	21–30	1	61–70

How Long Did These Patients Continue to Harbor Typhoid Bacteria in the Bile?

4 cases, or 3 per cent of the typhoid patients, showed typhoid bacilli in the bile 4 months after the fever had ceased (Table VI). It is interesting to refer to the findings in the stool cultures of these carriers at the same date (Table VII). It is noted that even in pronounced carriers duodenal cultures are much more reliable than stool cultures. Naturally, once it is ascertained or even suspected that a particular convalescent typhoid patient is excreting typhoid bacilli in the feces, continued patience with the stool examinations expressed by large numbers of cultures may

TABLE VI.

Name.	1st duodenal culture.		2nd duodenal culture.		3rd duodenal culture.		4th duodenal culture.	
	Interval after normal temperature.	Result.	Interval after normal temperature.	Result.	Interval after normal temperature.	Result.	Interval after normal temperature.	Result.
	days		*days*		*days*		*days*	
Ericksen	29	+	63	+	97	+	128	+
Schaefer	11	+	39	+	69	+	106	+
Hoffman	30	+	50	+	81	+	122	+
Mueller, E.	14	+	55	+	82	—	111	—
Ziska	9	+	42	+	72	—	109	—
Gutte	62	+	74	+	90	—	127	—
Lehman	8	+	108	+	111	+	143	+

demonstrate the bacteria. However, one has no guide as to those who are more likely to become typhoid carriers. And since it is impracticable to undertake a long special feces investigation on every patient, particularly when one is dealing with a typhoid epidemic, main reliance should be placed upon bile cultures; for, as has been seen, the usual routine stool examinations during convalescence missed 15 per cent of carriers, and of these, 3 per cent were carriers for over 4 months, possibly permanent carriers.

TABLE VII.

		Ericksen.	Schaefer.	Hoffman.	Lehman.
Feces.	No. of Endo plates made from feces.	8	8	6	60
	No. of plates showing typhoid colonies.	8	0	4	4
	Proportion of typhoid to colon colonies on these plates.	1 to 75 or 100	0	1 to 50 or 75	1 to 50 or 75
Bile.	No. of typhoid colonies on Endo plates from bile of same date.	340 to 450 colonies in pure culture on each plate.	5 to 10 colonies in pure culture on each plate.	350 to 500 colonies in pure culture on each plate.	40 to 50 colonies on each plate.

How Numerous May the Typhoid Bacteria Be in the Bile?

In general, if the typhoid bacteria are present in the bile in great numbers, many typhoid colonies are seen on the original Endo plate made directly from several drops of the bile. On the other hand, if the number is not so great, the growth is obtained principally in the broth and only very few colonies or none at all are noted on the original Endo plate. Thus, 2 patients who showed a heavy growth on the original Endo plate at the first culture showed a growth only in the broth and not on the Endo plate at the reexamination of the bile 1 month later. Further examination after several weeks' interval, gave no typhoid bacilli even in the broth culture. In 3

other patients the growth was just as heavy at the second and third examinations as at the primary culture. It was difficult to get accurate figures concerning the actual number of colonies in the bile because pour plates from Endo's medium do not distinctly differentiate typhoid from other types of organisms. Approximate estimations were made by spreading 0.5 cc. of the bile very carefully over the entire surface of an Endo plate. In many instances the colonies were in such great numbers that they were confluent and difficult to count. As many as 32,000 colonies per cc. have been estimated by this method and this in a man (Ziska) in whom the stool culture was negative. It must be remembered that this number of colonies would represent a far greater number of bacteria.

In the Cases with Positive Duodenal Cultures Are Typhoid Bacilli Constantly Present in the Bile or Is the Excretion an Intermittent One?

A positive solution to this question is important in order: (1) to establish whether there was any best time for culturing the bile; (2) to explain the apparent irregularity in the excretion of the typhoid bacilli in the feces; (3) to ascertain whether 1 negative duodenal culture was sufficient to exclude the existence of a carrier condition.

1. The question of *hourly intermittency* was studied. Two carriers (for a pecuniary consideration) retained the duodenal tube for 24 hours without taking food, and specimens of pure bile were examined every 2 hours. After each sample was collected, sterile distilled water was forced through the tube in order to prevent the bile from stagnating and mixing with the next specimen. It was found that typhoid bacilli were constantly present, as is noted in Table VIII.

2 and 3. When duodenal cultures were repeated in carriers at various intervals over a longer period of time, it was found that as a general rule cultures would either consistently continue positive, or if they once became negative they would remain so. This is very different from the feces findings in these carriers. Bacteria are often reported to be absent from the feces for months, only to recur again later on. It seems to be almost definitely established in the literature that the excretion of typhoid bacilli in the feces is intermittent.

TABLE VIII.

Name.	Bile obtained.				Typhoid present.
	Time.	Quantity.	Transparency.	Color.	
	a.m.	*cc.*			
Haak.....	9.00	4	Turbid.	Yellow-green.	+
	11.00	12	Clear.	" "	+
	p.m.				
	2.00	13	"	" "	++
	4.00	16	Slightly turbid.	" "	++
	6.30	21	Clear.	" "	++
	9.00	6	"	" "	++
	a.m.				
Ericksen.....	9.30	3	"	" "	+
	11.30	10	"	" "	+
	p.m.				
	2.00	9	Slightly turbid.	Orange.	+
	4.00	15	Clear.	Yellow-green.	+
	6.30	8	"	" "	+
	9.00	22	"	Orange-yellow.	+

Semple and Greig (13), who published a thorough bacteriological investigation of typhoid convalescents, show that carriers may be readily overlooked if the routine feces examination is limited to 3 or 4 cultures during the 6 weeks following defervescence. Klinger (14) published most interesting results in this respect.

Date.	Stool culture.	Date.	Stool culture.	Date.	Stool culture.	Date.	Stool culture.	Date.	Stool culture.
1904		*1904*		*1904*		*1904*		*1905*	
Jan. 30	−	May 3	−	Aug. 10	−	Oct. 11	+	Feb. 13	−
Feb. 10	+	" 10	−	" 16	−	" 29	−	" 18	+
" 11	+	" 19	+	Sept. 12	+	Nov. 2	+	Mar. 24	+
Mar. 26	−	June 8	+	Oct. 1	−	" 24	+	Apr. 26	+
Apr. 3	+	July 2	+	" 5	−	Dec. 22	+	May 12	+

+ indicates typhoid bacilli present; −, typhoid bacilli absent.

The writer is convinced that this intermittency is only apparent; were duodenal cultures made during those periods when the stool is apparently free from bacteria, typhoid bacilli would undoubtedly be

present in the bile. There are, however, exceptions to this uniformity of excretion in the bile. Occasionally a negative duodenal culture was interspersed among positive ones. This intermittency was rare and the negative findings would not persist for any length of time; often as early as the next day a positive culture would again be noted. No definite explanation can be offered for this variation. In two patients there was an associated cholelithiasis, so that possibly the stone obstructed the cystic duct and the bile at the time of culture was obtained from a non-infected liver. That such conditions exist was proved by operation and by postmortem examination, and they are reported by the writer further on. In another case, an occasional negative culture amongst numerous positive ones was noted after a cholecystectomy failed to cure the carrier state. In these liver infections, intermittency can only arise if the particular negative specimen happens to be drained from a part of the liver which was not infected. Taking into consideration such occasional irregularities, the author requires two consecutive negative bile cultures made at intervals of one or more days instead of a single negative bile culture, before a patient is safely considered free of typhoid bacilli. This precaution applies especially to three groups of cases: (1) those who once showed a positive typhoid culture in the bile; (2) those in whom a surgical operation (cholecystotomy or cholecystectomy) had been performed for the cure of the carrier condition; (3) those who during the acute illness or convalescence manifested symptoms of cholecystitis or cholelithiasis. As will be seen later on, it is these patients who usually become carriers.

Should Stool Examinations Be Entirely Discarded in Favor of Bile Cultures or Is There a Type of Pure Intestinal Typhoid Carrier Whose Bacteria Do Not Lodge in the Bile but in the Intestine and Are Therefore Detected Only by Stool Examinations?

The existence of a pure intestinal carrier has been a disputed question but a very important one, especially from the standpoint of surgical therapy; for excision of the gall bladder in these cases will not effect a cure. Of our entire series of 164 typhoid cases studied, there was only one patient who from the very beginning of his convales-

cence, 5 days after normal temperature, began and continued to show typhoid bacteria in the stools in almost pure culture. In spite of this, 4 duodenal cultures were made during the first 2 months after normal temperature, and in none of them were typhoid bacilli isolated. The only bacteria found were colon bacilli. There was nothing unusual in this patient's clinical history. He was 54 years old, and reported sick Aug. 2, 1918, with the usual symptoms of headache, lassitude, and fever. He had one typhoid inoculation on July 26, 1918. He ran a comparatively mild typhoid, with fever for approximately 3 weeks. There were no complications and he convalesced without interruption.

One cannot deny that types of pure intestinal carriers do exist, although they comprise only about 2 per cent (1 in 53) of all typhoid carriers, and only about 0.6 of 1 per cent of all typhoid patients. A detailed study of the different types of carriers is taken up in Section III, but it may be said here that an intestinal carrier is very readily differentiated from a bile carrier by stool examinations. In the former, there is no difficulty in detecting the typhoid bacteria. They begin to appear in the stools early during convalescence, and are found practically in every specimen in very great numbers and in almost pure culture. In the bile carriers, if typhoid colonies are found in the stool cultures at all, they appear there with great irregularity (in 1 out of 3 to 5 cultures) and in fewer numbers than in intestinal carriers. For example, on the Endo plate made from the stool of a bile carrier, the ratio between the typhoid and the colon colonies would be approximately 1 to 25 or 75, while in the intestinal carrier this proportion would be reversed. In the latter, it seems as if the flora of the intestine are changed from colon to typhoid. A more detailed discussion of this topic is undertaken in Section III on Typhoid Carriers.

What Other Types of Bacteria Were Found in the Duodenal Cultures, either Alone or in Association with the Typhoid Bacillus?

Out of 39 duodenal cultures which showed typhoid bacteria, 32 (82 per cent) were pure cultures, 4 (10 per cent) were mixed with colon bacilli, and 3 (8 per cent) with the *Staphylococcus albus*. The more persistent carriers had pure cultures. However, even in the

mixed cultures the typhoid colonies stood out prominently on the Endo plates and were isolated with no difficulty. Table IX shows other bacilli found in 132 cultures which did not contain the typhoid bacillus.

TABLE IX.

No. of cases.	Per cent of cases.	Bacteria found.
42	32	Colon bacillus.
13	10	*Staphylococcus albus.*
5	4	" *aureus.*
12	10	" and colon bacillus.
8	6	An intermediate " "
2	0.1	*Bacillus proteus.*
50	38	Sterile.

The items of interest in the above figures are, first, the high percentage of pure typhoid growths, and, secondly, the high percentage of sterile cultures. The last was especially surprising in view of many obstacles which were encountered and which interfered with the usual rules observed in taking sterile cultures.

III. TYPHOID FECES CARRIERS, WITH SPECIAL REFERENCE TO A CLASSIFICATION OF THE VARIOUS TYPES AND THEIR SURGICAL TREATMENT.

Temporary Versus Permanent Typhoid Carriers.

Every typhoid patient who continues to show typhoid bacteria in the excreta (urine or feces) after the acute disease is over, should be classed as a "carrier." This carrier state may ultimately cease, in which case the individual is considered a "temporary carrier," or it may continue the rest of the patient's life and then the individual is classed as a "permanent carrier." Temporary carriers are usually such for only weeks or months and then clear up; occasionally they persist for a year or even longer and then they are known as "chronic" carriers. It is safest, however, to consider every carrier a permanent one until he is proved otherwise.

It is difficult to determine how long a time must elapse before a temporary carrier can be classed as a chronic or probably permanent one. It was found that those patients who showed typhoid bacteria in the stool for longer than 3 to 4 months after normal temperature, usually became chronic or permanent carriers.

Diagnosis of Feces Carriers.

We advise the use of the general term "feces carrier" to include all patients who show typhoid bacteria in the feces, rather than the term "intestinal carrier," because the latter designation should be reserved for a special class of feces carriers.

The finding of typhoid bacilli directly in the bile has formed our basis for the designation of feces carriers. This method determines much more readily than stool cultures the probable presence of typhoid bacteria in the intestinal tract, especially during the early part of convalescence from typhoid fever. In our series of 136 cases, 13 feces carriers would have escaped detection if duodenal cultures had not been made. Cultures of the stool alone may suffice for the detection of "chronic" or "permanent" feces carriers because in

these the typhoid bacteria have reached to great numbers in the intestines. During the first weeks or months after normal temperature, however, and this is just the important time for the detection of the carrier, it has been demonstrated that, while the bile may be full of typhoid bacteria, few or no typhoid colonies will be seen in the routine stool cultures of some patients.

No feces carrier should be dismissed as having been only a temporary carrier until repeated duodenal cultures show no typhoid bacilli. It is fully realized that this method is more troublesome for the patient than stool cultures; on the other hand, it is much safer for the community.

In the following study of 164 typhoid patients, at least 4 stool examinations by the Endo method were made on each patient. In addition, duodenal cultures were made on 136 of them. It is to be kept in mind that these tests were only started *during convalescence*, after the patient was free of fever and had remained so; in other words, at a time when the laity and some physicians have a tendency to leave off the usual precautions taken during the acute stage of the infection in order to prevent its spread.

Frequency of Various Types.

Of these 164 typhoid cases, 39, or 21 per cent, showed typhoid bacilli in the feces during convalescence. In addition, there were 14 cases in whom 3 consecutive stool examinations proved negative, while duodenal cultures showed numerous typhoid bacteria. Thus, adding these together, we find that 53, or 32 per cent, continued as feces carriers after the temperature reached normal.

This is a very high percentage in comparison to the figures reported by other writers.

	per cent
Schröder (15)	7.9
von Drigalski (11)	11.0
Lentz (16)	4.0
Klinger (17)	11.6
Semple and Greig (13)	11.6

The typhoid bacilli persisted in the stools of these patients for varying lengths of time. Results in the 53 positive cases are shown in Table X.

TABLE X.

No. of cases.	Per cent of all positive cases.	Per cent of all typhoid cases.	Duration of carrier state after normal temperature.
			weeks
3	5.5	1.8	1
9	17.0	5.4	2
10	19.0	6.0	3
7	13.0	4.2	4
4	7.0	2.4	5
3	5.5	1.8	7
6	11.0	3.7	8
2	3.7	1.2	9
1	2.0	0.6	10
1	2.0	0.6	11
1	2.0	0.6	12
2	3.7	1.2	17
1	2.0	0.6	18
1	2.0	0.6	19
2	3.7	1.2	21*

Summary in terms of months.

			months
29	55.0	17.4	1
13	24.0	7.9	2
5	10.0	3.0	3
6	11.0	3.6	4*

* And longer.

At the time of writing, the typhoid bacilli had cleared up in all but 7 carriers who had been observed for 12, 17 (2 cases), 18, 19, and 21 (2 cases) weeks respectively after normal temperature.

Judging by the stool cultures, surely 4, if not all of them, will undoubtedly remain chronic or permanent typhoid feces carriers; this makes *2.4 per cent* to *4.2 per cent* of all typhoid cases. These figures correspond with the findings of others.

	per cent
Hetsch (18)	4.62
Fornet (19)	0.9
Kayser (20)	5.0
Park (21)	5.0
Kirchner and co-workers (22)	5.0
Frosch (23)	2.47
Mayer (24)	4.0

TYPES OF FECES CARRIERS.

The gall bladder, or rather the bile coming from the gall bladder, has been conceded by all as the source of the typhoid bacteria in the feces. By means of duodenal cultures, a finer classification is possible. Feces carriers may primarily originate as one of three types: (*a*) intestinal carriers, (*b*) gall bladder (bile) carriers, (*c*) liver or duct (bile) carriers.

Intestinal Carriers.

An intestinal carrier is one in whom the typhoid bacteria in the feces come primarily from the intestines and not from an infected gall bladder or liver by means of the bile. The existence of a true intestinal type of carrier has never been definitely accepted. It is even denied in the recent article of Nichols (41). A negative proof was presumably offered by those cases in the literature in whom cholecystectomy with excision of the cystic duct, had failed to cure the carrier state. Such evidence in favor of the existence of intestinal carriers is insufficient, because, as will be seen further on, the typhoid bacteria in the feces of patients after cholecystectomy, may originate higher up than the gall bladder, in the liver or gall ducts. Positive evidence for the existence of a pure intestinal carrier type is afforded by means of duodenal cultures and was proved in only one case of our entire series. This was a patient 44 years old who reported sick Aug. 2, 1918, with the usual symptoms of headache, lassitude, and fever. He had had an antityphoid inoculation 7 days previously. He ran a comparatively mild typhoid fever for approximately 4 weeks. There were no complications and he reached normal temperature and convalesced without interruption. The first routine stool culture made 5 days

after normal temperature revealed the great proportion of colonies on the Endo plate to be typhoid colonies. The subsequent specimens of feces continued to show almost a pure culture of typhoid bacteria. This has persisted up to the time of writing (Feb. 20, 1919), 21 weeks after normal temperature and will probably remain so.[2] During the first 2 months of convalescence, however, 4 duodenal cultures revealed no typhoid bacteria in the large quantities of pure bile obtained. Actual dates of examinations are shown in Table XI.

TABLE XI.

Date of normal temperature.	Dates of positive stool cultures.	Dates of negative duodenal cultures.
Aug. 28	Sept. 2	
	" 14	
	" 23	
		Sept. 25
	Oct. 10	
		Oct. 15
	" 19	
		" 23
	" 26	
		" 29
	Nov. 16	

Thus it can be assumed that types of pure intestinal carriers exist. They comprise only about 0.6 per cent (1 out of 164) of all typhoid patients and 2 per cent (1 out of 53) of all feces carriers, temporary and permanent. Of the permanent feces carriers, 1 out of 5 or 6 may originally have been a pure intestinal carrier. The fact that there is a type of intestinal carrier is extremely important to know, for, as will be discussed more fully further on, cholecystectomy in this type of case is of no value. It is possible to differentiate with reasonable certainty an intestinal carrier from a bile carrier, by stool and duodenal cultures, especially in the early period of the carrier state. The intestinal carrier begins to show the typhoid colonies in the feces plate cultures during early convalescence and they persist in almost every stool specimen in very great numbers and in almost pure

[2] This prediction came true. The patient was operated upon several months later. Cholecystectomy was performed, but the carrier condition has continued.

TABLE XII.

Name.	Date of normal temperature.	Dates of cultures.	Feces cultures.	Duodenal cultures.
Ericksen.....	Aug. 27	Sept. 11	—	
		" 20	—	
		" 26	+	+
		Oct. 3	—	
		" 11	—	
		" 18	—	
		" 29		+
		Dec. 2		+
Schaefer.....	Sept. 24	Sept. 14	—	
		" 21	—	
		" 28	—	
		Oct. 5		+
		" 8	—	
		Nov. 2		+
		Dec. 2		+
		" 31	—	
Hoffman.....	" 10	Sept. 13	+	
		" 24	—	
		" 30	—	
		Oct. 4	—	
		" 11		+
		Nov. 1		+
		Dec. 2		+
		Jan. 2		+
		" 12	—	
		" 20	—	
Lehman.....	" 12	Sept. 5	—	
		" 11	—	
		" 17	—	
		" 20		+
		" 21	—	
		" 23	—	
		" 30	—	
		Oct. 7	—	
		" 20	—	
		" 27	—	
		Jan. 6		+
		" 9		+

+ indicates typhoid bacilli present; —, typhoid bacilli absent.

culture. It seems as if the entire intestinal flora are changed from colon to typhoid. On the other hand, in those cases where the typhoid bacilli in the intestines originate in the bile, the typhoid colonies on plate cultures made from the stool during early convalescence are present either in very small numbers (1 typhoid colony to 50 or 75 colon colonies) or are absent entirely. The diagnosis of the bile carrier state is possible only by duodenal culture and not by feces culture, as is seen in Table XII. The results in this table are in marked contrast to the findings in the intestinal carrier quoted previously (Table XI).

Bile Carriers.

The bile may transport the typhoid bacilli into the intestines from either one or both of two sites: (*a*) the gall bladder, (*b*) the liver or bile ducts.

An artificial experiment could not have been planned to differentiate between these two sources better than the solution of nature offered by two cases of the series. Two patients, Karpinsky and Lehman, manifested symptoms referable to the gall bladder during their convalescence.

At this time, direct culture of the bile by means of the duodenal tube showed numerous typhoid bacilli in Lehman and none in Karpinsky. Cholecystectomy with complete excision of the cystic duct was done in both (Major Kammerer). A pure culture of typhoid bacteria was obtained from the contents of each gall bladder. The apparent discrepancy in the case of Karpinsky of the negative bile culture before operation and the positive culture from the gall bladder was explained at the time of operation. A large stone was fixed in the cystic duct which completely occluded this passageway. The gall bladder was contracted, had a very much hypertrophied wall and contained a small amount of thick, greenish mucous fluid. The bile we had cultured from the duodenum before operation had come directly from the liver which was not infected and did not enter the gall bladder which was the only seat of infection. The mucoid bilious fluid in the gall bladder escaped into the duodenum probably only at irregular intervals as was evident by 1 stool examination when few typhoid colonies were present. Far different, however, were the conditions in the other patient (Lehman) in whom typhoid bacteria were found in the bile from the duodenum before cholecystectomy and also in the bile of the excised gall bladder. At operation there was discovered a large chronically inflamed gall bladder, abnormally thickened and with numerous stones but all free so that there was no interference with the lumen of the cystic duct. After complete healing of the abdominal wounds in both patients, duodenal cultures were repeated in each. In Karpinsky (where the bile coming from the liver was sterile) it was found that typhoid bacteria

remained absent, and the patient was cured. In Lehman, however, n spite of complete excision of the gall bladder and the cystic duct, repeated duodenal cultures were positive; probably, even before the operation, the bile which came from the liver and before it entered the gall bladder. was already infected and remained infected after cholecystectomy.

Detailed study shows that it is this condition of hepatic carrier rather than the condition of intestinal carrier which in most cases accounts for the persistence of the carrier state after removal of the gall bladder and cystic duct. That our patient Lehman was not an intestinal carrier can be readily noted by the results of the stool cultures. Seven routine stool examinations made at intervals of 5 to 7 days from Sept. 5 to Oct. 18 (the date of operation) showed no typhoid colonies, although duodenal examinations on Sept. 20 showed a pure culture of very numerous typhoid bacilli. Again after operation, isolated specimens of stool showed no typhoid organisms while duodenal cultures repeatedly demonstrated a heavy growth of typhoid bacilli. A systematic search in the stool was then undertaken and out of 163 Endo plates (embracing a period of 2 weeks) prepared from various dilutions of daily stool specimens, 11 plates were found which showed few scattered typhoid colonies. As was discussed above, such findings in the stool are characteristic of a gall bladder or liver carrier and not an intestinal carrier. Were this patient an intestinal carrier, there would have been no difficulty in detecting typhoid in the stool in great numbers both before and after operation, as was the case in our one example. A more detailed history of these two patients is here given for those who may be interested in any particular data.

Case 1.—Alfred Karpinsky, Register No. 571, age 42 years; born in Germany; sailor.

Past History.—Nothing of importance is noted in his habits, family history, or venereal history. Has never been sick before.

Present Illness.—Admitted to U. S. A. General Hospital No. 12 on Aug. 14, 1918, having been sick with headache and fever for about 8 days before this date at Hot Springs, N. C. Had received one antityphoid inoculation there before admission to the hospital.

Examination on Admission to U. S. A. General Hospital No. 12 by Lieutenant Sanders. The patient had no complaints. Roseola in great numbers over thorax, abdomen, back, and thighs. *Lungs.*—Occasional râles. *Heart.*—Slightly enlarged to left; blood pressure 100/70. *Abdomen.*—Not distended, not tender.

Liver.—Edge felt 1 inch below costal margin. *Spleen.*—Edge palpable ½ inch below costal margin. *Temperature course.*—Fluctuated from 102–103.6° reaching 104°F. only once; temperature continued from Aug. 14, date of admission, to Aug. 27, when it reached normal and continued so (total fever course approximately 21 days).

Early Laboratory Examinations.—Blood culture.—Aug. 18, negative. *Widal test.*—Positive. *Blood count.*—White blood cells, 10,550: polymorphonuclears, 58 per cent; lymphocytes, 42 per cent. *Urine.*—Trace of albumin, few erythrocytes.

Further Course of Disease.—(Complication.) Patient did not feel sick during fever course. On Sept. 1, 4 days after the beginning of normal temperature, patient complained of pain in abdomen and on examination there was obvious tenderness in right upper quadrant and muscular rigidity. The temperature suddenly rose to 105°F., but came down in 2 days. *Blood count.*—White blood cells, 11,800: polymorphonuclears, 55 per cent. *Diagnosis.*—Acute cholecystitis. The abdominal symptoms subsided entirely in 8 days and patient felt well again. On Oct. 8, 1918, about 1 month after the first attack, the patient again complained of pain in abdomen and again there was rigidity and tenderness over the gall bladder region. *Blood count.*—White blood cells, 11,750: polymorphonuclears, 56 per cent; lymphocytes, 44 per cent. *Duodenal culture.*—No typhoid bacilli, few colon bacilli, and many leucocytes. This attack was not as severe as the first; the temperature only rose to 101°F. for several days. The patient consented to operation.

Operation Report by Major Kammerer, Oct. 9, 1918.—Cholecystectomy. Many old adhesions about large gall bladder. Riedel's lobe present. Separation of gall bladder from bed of liver somewhat difficult. Gall bladder contained 21 large and small calculi varying from ¼ to 1 inch in diameter, with 1 of the larger stones in the cystic duct. Wall of the gall bladder about ½ inch thick; cystic duct ligated at junction with hepatic. Considerable hemorrhage from liver bed controlled by tampon. *Cultures from gall bladder contents showed pure growth of Bacillus typhosus.*

Surgical Course after Operation.—About 1 week after the operation (Oct. 16, 1918), the patient became jaundiced and there was a slight rise in temperature. There seemed to be an obstruction to the flow of bile because the stools became clay colored. The tampons were removed from the wound after which there was a profuse drainage of bile for about 3 weeks. Then the patient's jaundice disappeared; the stools resumed their normal color; the drainage ceased almost entirely; the wound began to close. It was healed entirely on Jan. 10, 1919.

Results of stool, urine, and duodenal cultures are shown in Table XIII.

Condition at Time of Writing (Feb. 20, 1919).—Patient is feeling perfectly well; is up and about and is to be sent to Fort Oglethorpe.

Case 2.—Herman Lehman, Register No. 505, age 34 years; born in Germany; barber.

Past History.—Had volvulus at age of 15; otherwise nothing of importance is noted in personal or past history.

Present Illness.—Began about July 27, 1918, with weakness, loss of appetite, and slight fever.

Examination on Admission to U. S. A. General Hospital No. 12 by Lieutenant Sanders, Aug. 14, 1918. Patient complained of headache, abdominal pains, and deafness in left ear. General condition fair; scattered roseola over body. *Heart.*—Action good; pulse slow; blood pressure 100/70. *Abdomen.*—Median scar from old volvulus operation; pain, tenderness, and rigidity in right upper quadrant. *Spleen.*—Just palpable. *Diagnosis.*—Acute cholecystitis complicating typhoid fever. *Temperature course.*—Patient came in with irregular temperature of 102–104°F., which gradually subsided in 1 week (Aug. 28, 1918). After several days of normal temperature, the fever slowly rose to 105°F. and then gradually came down again to normal (Aug. 25 to Sept. 14).

TABLE XIII.

Dates of cultures.	Feces cultures.	Urine cultures.	Duodenal cultures.
1918			
Sept. 1	—	+	
" 10	+	+	
" 21	—	—	
" 27	—	—	
Oct. 3	—	—	
" 10	—*		—
" 24	—*	—*	
1919			
Jan. 1		—*	
" 2	—*		—*
" 9	—*		—*

* Subsequent to operation.

Early Laboratory Examination.—Blood culture.—Aug. 18, negative; Aug. 30, positive. *Widal test.*—Positive. *Blood count.*—Aug. 14, white blood cells, 4,850: polymorphonuclears, 68 per cent; lymphocytes, 32 per cent. *Urine.*—Very faint trace of albumin, occasional hyaline cast.

Further Course of Cholecystitis.—This attack lasted for 3 weeks and gradually subsided by Sept. 5, 1918, when the patient was entirely free of gall bladder symptoms.

Summary.—Apparently the patient was first seen towards the end of the course of a typhoid infection, at which time there was also an involvement of the gall bladder. This inflammation subsided, but the patient had a relapse of the typhoid as was evidenced by a positive blood culture on Aug. 30.

Further Course of Disease.—With the subsidence of the fever on Sept. 14, the patient began to feel well and had no further complaints either subjectively or objectively until Oct. 12 (almost a month after normal temperature), when he

had a typical attack of cholelithiasis requiring hypodermic administration of morphine. The following day he was slightly jaundiced; he had local symptoms of cholecystitis and consented to operation. *Blood count.*—White blood cells, 8,800: polymorphonuclears, 44 per cent; lymphocytes, 56 per cent.

Operation by Major Kammerer, Oct. 18, 1918.—Small gall bladder entirely enclosed within old adhesions which were in part ligated and in part torn away. Cystic duct ligated as closely as possible to entrance into common bile duct. Concentric hypertrophy of gall bladder; very small lumen; contained 1 large and several small irregular stones; gall bladder wall about $\frac{1}{8}$ inch thick; cystic duct free. *Culture from gall bladder contents showed Bacillus typhosus.*

TABLE XIV.

Dates of cultures.	Feces cultures.	Urine cultures.	Duodenal cultures.
1918			
Sept. 5	—	—	
" 11	—	—	
" 17	—	—	
" 20			+
" 21	—	—	
" 23	—	+	
" 30	—	—	
Oct. 7	—	—	
" 20	—*	—*	
" 27	—*	—*	
1919			
Jan. 1	—*		
" 6			+*
" 9			+*
Feb. 10			+*
" 27			+*
May 11			—*
" 18			—*

* Subsequent to operation.

Course after Operation.—Patient showed effects of shock after operation but soon recovered. About 1 week later, the wound showed some drainage of bile which kept up for 2 weeks and then ceased. On Nov. 16, 1918, the wound was entirely healed. Nov. 20, 1918, acute phlebitis of left leg which continued for 2 weeks but then subsided; slight edema of left leg remained. Dec. 22, 1918, slight adenitis of left inguinal glands for 6 days.

Results of stool, urine, and duodenal cultures are shown in Table XIV.

Condition at Time of Writing (Feb. 20, 1919).—Patient is feeling perfectly well, no trouble of any kind; has gained 17 pounds in weight, but has remained under isolation as a carrier. (He ultimately cleared up.)

While, as was seen above, it is possible to differentiate the intestinal carrier from the bile carrier, it is impossible by laboratory means to separate the two types of bile carriers, although it would be extremely important for therapeutic purposes to be able to do so.[3] Then, too, one cannot state absolutely whether in the liver carriers, the bacteria are located in the liver tissue or hepatic ducts. During typhoid fever the damage to the hepatic parenchyma in the form of the small areas of focal necroses might speak for the deposit of the bacteria in the liver; bacteria have been isolated from the walls of the larger divisions of the hepatic duct as well as from the liver substance itself.

The most important advice is to try to establish the type of carrier during the *early convalescence* of the typhoid patient; *i.e.*, at the beginning of the carrier state. At this stage, the intestinal carrier is readily differentiated from the bile carrier. If the carrier state continues for a long time, various combinations of these three types may result, thus masking the characteristic features of the original type. An original bile carrier, for example (either gall bladder or liver), may ultimately resemble (although not really be) an intestinal carrier; that is, the bacteria would become so numerous that the feces would constantly show large numbers of them; possibly, too, the bacilli may lodge in the intestines on their way down and actually multiply there. A case in point occurred in our series. This patient (Ericksen) had the ordinary type of typhoid without any complications and ran a moderately high temperature for about 23 days. Of the usual number of stool cultures, only one specimen showed a few typhoid colonies on the Endo plate about 1 month after the beginning of normal temperature. During the following weeks, the stool cultures were again negative, although repeated duodenal tests revealed numerous typhoid bacteria in pure culture. After several months, the stools too began to show typhoid colonies in greater numbers, and then these gradually

[3] By the use of magnesium sulfate instillation into the duodenum, Lyon (25) has lately devised a method by which it is attempted to collect separately bile from different parts of the biliary apparatus. This should be tried in the future, and if proved practical, it may help us greatly in this differentiation of carriers. Instead of magnesium sulfate Stepp (26) injects a peptone solution into the duodenum; these agents are supposed to cause a contraction of the gall bladder and expulsion of the bile into the duodenum.

increased so that soon the larger proportion of the colonies on the Endo plates were typhoid; in other words, the picture resembled the characteristics of an intestinal carrier type. A more detailed history of this patient follows.

Case 3.—Christen Ericksen, Register No. 518, age 25 years; born in Germany; sailor.

Past History.—Nothing of importance is noted in personal history.

Present Illness.—Started 4 weeks before admission to hospital with headache, pains in the abdomen, and constipation.

Examination on Admission to U. S. A. General Hospital No. 12 on Aug. 13, 1918, by Lieutenant Sanders. The patient had no complaints except constipation; general condition good; tongue coated in center; scattered roseola over body. *Heart.*—Normal; pulse slow; blood pressure 105/65. *Abdomen.*—Flaccid. *Liver.*—Edge palpable just below costal margin. *Spleen.*—Palpable at costal margin. *Temperature course.*—Irregular fever from 101–104°F. for 13 days after admission; temperature reached 104° only twice (total fever course approximately 24 days).

Early Laboratory Examination.—Blood culture.—Aug. 18, negative. *Widal test.*—Negative. *Blood count.*—White blood cells, 5,650: polymorphonuclears, 59 per cent; lymphocytes, 41 per cent. *Urine.*—No albumin or casts.

Further Course of Disease.—Patient did not feel very sick during the disease. After his temperature reached normal (Aug. 27, 1918) convalescence progressed rapidly and uneventfully.

Results of stool, urine, and duodenal cultures are shown in Table XV.

Condition at Time of Writing (Feb. 20, 1919).—Patient is feeling perfectly well; has gained 20 pounds in weight; has remained under strict isolation as a carrier.[4]

It thus seems plausible to assume that in this case the intestinal carrier condition was secondary to the biliary infection. The reverse condition may occur too; that is, an original intestinal carrier with no bacteria in the bile cultures made early during convalescence may ultimately show typhoid bacilli also in the bile. This transition will be explained in detail in the discussion of ascending infections of the gall bladder.

[4] On Mar. 24, 1919, cholecystectomy was performed. Cultures from the gall bladder contents showed *Bacillus typhosus*. Stool cultures were positive only once, 5 days after the operation, and subsequent stool and duodenal cultures were negative.

TABLE XV.

Dates of cultures.	Feces cultures.	Urine cultures.	Duodenal cultures.
1918			
Sept. 11	—	—	
" 20	—	—	
" 26	+	—	+
Oct. 3	—	—	
" 11	—	—	
" 18	—		
" 29			+
Dec. 2			+
" 31	+		
1919			
Jan. 2	+		+
" 6	+		
" 12	—		
" 21	+		
Feb. 4			+
" 11	+		

How Do the Typhoid Bacilli Reach the Gall Bladder?

Three possibilities can be given as answers.

1. The generally accepted path for the conveyance of the typhoid bacillus to the gall bladder is by way of the blood to the liver and then into the bile. The damage to the hepatic parenchyma during the typhoid illness may more readily allow the passage of the bacillus into the bile. This injury to the liver tissue is not, however, essential for it has been repeatedly shown (Blachstein (27), Welch (28), and Doerr (29)) that in rabbits which have been inoculated intravenously by typhoid bacilli, the bacteria regularly appear in the gall bladder and often as early as 8 hours after inoculation. Doerr demonstrated that when the hepatic duct was ligatured the bile invariably remained sterile. Also when the cystic duct was ligatured, no bacilli could be demonstrated in the gall bladder. Once the bacteria reach the gall bladder, they grow there as readily as in a culture tube, finding a suitable medium which is periodically renewed and eliminated. If the foci in the liver (or ducts) clear up, then the carrier remains only a gall bladder (bile) carrier; on the other hand, the liver infections may continue and then the patient persists also as a liver (bile)

carrier. The factors which govern this are unknown. While the above experiments proved that the bacilli travelled by the bile and not by the blood vessels of the gall bladder itself, there are observers who believe that:

2. The typhoid bacilli may enter the bile by the *blood capillaries in the submucosa of the gall bladder wall* where they possibly form emboli. In a patient who died during the first weeks of his typhoid illness, Koch (30) by careful histological examination found nests of typhoid bacilli in the papillæ of the inflamed mucosa of the gall bladder wall. These bacteria lay in close proximity to the capillaries and were therefore assumed to be capillary emboli. These findings were also corroborated in animals (Chiarolanza (31)).

3. The *ascending route of infection* of the gall bladder by way of the common bile duct has not been received with general favor. The author, however, desires to record the following case as evidence that this mode of infection is possible.

Case 4.—Gustave Haak, Register No. 524, age 44 years; born in Germany.

Past History.—Nothing of importance is noted in his habits, family history, or venereal history. Has never been sick before.

Present Illness.—Was at the hospital at Hot Springs since Aug. 2, 1918, with headache and fever. On July 26 had received 1 antityphoid inoculation.

Examination on Admission to U.S.A. General Hospital No. 12 on Aug 13, 1918, by Lieutenant Wenner. General condition good; tongue dry and coated. *Heart.*—Sounds fair; dicrotic pulse. *Lungs.*—Numerous râles. *Abdomen.*—Soft. *Liver.*—Edge felt one finger's breadth below costal margin. *Spleen.*—Just palpable and tender. *Temperature course.*—On admission, temperature was 104.6°F., but the fever came down gradually so that it reached normal on Aug. 28, 1918, and remained so (total fever course approximately 26 days).

Early Laboratory Examination.—Blood culture.—Aug. 19, negative. *Blood count.*—White blood cells, 12,000: polymorphonuclears, 64 per cent; lymphocytes, 36 per cent. *Urine.*—Faint trace of albumin, occasional hyaline cast.

Further Course of Disease.—Patient did not feel sick during fever course. After the temperature reached normal, convalescence progressed rapidly and uneventfully.

Results of stool, urine, and duodenal cultures are shown in Table XVI.

Condition at Time of Writing (Feb. 20, 1919).—Patient is feeling perfectly well; has gained 31 pounds in weight.

In recapitulation, we had a patient who from the very beginning of his convalescence, 5 days after normal temperature, began to show an almost pure culture of typhoid bacilli in the feces which continued thus right along for 6

months (up to the time of writing). In spite of these early positive stool cultures, 4 duodenal cultures were made during the first 2 months after normal temperature and in none of them were typhoid but only colon bacilli found, although large quantities of pure bile were obtained for each culture.

TABLE XVI.

Dates of cultures.	Feces cultures.	Urine cultures.	Duodenal cultures.
1918			
Sept. 2	+	—	
" 11	—	+	
" 14	+	—	
" 23	+	—	
" 25			—
" 30	—	—	
Oct. 10	+	—	
" 12	+	—	
" 15			—
" 19	+		
" 23			—
" 26	+		
" 29			—
Nov. 9	—		
" 16	+		
" 25	—		
Dec. 2	+		+
" 16	+		
1919			
Jan. 2	+	—	—
" 6	+		+
" 12	+		
" 21	+		
" 23			+
Feb. 2	+		
" 4			+
" 11	+		
" 20			+

These findings proved the patient a true intestinal carrier. The fifth duodenal culture, however, which was repeated in 1 month showed few typhoid colonies amongst the colon bacteria. 1 month later, the typhoid colonies in the bile were more numerous and the future duodenal cultures continued to show a profuse pure growth of typhoid bacteria.

Those opposed to the general principle of ascending infections of the gall bladder, may voice the criticism that the typhoid bacteria cultured from the duodenum in the case described did not come from the gall bladder, but had found their way from the large intestine into the duodenum and were only washed out from the duodenum by the bile which came from a non-infected gall bladder. Naturally, the only positive proof in answer to this criticism would be a culture directly from the gall bladder.[5] However, the writer feels that the complete absence of typhoid bacilli in the bile at first, then the slight invasion, and finally the almost pure culture, speaks for the infection of the bile by the ascending route. In this way, a primary intestinal carrier becomes also a bile carrier.

Similarly, the principle of ascending infections may possibly account for a small percentage of liver carriers who originally may not have been liver carriers, but only gall bladder carriers. Since bile is produced in the liver almost constantly, it is present in the ducts all the time but in a stagnant condition, because it flows into the duodenum only intermittently. It should not be difficult therefore for bacteria to invade the bile ducts of the liver or the liver tissue itself when some infected bile from the gall bladder is dammed back through the cystic duct into the hepatic ducts and liver. Especially is this a possibility the longer the bacteria have been harbored in the gall bladder. This view was brought out forcibly in three patients at the Walter Reed Hospital kindly shown to the writer by Lieutenant-Colonel Nichols. They were all bile carriers; two of them had persisted for 12 and 13 years and one for only 12 months. Cholecystectomy and excision of the cystic duct cured the patient with the more recent carrier condition but had no effect whatever upon the old carriers. The duodenal contents of the latter showed as many typhoid bacteria after as before the cholecystectomy. The author does not wish to give the impression that the ascending route of infection,

[5] Since the time of this original manuscript, Patient Haak was operated upon. On Mar. 18, 1919, a cholecystectomy (without drainage of the common bile duct) was performed by Captain William S. Long. As was predicted, the gall bladder contents showed a pure growth of typhoid bacilli. 1 month later (Feb. 21 and 24), both the stool and duodenal cultures were still positive for *Bacillus typhosus*. Apparently the infection by this time had extended higher up into the liver or bile ducts.

either for the gall bladder or liver carriers, is the frequent or the general occurrence, but merely wishes to point out that this path of infection is undoubtedly a possible one.

Surgical Treatment of Carriers.

Dehler (32) was the first to suggest operative interference directed towards the cure of the carrier state. In his first 2 patients only drainage of the gall bladder (cholecystostomy) was resorted to. In one of these patients typhoid bacilli were isolated in small numbers from the feces several months after operation.

Grimme (33) next reported that he had had the operation of cholecystectomy performed in a female asylum carrier. 15 days after the operation, *Bacillus typhosus* was found in the feces, but not at a later period.

Since then, cholecystectomy has been adopted as the routine surgical procedure for the cure of carrier conditions, but the results have not been uniformly successful (Lorey (34), Loele (35), Huismans (36), Kamm (37), Fromme (38), Dehler (39), Leary (40), Nichols, Simmons, and Stimmel (41)). The cause for the failures is evident if the classification of the various types of carriers is kept in mind.

Formerly when cholecystectomy did not cure a typhoid carrier, it was usually attributed to the fact that the cystic duct had not been completely excised. The bacteria were supposed to continue to multiply in the duct as a pocket where bile was retained, simulating a gall bladder but on a very much smaller scale. While this explanation may account for the failures in some of the early cholecystectomies, in recent times the surgeons are certain to excise the cystic duct completely. The author has personally observed 2 such complete excisions where the carrier state remained unaffected. The present study has shown that there are two additional reasons which may account for the failure: (*a*) Hepatic (or duct) carriers. These patients will not be relieved by cholecystectomy because the infection continues higher up. (*b*) Intestinal carriers. These too will be influenced in no way by complete excision of the gall bladder. The bacteria will continue to grow somewhere in the intestines.

Therefore, if a carrier is diagnosed as an intestinal one, operative treatment should not be resorted to. Only bile carriers should be

treated surgically and the type of bile carrier more amenable for operation is the gall bladder type. Unfortunately, it is impossible to differentiate absolutely between the gall bladder and hepatic types.[3] Taking this into consideration, the author advises instead of cholecystectomy alone, a cholecystectomy plus long continued drainage of the liver through the hepatic duct. The reasons for this are both diagnostic and therapeutic. After the drainage of the bile has set in, it is a simple matter to take cultures of the bile coming from the liver through the rubber drainage tube and ascertain whether typhoid bacteria are present or not. At the same time, the stool should be examined as control. If repeated cultures from the bile prove that the liver or ducts are not infected, drainage may be stopped and the wound may be allowed to close quickly. On the other hand, if the bile is proved to contain typhoid bacteria, drainage should be prolonged with the hope that in this way the infection may clear up.

Drainage of the common bile duct outside of the body has the advantage over the natural flow into the intestines in that it keeps the intestines free of typhoid bacteria and in that a greater quantity of bile is drained. Physiology of bile excretion teaches us that although bile is formed more or less continuously, it enters the duodenum only periodically during the time of digestion. The bile during the intervening periods is prevented from entering the intestines because the opening of the common bile duct into the duodenum is closed by a sphincter. The secretion, therefore, backs up into the liver. No bile appears in the duodenum as long as the stomach is empty. When a meal is taken, the entrance of the chyme into the duodenum is followed by an ejection of bile. It would seem therefore that each gush of chyme into the duodenum excites by reflex action an inhibition of the sphincter and the opening of the common bile duct. By means of hepatic drainage, the bile which is formed all the time is also excreted all the time, thus constantly flushing the infected area and allowing of no stagnation where the bacteria can grow more readily. The possibility of ridding the liver or ducts of bacteria seems in this way a little more hopeful.

Another important consideration is the time of operation. When surgical measures are contemplated, they should be done early when the original type of the carrier state remains uncomplicated. If the

typhoid carrier presented no clinical symptoms referable to the gall bladder during the acute stage of the disease or convalescence, 6 months from the onset of normal temperature is a reasonable time to wait for nature to cause the disappearance of typhoid bacteria from the bile before operating. On the other hand, if symptoms of cholecystitis or cholelithiasis[6] were present during any stage of the illness and duodenal cultures persistently showed typhoid bacteria, operative interference may be undertaken even earlier, because these patients usually become chronic carriers. It is to be remembered, however, that not all patients who present gall bladder symptoms during typhoid fever become carriers, so that operation cannot be justified from the carrier standpoint unless duodenal cultures show typhoid bacteria.

The author observed 4 carriers in each of whom cholecystectomy (without drainage) was performed. In 2 (Karpinsky and Ericksen) the carrier condition disappeared immediately after operation. In 1 of these (Ericksen) only 1 stool culture made 5 days after operation showed typhoid bacteria, while subsequent cultures both of the feces and duodenum were negative. These were examples of pure gall bladder carriers, the type most amenable for operation. In the third patient (Lehman) the duodenal cultures remained positive for four months after operation, although repeated stool examinations were negative. Except for the duodenal cultures, this case would have passed as a surgical cure and been permitted to leave the hospital before he ceased to be a menace to the community. This is an example of a liver carrier, usually not helped by operation. (The writer is informed that ultimately, 8 months after operation, 2 consecutive negative duodenal cultures were obtained.) In the fourth patient (Haak) both duodenal and stool cultures continued positive after operation. This is an example of an original intestinal carrier with secondary bile infection.

It is to be assumed that in many instances consent for operation will not be obtained from the apparently healthy carriers; but when it is, our classification and study of the various types should give us better results than we have had in the past, or a better understanding of our failures.

[6] The relationship between symptoms of cholecystitis during the typhoid fever and a later carrier state is more fully discussed in the next section.

IV. PREDISPOSING FACTORS TO THE TYPHOID CARRIER STATE WITH SPECIAL REFERENCE TO CHOLECYSTITIS AND CHOLELITHIASIS.

Why Do Some Patients Harbor Typhoid Bacteria in the Gall Bladder Longer Than Others?

For this study, the 21 patients who continued to show a positive bile culture were selected. A detailed analysis was made of the clinical course of their disease, in order to discover any possible predisposing elements to the carrier state. The following factors were investigated: (*a*) blood culture, (*b*) length of illness, (*c*) severity of illness, (*d*) prophylactic inoculation, (*e*) cholecystitis, (*f*) cholelithiasis.

Did These Patients with Positive Duodenal Cultures Have a Positive Blood Culture during the Active Stage of the Disease?

This question is taken up because some readers may ask it. The author, however, feels that all typhoid cases at one time or another during the active stage of the disease have had a bacteremia; and especially those who later developed a positive bile culture. The bacteria must reach the gall bladder by way of the blood (the only exception to this is possibly the bile carrier due to ascending infection). It is surprising to note, nevertheless, that in only 4 of 19 cases was a positive blood culture reported (2 patients had normal temperature and no blood culture was taken). Table XVII shows the time during the course of the disease when these negative blood cultures were obtained.

TABLE XVII.

No. of cases.	No. of cultures.	Week of disease when culture was taken.
3	2	1st
3	2	2nd
7	1	3rd
2	1	4th

While a negative blood culture did not mean that there were no bacteria in the circulation, it did imply that there were no excessive numbers of them and that they did not persist in the blood over an extended period.

Of the positive cultures, there were three obtained during the second and one during the third week.

No relationship, therefore, can be discovered between a positive or negative blood culture as a predisposing factor towards the typhoid carrier state. Only if a great majority of the cases had shown a positive culture and especially at a stage of the disease when a positive culture is unusual, would it have been permissible to draw any conclusions.

Does the Length of the Disease Have Any Bearing upon the Tendency of the Bacilli to Persist in the Bile?

The length of the illness was reckoned in terms of the number of days of fever; *i.e.*, from the day when the patient went to bed to the day when the temperature came down to below 100° and stayed there. These fixed dates have always seemed to the author a much more reliable method for determining the actual length of the disease than the way usually estimated; namely, from the time the patient first began to feel ill, to some arbitrary day during convalescence, such as when the patient was able to get out of bed or leave the hospital, etc. In our 21 cases the duration of fever is shown in Table XVIII.

TABLE XVIII.

No. of cases.	Duration of fever.	No. of cases.	Duration of fever.
	weeks		*weeks*
3	2	4	5–6
7	3–4	1	6–7
2	4–5	4	7–8

The average case of typhoid fever runs a temperature for about 4 weeks. It is interesting to note that the majority of the typhoid carriers did not have a shorter acute illness but rather a longer one than the general average. The possibility must be considered that

this protracted course is not a predisposing factor to a carrier condition but one resulting from the ever existing infected bile in the biliary tract and intestines. In this connection it may be mentioned that among these 21 patients there were 4 cases with relapses and 4 cases with recrudescences; *i.e.*, 38 per cent. This percentage is rather high when compared with the 18 relapses and 18 recrudescences (22 per cent) which occurred amongst the total number of cases (164). From the same figures it may be seen that 22 per cent of all the patients who suffer from either a recrudescence or relapse tend to become typhoid carriers, temporary or permanent (8 out of 36).

Does the Severity of the Disease Have Any Bearing upon the Predisposition to the Typhoid Carrier State?

We differentiated strictly between the length of disease and severity of infection. One may have a long, but at the same time mild infection and *vice versa*. The same holds true for a short illness. In grading the degree of each patient's illness, we merely took into consideration the severity of the infection during the acute stage. A ++++ scale was used.

++++ signifies the severest type of infection where prognosis was very grave from the start and the patient died.

+++ signifies a seriously ill patient with a doubtful prognosis during his illness but one who finally recovered.

++ is the usual sick typhoid.

+ means a very mild infection.

− means that the patient was clinically not ill at all; were it not for the laboratory findings, the disease would have remained unrecognized.

Of the 21 patients with positive duodenal cultures:

1 was graded − (not sick at all.)
11 were " +
5 " " ++
4 " " +++

Of the 4 patients who became permanent typhoid carriers:

3 were graded +
1 was " ++

It is evident that 16 of the cases, approximately 80 per cent, were only + and ++ types of infection. This proportion is practically

the same as existed between the entire number of + and ++ cases and the total number of typhoid cases at the hospital. The point of importance that the author desires to emphasize is that it is not necessarily the severe types of infection that are prone to become carriers.

Are Patients Who Have Received Antityphoid Inoculations and Have Been Infected in Spite of the Inoculations Less Liable to Become Typhoid Carriers?

Of the total number of typhoid patients treated at the hospital:

38.0	per cent had received	no inoculation.
41.0	" " " "	only 1 "
11.8	" " " "	2 inoculations.
2.2	" " " "	3 "
8.0	" "	unknown.

Of the 21 patients who persisted with typhoid bacteria in the bile: 45 per cent had received no inoculations; 40 per cent had received only one inoculation; and 15 per cent had received two inoculations. None had a complete course of inoculations. Those who had received only one or two prophylactic injections did not complete their third injection because the illness had already set in, or set in immediately after the last inoculation. While the above figures may speak somewhat for the beneficial influence of inoculations, conclusions as to their value cannot be drawn too closely.

Cholecystitis.

In a study of the cases which manifested symptoms of cholecystitis either as a complication during the disease or as a sequel during convalescence, a very close relationship to the carrier state was noted. Out of 178 proved typhoid cases, 8 (4.5 per cent) had definite clinical evidence of cholecystitis during the acute illness.

A tabulation of the findings in 7 of these patients is given in Table XIX (the eighth died of pneumonia several days after admission before the laboratory examinations could be made).

TABLE XIX.

Case No.	Name.	Feces culture.	Duodenal culture.	Direct culture from gall bladder.	Cholelithiasis.	Carrier condition.	Results.
1	Karpinsky..	+	—	+*	+	+	Cured (cholecystectomy).
2	Lehman.....	—	+	+*	+	+	Remained carrier even after cholecystectomy, due to liver infection.
3	Kobe.....	—	—	+†	+	+	Died (cholecystitis and complicating myocarditis and pneumonia).
4	Ziska.....	+	+	0	0	+	Cured (temporary carrier).
5	Bergenthal...	—	+	0	0	+	" " "
6	Kussner.....	+	0	0	0	—	" " "
7	Jannssen....	—	0	0	0	—	" (never proved a carrier).

* Operation.
† Post-mortem.

Thus it is noted that of the 7 patients who manifested symptoms of cholecystitis 6 ultimately became typhoid carriers. The bacilli were found in the excised gall bladder of 3, in the duodenal contents of 2, and in the feces of 1. Some of the discrepancies of the above table require explanation.

Case 1 (*Table XIX*) showed no typhoid bacilli in the duodenum because as was proved by operation the cystic duct was occluded by a large stone. At the time when the *positive stool* was obtained, it was probable that the cystic duct was either not yet completely occluded or was only intermittently occluded.

Case 3 (*Table XIX*) never showed typhoid bacilli in either the feces or duodenum. The patient had recurrent attacks of cholecystitis and died during one of these periods of a myocarditis and a complicating pneumonia. A short review of this man's history will disclose the evidence of the carrier condition.

He was admitted to U.S.A. General Hospital No. 12 on Aug. 13, 1918; had been at the hospital in Hot Springs since Aug. 1, 1918; had typical clinical typhoid with marked intestinal hemorrhages and definite myocarditis.

Aug. 13 to 26. Temperature gradually came down to normal.

Aug. 26 to Sept. 1. Temperature normal.

Sept. 1 to Sept. 21. Typical relapse with blood in stools and temperature as high as 104.8°F., gradually coming down.

Sept. 21 to Oct. 9. Temperature normal; patient out of bed.

Oct. 9 to Oct. 18. Another relapse but milder than the first.

Oct. 18 to Nov. 15. Temperature normal; patient out of bed.

Nov. 15 to Nov. 21. Temperature up to 103°, condition simulating relapse, with symptoms of cholecystitis for the first time.

Nov. 21 to Jan. 2. Temperature normal; patient out of bed.

Jan. 2 to Jan. 30. Temperature up to 103.5°, with renewed symptoms referable to cholecystitis associated with lobar pneumonia; death.

Laboratory Findings.—Widal test.—Positive on admission. (Patient had received no inoculation.) *Blood cultures.*—6 negative. *Stool.*—At no time positive for typhoid bacilli. *Urine.*—Typhoid bacilli found only once, Nov. 22, 1918. *Duodenal culture.*—Oct. 10, negative. *Autopsy report.*—The gall bladder region presented old dense fibrous masses which bound the liver to the diaphragm and formed one large mass of the gall bladder, pylorus, duodenum, and pancreas. After very difficult dissection, the gall bladder was found deeply buried. It was the size of a pigeon's egg and was contracted down upon four yellow apparently cholesterol stones which entirely filled the cavity. The gall bladder contained a little mucus but no bile. The cystic duct was obliterated.

Cultures from the interior of the gall bladder showed *Bacillus typhosus;* at the same time, cultures from the duodenum, large intestine, liver, spleen, lungs, abdominal fluid, etc., showed no typhoid bacilli. The apparent discrepancy between the positive culture from the gall bladder interior and negative culture from the duodenum before and also after death are readily explained by the complete shutting off of the cystic duct both by the stones and adhesions. The bile which was examined during life did not come through the gall bladder but directly from the liver which was not infected. The bacteriological conditions in this patient were identical with those of patient Karpinsky discussed in the chapter on bile carriers.

Cases 4 and 5 (*Table XIX*) showed typhoid bacteria in the bile by duodenal culture.

Cases 6 and 7 (*Table XIX*) had the most severe attacks of cholecystitis. Repeated attempts at duodenal cultures were unsuccessful. In Case 6 the duodenal tube was passed four times but on no occasion was bile obtained. Similarly, in Case 7 (Table XIX), three attempts were made and on one occasion only bile-stained fluid, but not true bile, was recovered. This did not show typhoid bacilli. It is very unusual to meet with such difficulty. The failures were probably due either to reflex spasms of the pylorus or true pyloric obstruction from adhesions of the gall bladder. The frequent vomiting which these patients suffered from substantiated this view. The fact that the stool examinations in Case 7 did not reveal typhoid bacilli does not, in the light of experiments already discussed, exclude the possibility of their existence in the bile. It is possible, too, that here again we were dealing with a contracted and obliterated gall bladder where the typhoid bacilli could be recovered only from the interior of the gall bladder.

In recapitulation, it is noted that of 7 patients who had attacks of cholecystitis during the course of their typhoid fever, 6 became definite typhoid carriers, either temporary or permanent, with typhoid bacteria in the bile or feces; the 1 other patient was inadequately examined.

These facts assume importance in the light of the surgical therapy in carriers. Cholecystectomy (plus drainage of liver) for typhoid cholecystitis becomes warranted very much earlier when we have the additional knowledge that a typhoid carrier state results in most cases of cholecystitis. Once the symptoms of cholecystitis have manifested themselves, recurrent attacks of pain and fever are almost bound to arise as long as live typhoid bacteria remain in the gall bladder. This is probably the state of affairs that exists in patients operated upon for cholecystitis many years after the typhoid illness, in whom typhoid bacilli are unexpectedly found in the gall bladder. These individuals had undoubtedly been carriers during all that interval. Especially in these patients should duodenal cultures be made as soon after the operation as feasible, in order that a liver carrier may not be overlooked.

If operation for typhoid cholecystitis is delayed and attacks recur, very marked adhesions are developed which may make surgical interference very much more difficult or even dangerous. Such would have been the case in our patient Kobe who died of a complicating pneumonia and myocarditis before the operation was undertaken. Even at post-mortem it was impossible to dissect out the gall bladder without great damage to the related organs.

Many of the so called prolonged cases of typhoid fever or the cases with frequent relapses are probably typhoid infections of the gall bladder, although the local process may be so mild that definite symptoms of cholecystitis are either overlooked or missing.

On the other hand, not all patients of our series who became carriers had manifested symptoms referable to the gall bladder at sometime during the course of the acute disease. Of our 21 patients with persistent positive bile cultures, only 7, or 33 per cent, had presented gall bladder symptoms during the typhoid illness. Amongst the 14 others who did not show such symptoms, 2 were the most persistent carriers.

Pathology of the Gall Bladder in Carriers.

This division of carriers on the basis of former gall bladder involvement explains very logically the two types of gall bladders met with in carriers. In those who do not manifest any gall bladder disturbances, the gall bladder may show only very slight pathological changes. The normal glistening appearance of the peritoneal coat may be dulled, the wall may be slightly thickened, the entire organ may not be enlarged, and on being cut open the mucous membrane may present just a thickened or congested appearance. Microscopically, the different layers of the wall may be sharply outlined, but infiltrated by lymphocytes and few leucocytes. In these cases the gall bladder acts purely as a test-tube containing the bile medium in which the typhoid bacteria propagate without affecting the gall bladder itself. In the second type, the gall bladder may present the inflammatory changes of various grades of cholecystitis. The entire organ may be buried in dense adhesions, the walls may be $\frac{1}{2}$ to $\frac{3}{4}$ inch in thickness, and on section the entire normal gall bladder appearance may be obliterated and replaced by a mass of fibrous tissue infiltrated by lymphocytes and polynuclear leucocytes. In between these two extremes, all degrees of pathological changes may exist.

Cholelithiasis.

The relationship between cholelithiasis and typhoid fever has already stimulated such a vast amount of literature that only the outstanding features can be reviewed here. The discovery of the typhoid bacillus in cases of cholecystitis and cholelithiasis long after recovery from the primary infection, was made many years previous to the recognition of the typhoid carrier. In 1892 Naunyn (42) had observed that gall stone troubles frequently occurred in persons who had suffered from typhoid fever. Lentz (43) in 1905 was the first to direct attention to the *association of gall bladder complaints with the carrier state.* Since then it has been frequently shown that quite a large number of carriers suffer from gall stones, while in others, though symptoms may have been absent, a condition of cholelithiasis has been very frequently found, whether on examination, post-mortem, or at operations directed towards bacteriological cure of the carrier condition.

In our 21 carriers there were 3 (14 per cent) in whom gall stones were discovered.

(1) *Karpinsky.*—Operation about 7 to 8 weeks after onset of illness; 21 stones; 4 very hard and about 1 inch in diameter; 1 about ¾ inch in diameter; 5 about ½ inch in diameter; the rest about ¼ inch in diameter; they were all hard and faceted.

(2) *Lehman.*—Operation 2 months after onset of illness; 1 large, hard, mulberry-shaped gall stone about 1¼ inches in diameter and several very small softer stones (all calculi of cholesterol).

(3) *Kobe.*—Post-mortem about 6 months after admission to hospital; 4 stones (cholesterol); 2 large stones each about 1 inch in diameter and 2 smaller ones about ½ inch in diameter.

Förster (44) in an analysis of several hundred carriers found the same percentage (14 per cent) of gall stone sufferers. The remaining 85 per cent undoubtedly have some disease of the gall bladder, although they give no clinical indication thereof. This accords well with our knowledge that 90 per cent of all gall stone cases present no symptoms during life. These facts brought to the writer's consideration the question whether it is the chronic carrier infection of the gall bladder which gives rise to the formation of the stones, or *vice versa* whether it is a preexisting cholelithiasis which predisposes the typhoid patient to the development or continued existence of the carrier state. X-ray examination of the gall bladder region was made in each of our 21 carriers, but in only 2 were the x-ray findings suspicious of stones.

These negative results agree fully with the fundamental findings of Blachstein (27) and Welch (28) who showed that no *locus minoris resistentiæ* in the gall bladder was essential for the development of the carrier condition; mere intravenous inoculation of typhoid bacilli in rabbits is followed by the presence of these organisms in the gall bladder for a very long while—in one instance for 128 days. While these experiments disprove the absolute necessity of a previous gall bladder injury for the formation of a carrier condition, they do not speak against the logical assumption that those patients with a previous cholelithiasis are more prone to become carriers, or to remain such for a longer period of time.

As for the relationship between the typhoid bacillus and actual gall stone formation, here also a division of opinion exists. While

the typhoid bacillus has been repeatedly isolated from the center of gall stones both in cases of cholelithiasis operated on by the surgeon and in chronic carriers either at operation or at autopsy (Anton and Fütterer (45), Droba (46), Blumenthal (47), Levy and Kayser (48)), no definite decision has yet been reached as to whether the typhoid bacillus directly excites the formation of the gall stone and forms a nucleus for it or whether the stone is preformed and later penetrated by the typhoid bacillus. Conforming with these opposing views, some authorities (*e.g.* Bacmeister (49)) believed that organisms were only to be found in old stones into which they had penetrated, while other observers (*e.g.* Cushing (50)) held that only recently formed stones contained bacilli. As is so often the case in medicine, experimental data were furnished by both sides in support of their contentions.

On the other hand, gall stones were placed in broth and bile cultures of the *Bacillus typhosus* and also of *Bacillus coli*, and these bacteria were then recovered from the center. Gilbert and Fournier (51) found that bacilli wandered in when stones were of cholesterol and not in the case of other stones; but then, it is usually the cholesterol stone which develops during typhoid fever. The Aschoff school has recently shown that the cholesterol content of the blood is increased in all long continued acute and subacute septic or pyemic processes and therefore concludes that in typhoid the stones are found in the gall bladder because of stagnation of the bile. The bacteria may later travel into the stone and thus bear no etiological relationship to the stone.

On the other hand, embryonic gall stone formation may be observed in test-tubes containing bile cultures of either typhoid or colon bacilli. Bacmeister found that *Bacillus coli*, *Bacillus typhosus*, *Bacillus proteus*, and especially *Bacillus pyocyaneus* could cause a precipitation of cholesterol from bile. Even true concretions were found after prolonged growth of the bacteria in this medium.

Then, too, gall stone formation has been observed after injecting heated bouillon cultures of typhoid bacilli into the gall bladders of rabbits (Gilbert and Fournier (52)). Doerr (53) has met with two concretions of the size of a lentil in a rabbit which had been injected intravenously with typhoid bacilli 40 days before. Gay (54) noted

gall bladder concretions in some of his carrier rabbits, and Richardson (55) succeeded in experimentally producing concretions in rabbits by the injection into the gall bladder of agglutinated typhoid bacilli.

In the light of these varied experimental results, there can be no doubt that an attack of typhoid fever predisposes a patient to the formation of gall stones, but at present one cannot be certain how far this formation is contributed to by other factors. It is probable that in certain instances the precipitation of cholesterol comes first, to be followed by the entrance of bacteria, while in other instances the stagnation of the bile may cause a precipitation of the cholesterol around a nucleus of epithelial cell debris and typhoid bacteria.

The stones formed during typhoid fever may reach a large size in a comparatively short time. In one of our carriers (Kobe) 4 cholesterol stones were removed from the gall bladder 6 months after admission for the acute typhoid. 2 were about 1 inch in diameter and 2 about $\frac{1}{2}$ inch in diameter; typhoid bacteria were recovered from the center of all. The size of the stones seemed very much larger than would be expected to develop in 6 months.

One should not, however, fall into the fallacy of attributing to the typhoid infection all stones found in a patient who gives the history of a recent or old typhoid. In Case 2, the large stone-hard cholesterol calculus about $1\frac{1}{4}$ inches in diameter undoubtedly existed long before the typhoid illness which was only 2 months old. The 2 smaller and softer stones, also of cholesterol, were probably of typhoid origin. Unfortunately the stones were dropped into formalin and cultures made from the center of the stones the next day were found sterile. It may be that in typhoid patients with preexisting cholelithiasis, the tendency to the formation of stones being already present, the added element of the typhoid infection predisposes the patient to additional and more rapid gall stone formation.

V. TYPHOID URINE CARRIERS.

A. Irregularity and Intermittency of Bacilluria; New Method for Detecting Urine Carriers.

The excretion of typhoid bacilli in the urine from typhoid convalescents has been studied in 164 patients and entailed approximately 2,000 urine cultures. According to the army rule, every patient was kept under strict typhoid precautions until 3 consecutive urines and stools at intervals of 6 days were free of typhoid bacteria. The first culture of the urine was generally made when the patient's temperature was nearing normal or had already reached normal. It was found that the excretions of typhoid bacilli in the urine during convalescence followed one of three courses:

(*a*) *Urines which were at no time positive* (3 or more consecutive urines at intervals of 6 days being negative). This comprised 51 per cent of the patients (84 out of 164).

(*b*) *Urines* which were at *first positive* and remained so for a shorter or longer time, *then* became *negative* and remained so. This comprised 25 per cent of the patients (41 out of 164). For example, patient Gutte: Sept. 13, 18, 29, positive; Sept. 30, Oct. 7, 26, Nov. 2, negative.

(*c*) *Urines which changed*, sometimes positive, at other times negative, then positive only to become negative again, etc. This comprised 23 per cent of the patients (39 out of 164). As typical of such irregularity of findings in the routine urine specimens, results with 4 patients are cited in Table XX.

It is these changing urines which particularly interested the writer. The question arose whether it was possible that 3 isolated specimens of urine examined at intervals of 6 days could show no typhoid bacilli, while other specimens taken in between or even later on contain typhoid bacteria. The great danger of overlooking urine carriers would thereby be offered. As the routine cultures were usually made of specimens passed in the morning, the first question to decide was whether *the time of voiding had any influence upon the result.* 12

patients were selected and every specimen voided by each patient was carefully collected and cultured separately. All precautions for obtaining a sterile specimen were observed: the head of the penis was washed with bichloride solution; the first part of the urine voided was not kept but used for washing out the urethra; the last part was voided directly into a sterile bottle. At this time, cultures were made only on Endo plates; 1 cc. of each specimen was spread in a thin layer over the entire surface of the media and incubated for 24 hours.

TABLE XX.

Name.	Urine examinations.		Name.	Urine examinations.	
	Dates.	Results.		Dates.	Results.
Adam.....	Sept. 13	+	Streitzel.....	Sept. 12	+
	" 17	—		" 17	+
	" 19	+		Oct. 5	—
	" 25	—		" 21	+
	Oct. 1	+		" 28	+
	" 18	+		" 30	—
	" 20	—		Nov. 4	—
	" 26	—		" 18	+
				" 18	—
Bier.....	Sept. 7	+	Kussner.....	Oct. 8	—
	" 16	—		" 15	+
	" 21	+		" 21	+
	" 30	—		" 30	—
				Nov 6	—
				" 22	+
				" 29	—

+ indicates typhoid bacilli present; —, typhoid bacilli absent.

Every suspicious growth was identified by the Russell double sugar medium and then by serum agglutination. The results in 3 of the 12 cases thus examined are cited in Table XXI.

We sought the explanation for the striking results shown in Table XXI. Keeping in mind the general laboratory experience that sterile cultures are often seen on solid media when growths occur in broth, we continued the above plan of separately examining every specimen voided by 5 patients, but this time cultures were made not only on Endo plates, but also in broth (from 1 to 2 cc. of urine). The

TABLE XXI.

Name.	Urine examination.			Name.	Urine examination.		
	Date.	Time.	Result.		Date.	Time.	Result.
		a.m.				*p.m.*	
Schubert.....	Sept. 13	2	—	Trautman ...	Sept. 13	2	+
		p.m.				5	—
		4	+			8	+
		7	+		" 14	7	+
		10	—			10	+
		a.m.				*a.m.*	
	" 14	11	—		" 15	5	+
		p.m.				8	+
		3	+			*p.m.*	
		9	+			2	+
		a.m.				7	+
	" 15	4	—			11	+
		8	+			*a.m.*	
		p.m.			" 16	5	+
		1	—			*p.m.*	
		3	—			1	+
		11	—			5	+
		p.m.				8	+
	" 17	9	—			10	+
		a.m.				11	—
	" 18	3	+			*a.m.*	
		n.			" 17	5	—
	" 19	12	—			8	—
		p.m.				*p.m.*	
		8	—			2	—
		a.m.				6	—
	" 20	4	+			9	—
		p.m.				10	+
	" 22	7	+			*a.m.*	
		a.m.			" 18	6	+
	" 23	3	+			*p.m.*	
	" 24	4	+			2	—
	" 25	1	+			6	+
		6	+			9	—
		p.m.				11	—
		2	+			*a.m.*	
		8	+		" 19	5	—
		11	—				
		a.m.					
	" 26	4	+				
		6	+				

TABLE XXI—*Concluded.*

Name.	Urine examination.			Name.	Urine examination.		
	Date.	Time.	Result.		Date.	Time.	Result.
		p.m.				*p.m.*	
Muza.....	Sept. 13	9	+	Muza.....	Sept. 19	5	+
		a.m.				8	+
	" 14	2	+			*a.m.*	
		6	+		" 20	4	+
		p.m.				*p.m.*	
		4	—			4	—
		a.m.				9	—
	" 15	2	—			*a.m.*	
		9	—		" 21	8	+
		p.m.				10	—
	" 16	9	+		" 22	4	—
		a.m.				7	—
	" 17	4	+			*p.m.*	
		p.m.				2	—
		5	—			5	—
		7	+			8	—
		a.m.				*a.m.*	
	" 18	8	+		" 23	2	—
	" 19	7	+			5	—
		10	+			7	—
		p.m.					
		1	+				
		2	+				

results from 1 case are noted in Table XXII. The other 4 patients gave similar results.

It was thus definitely proved that the use of Endo plates alone for the purpose of detecting typhoid bacilli in the urine is insufficient unless the number of bacteria happens to be excessive. The safe procedure is first to obtain a 24 hour growth in broth and then to make an Endo plate therefrom.

The irregularity in the excretion of typhoid bacilli in the urine thus seemed to be explained as dependent upon the technique; *i.e.*, the use of solid media only. On further study, however, it was soon found that there was an actual intermittency in the excretion of the typhoid bacilli. That is, certain specimens of urine were found absolutely sterile (also in broth), while others voided within several hours were

full of typhoid bacteria. As examples, we may cite the following cases (Table XXIII).

Thus it is surprising to find, for example, a urine (Schubert, Table XXIII) full of typhoid bacilli at 3 p.m., sterile 4 hours later, and full of typhoid bacilli again 3 hours later. It only definitely proves that excretion of typhoid bacteria in the urine follows an intermittent curve.

TABLE XXII.

Name.	Date.	Time.	Cultures made on Endo plates.	Cultures grown in broth.	Name.	Date.	Time.	Cultures made on Endo plates.	Cultures grown in broth.
		a.m.					*a.m.*		
Schubert.....	Sept. 27	4	+	+	Schubert.....	Sept. 29	2	—	+
		9	+	+			6	—	+
		p.m.					8	—	+
		5	+	+			*n.*		
		8	+	+			12	—	+
		11	+	+			*p.m.*		
		a.m.					4	—	+
	" 28	3	+	+			9	—	+
		6	+	+			*a.m.*		
		p.m.				" 30	1	+	+
		2	—	+			8	+	+
		6	—	+			*p.m.*		
		10	—	+			4	+	+
		a.m.					11	+	+
	" 29	1	—	+					

During the entire study, it was noted that although practically the same quantity of urine was used for plating, the intensity of the growth on the Endo plate varied greatly. A *quantitative estimation* of bacteria in the urine was therefore undertaken in order to determine the approximate curve of excretion in any one patient. A pour plate in agar was made from 0.05 to 0.5 cc. of every specimen voided, incubated for 24 hours, and the number of colonies counted. Control cultures of each urine were made on Endo plates and in broth in order to ascertain any contaminating organisms. Results are shown in Table XXIV.

TABLE XXIII.

Name.	Date.	Time.	Culture made on Endo plate.	Culture grown in broth.	Name.	Date.	Time.	Culture made on Endo plate.	Culture grown in broth.
		a.m.					*p.m.*		
Schubert.....	Oct. 1	6	+	+	Mueller.....	Oct. 1	8	+	+
		10	+	+			*a.m.*		
		p.m.				" 2	10	+	+
		3	+	+			*p.m.*		
		7	—	—			1	+	+
		10	+	+			4	—	—
		a.m.					9	+	+
	" 2	1	+	+			*a.m.*		
		6	+	+		" 3	5	+	+
		11	+	+			*p.m.*		
		p.m.					2	+	+
		5	+	+			*a.m.*		
		10	+	+	Wietchel.....	Sept. 27	1	+	+
		a.m.					*p.m.*		
	" 3	3	+	+			3	+	+
		7	+	+			6	+	+
		p.m.					11	—	—
		3	—	—			*a.m.*		
		8	+	+		" 28	6	—	+
		11	+	+			*n.*		
		a.m.					12	+	+
	" 4	2	—	+			*p.m.*		
		8	+	+			2	—	+
Mueller	Sept. 29	8	+	+			7	—	+
		11	+	+			*a.m.*		
		p.m.				" 29	2	—	+
		4	+	+			7	—	+
		8	+	+			*n.*		
		a.m.					12	—	+
	" 30	6	—	+			*p.m.*		
		p.m.					2	—	+
		3	+	+			6	—	+
		8	+	+		" 30	6	+	+
		m.					9	+	+
		12	—	—			*a.m.*		
		a.m.				Oct. 1	7	—	—
	Oct. 1	6	+	+			*p.m.*		
		n.					1	—	+
		12	+	+					

TABLE XXIV.

Name.	Date.	Time.	Total No. of colonies per cc.	Name.	Date.	Time.	Total No. of colonies per cc.
		p.m.				*a.m.*	
Schubert..	Oct. 1	3	60,800	Schubert..	Oct. 8	*	0
		7	0		" 11	*	0
		10	6,260,000		" 12	*	0
		m.			" 14	*	0
		12	80,000			*p.m.*	
		a.m.			" 15	11	232
	" 2	6	25,100			*a.m.*	
		11	2,640,000		" 16	8	158
		p.m.			" 17	*	0
		5	2,400		" 18	*	0
		10	6,500		" 19	*	500
		a.m.				*p.m.*	
	" 3	3	520,000		" 20	9	300
		7	11,000			*a.m.*	
		p.m.			" 21	8	400
		3	0		" 22	*	0
		8	520,000		" 23	*	0
		11	2,240,000		" 24	*	0
		a.m.			" 25	*	0
	" 4	2	500,000		" 26	8	3,400
		p.m.			" 27	9	4,200
		2	10,370,000		" 28	8	2,000
		8	288		" 29	*	0
		11	3,200		" 30	*	0
		a.m.			" 31	*	0
	" 5	4	1,440,000		Nov. 1	*	0
		7	58,000		" 2	*	0
		p.m.			" 3	*	0
		11	20,800		" 4	*	0
		a.m.				*a.m.*	
	" 6	5	64		" 5	7	200
		p.m.			" 6	*	0
		2	1,200,000		" 7	*	0
		8	40		" 8	*	0
		a.m.			" 9	*	0
	" 7	1	0		" 10	*	0
		9	4,300				
		11	15,600				

* All specimens.

It is interesting to note the marked changes; *e.g.*, from 10,000,000 colonies to 200 in the course of 6 hours. Graphically represented, these figures are even more striking. The presence of several sterile specimens among the positive ones should be noted (Chart 1).

TABLE XXV.

Name.	Date.	Time.	Total No. of colonies per cc.	Name.	Date.	Time.	Total No. of colonies per cc.
		p.m.				*p.m.*	
Wietchel..	Sept. 30	6	24,000,000	Wietchel..	Oct. 5	11	0
		9	6,720,000			*a.m.*	
		10	28,000,000		" 6	8	0
		a.m.				*p.m.*	
	Oct. 1	7	0			2	0
		p.m.				3	0
		1	1,920			11	0
		6	18,200,000			*a.m.*	
		11	8,400,000		" 7	7	0
		a.m.			" 9	*	0
	" 2	7	64		" 10	*	0
		p.m.			" 11	*	0
		3	2,680,000		" 12	*	0
		7	3,600			*m.*	
		a.m.			" 12	12	800
	" 3	7	0			*a..m.*	
		n.			" 13	7	1,200
		12	36,400,000		" 14	*	0
		p.m.			" 15	*	0
		3	122,000		" 16	*	0
		6	11,600,000		" 17	*	0
		a.m.			" 18	*	0
	" 4	7	100		" 19	*	0
		p.m.			" 20	*	0
		9	4,140		" 21	*	0
		a.m.					
	" 5	7	216,000				

* All specimens.

Another patient (Wietchel) in whom a quantitative determination was undertaken proved of interest because the estimate was begun just before the urine became negative. It is observed that when the excretion of the typhoid bacteria ceases, it does not necessarily do so by a gradual downward curve but by an abrupt intermittent fluctuating one (Table XXV).

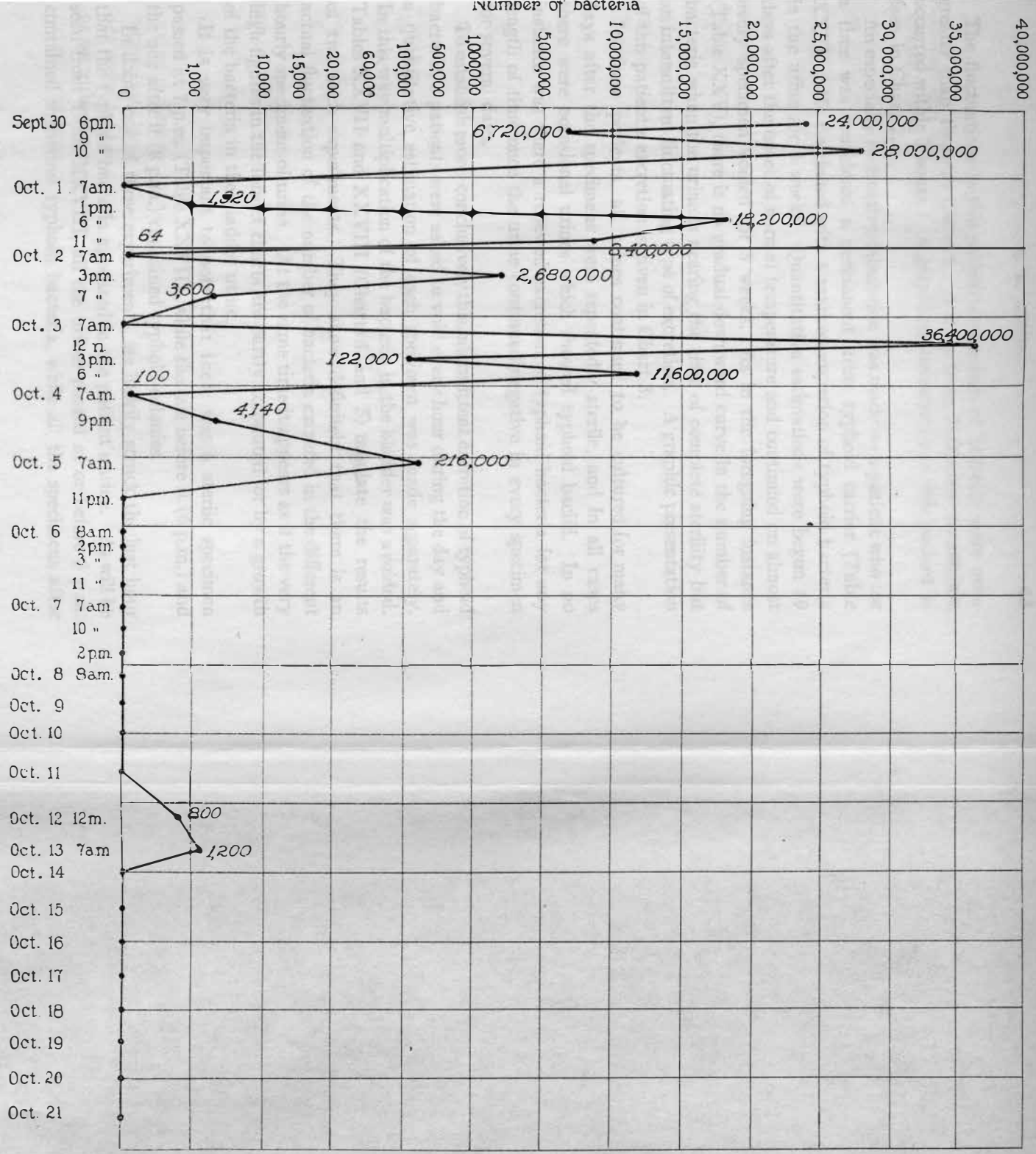

CHART 2. Quantitative estimation of bacteria in urine (Table XXV).

The fluctuations in this patient's excretion of bacteria were even greater than those in Chart 1. A drop from 36,000,000 to 100,000 occurred within 3 hours. A graphic representation of this patient is seen in Chart 2.

An especially instructive tabulation was made on a patient who for a time was considered a permanent urine typhoid carrier (Table XXVI). He continued with a marked excretion of typhoid bacteria in the urine for 6 weeks. Quantitative estimations were begun 19 days after the onset of normal temperature and continued on almost every specimen voided for 5 weeks. As in the foregoing instance (Table XXV), there is no gradual downward curve in the number of bacteria when the urine is nearing the time of complete sterility but an intermittent fluctuating type of excretion. A graphic presentation of this patient's excretion is given in Chart 3.

In these patients, all urines continued to be cultured for many days after the specimens were repeatedly sterile, and in all cases there were occasional urines which showed typhoid bacilli. In no instance was there a renewed excretion of typhoid bacteria for any length of time once the urine continued negative in every specimen for several days.

To establish more conclusively this intermittent excretion of typhoid bacteria, 3 patients were asked to void every hour during the day and a quantitative estimation of each specimen was made separately. In this way multiplication of the bacteria in the bladder was avoided. Tables XXVII and XXVIII (Charts 4 and 5) tabulate the results of two such experiments. They show definitely that there is an actual fluctuation of the number of bacteria excreted in the different hourly specimens of urine. At the same time it appears as if the very high figures in the former charts are partly accounted for by a growth of the bacteria in the bladder urine.

It is very important to note that there was a sterile specimen passed at 7 p.m. (Table XXVII), while the one before it (6 p.m.) and the one after it (8 p.m.) contained typhoid colonies.

In doing one of these experiments, we luckily struck the last hour that the typhoid bacteria appeared in the patient's urine. As will be seen from Table XXVIII, all the urines passed at or before 3 p.m. contained numerous typhoid bacteria, while all the specimens after

TABLE XXVI.

Name.	Date.	Time.	Total No. of colonies per cc.	Name.	Date.	Time.	Total No. of colonies per cc.
		p.m.				*a.m.*	
Mueller....	Sept. 30	8	1,330,000	Mueller...	Oct. 7	6	2,980,000
		m.			" 8	10	6,300,000
		12	0			*n.*	
		a.m.				12	2,000,000
	Oct. 1	6	150,000			*p.m.*	
		p.m.				2	1,280,000
		4	5,860,000			3	1,400,000
		8	2,160,000			7	141,000
		a.m.				9	1,260,000
	" 2	10	680,000			*a.m.*	
		p.m.			" 9	2	1,920,000
		1	1,800,000			6	740,000
		4	0			11	456,000
		9	1,050,000			*p.m.*	
		a.m.				3	1,740,000
	" 3	5	43,200,000			9	528,000
		p.m.				10	1,200,000
		2	10,300,000			*a.m.*	
		9	8,640,000		" 10	1	60,000
		a.m.				6	0
	" 4	9	12,300,000			11	10,400,000
		11	6,400,000			*p.m.*	
		n.				3	8,000,000
		12	15,800,000			8	1,400,000
		p.m.				10	980,000
		3	2,600,000			*a.m.*	
		5	8,400,000		" 11	1	1,120,000
		a.m.				7	1,320,000
	" 5	6	15,800,000			*p.m.*	
		11	10,000,000			2	1,400,000
		p.m.				5	760,000
		4	9,640,000			8	440,000
		5	1,680,000			*a.m.*	
		8	960,000		" 12	2	1,540,000
		m.				7	600,000
		12	8,600,000			*p.m.*	
		a.m.				2	1,200,000
	" 6	11	740,000			9	1,800,000
		p.m.				*a.m.*	
		2	560,000		" 13	6	2,420,000
		3	2,180,000				
		9	1,380,000				

TABLE XXVI—*Continued.*

Name.	Date.	Time.	Total No. of colonies per cc.	Name.	Date.	Time.	Total No. of colonies per cc.
		p.m.				*a.m.*	
Mueller....	Oct. 13	4	1,420,000	Mueller...	Oct. 20	1	840,000
		7	1,960,000			5	520,000
		a.m.				*n.*	
	" 14	3	1,620,000			12	1,880,000
		6	2,160,000			*p.m.*	
		11	1,240,000			3	1,820,000
		p.m.				7	1,420,000
		7	1,640,000			9	11,200
		11	1,280,000			11	1,120
		a.m.			" 21	6	9,000
	" 15	6	1,420,000			*a.m.*	
		10	1,260,000		" 22	9	1,880,000
		p.m.				*p.m.*	
		1	540,000			1	800,000
		4	860,000			2.30	3,800
		8	920,000			6	8,200
		a.m.				9	5,640
	" 16	2	760,000			*a.m.*	
		7	1,280,000		" 23	4	5,000
		11	940,000			8	2,200
		p.m.				*n.*	
		6	1,450,000			12	152,000
		a.m.				*p.m.*	
	" 17	1	750,000			3	52,600
		6	900,000			9	40,000
		11	1,240,000		" 24	*a.m.*	
		p.m.				6	3,920
		9	840,000			8	2,820
		a.m.				10	470.000
	" 18	6	750,000			*p.m.*	
		p.m.				2	94,000
		3	330,000			8	218,000
		8	1,060,000			11	300,000
		10	510,000		" 25	*a.m.*	
		a.m.				5	92,000
	" 19	4	720,000			*n.*	
		6	620,000			12	114,000
		p.m.				*p.m.*	
		1	400,000			2	2,920
		7	520,000			4	2,880
						9	180,000

TABLE XXVI—*Concluded.*

Name.	Date.	Time.	Total No. of colonies per cc.	Name.	Date.	Time.	Total No. of colonies per cc.
		a.m.				*p.m.*	
Mueller....	Oct. 26	1	158,000	Mueller...	Oct. 28	6	300
		5	240,000			9	50
		10.30	124,000			*m.*	
		p.m.				12	220
		6	1,000			*a.m.*	
		a.m.			" 29	10	240
	" 27	1	800,000		" 30	*	0
		7	1,860		" 31	*	0
		11	236,000		Nov. 1	*	0
		p.m.			" 2	*	0
		2	470,000		" 3	*	0
		8	166,000		" 4	*	0
		a.m.			" 5	*	0
	" 28	7	800		" 6	*	0
		10	650		" 7	*	0
		n.			" 8	*	0
		12	920				

* All specimens.

TABLE XXVII.

Name.	Date.	Time.	Specific gravity of urine.	Total No. of typhoid colonies per cc.
		a.m.		
Streitzel.....	Nov. 5	9	1.022	27,000
		10	1.020	8,200
		11	1.022	1,250
		n.		
		12	1.022	340
		p.m.		
		1	1.014	5,130
		2	1.016	100
		3	1.020	380
		4	1.016	10
		5	1.022	220
		6	1.026	20
		7	1.024	0
		8	1.020	170
		9	1.020	220

that showed no typhoid bacteria. From this particular patient, cultures were made separately of all urine specimens voided for the

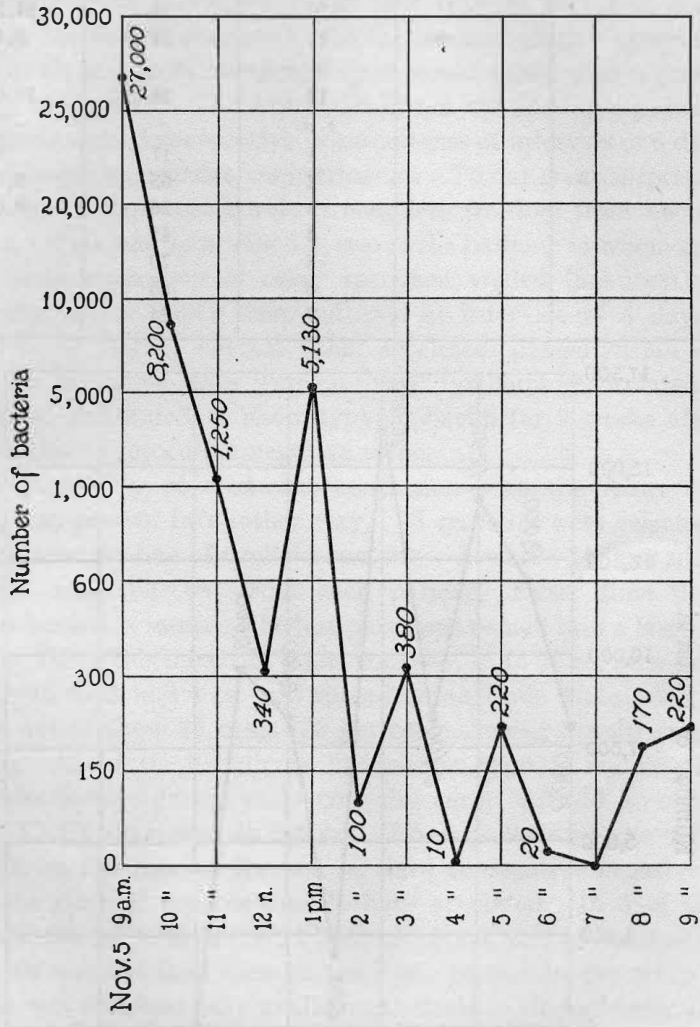

CHART 4. Hourly excretion of urine (Table XXVII).

16 days after that and not one of them showed typhoid bacteria. Such an abrupt and permanent cessation of the typhoid bacilluria is unusual.

TABLE XXVIII.

Name.	Date.	Time.	Acidity of urine in terms of cc. 0.1 N NaOH per 100 cc. of urine.	Total No. colonies per cc.
		a. m.	*cc.*	
Streitzel.....	Nov. 16	8	45	8,000
		9	26	12,000
		10	21	14,200
		11	24	8,400
		n.		
		12	26	16,000
		p. m.		
		1	17	880
		2	65	8,400
		3	26	9,600
		4	45	0
		5	20	0
		6	66	0
		7	54	0

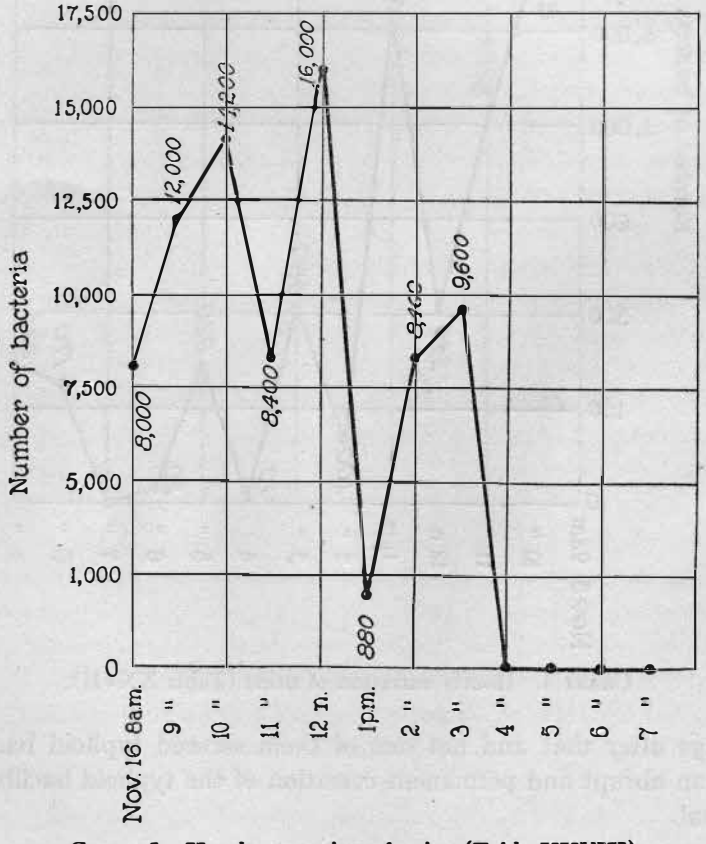

CHART 5. Hourly excretion of urine (Table XXVIII).

All experiments thus far described prove definitely that culturing a single specimen of urine for the presence of typhoid bacteria and concluding from a negative result that typhoid bacilluria does not exist, is absolutely erroneous. Even two consecutive examinations at intervals of a fixed number of days would surely miss a great percentage of carriers. The Army rule of not considering a patient free of bacteria until 3 consecutive urine cultures at intervals of 6 days are proved negative, appears very stringent. To our great surprise, even this is not a dependable rule if complete freedom from bacteria is sought. This was determined in one of the patients in whom cultures were made separately of every specimen voided (Schubert). The 3 routine urines which were cultured at intervals of 6 days happened to be sterile, whereas other specimens passed within several hours on the same days were positive; furthermore, isolated urine specimens continued to show typhoid bacilli for 2 weeks after the routine third consecutive negative urine.

The possibility of erroneous conclusion with the Army rule as guide, was proven in another way. 25 patients were selected who were apparently free of typhoid bacteria. A sterile 24 hour specimen of urine was collected from each patient. Every time that the patient voided, a sample (the last part) was passed into a large sterile bottle. From this mixed 24 hour specimen, 15 to 20 cc. were cultured in a broth flask and 1 cc. was spread on an Endo plate. We found that 5 out of these 25 cases (20 per cent) showed a positive typhoid growth. All of these patients had shown typhoid bacteria in the urine previously, during early convalescence. As will be seen from Table XXIX there was an interval of 5, 6, 8, 9, and 11 days respectively from the time of the last or third consecutive negative urine until the time of the positive 24 hour specimen. In 3 of these 5 patients, the bacteria in the 24 hour specimen were so numerous that a growth was obtained even on the Endo plate. In the other 2, the growth was obtained only in the broth flasks. Undoubtedly these 5 patients were excreting typhoid bacteria in the urine intermittently during the time of the 3 consecutive negative urines and even after that, although the urines had been considered no longer infectious.

TABLE XXIX.

Name.	No. of previous positive urine cultures during early convalescence.	Date of last positive urine culture.	Date of last negative urine culture.	Date of 24 hour specimen of urine.	Original Endo plate from 24 hour specimen.	Broth culture from 24 hour specimen.	Interval between last isolated negative urine culture and the positive 24 hour specimen.
							days
Buschendorf.	3	Sept. 23	Oct. 7	Oct. 15	+	++	8
Ackerman...	2	" 11	" 7	" 15	Few staphylococci.	Many staphylococci.	
Bolt.....	2	" 11	" 1	" 7	" "	None.	
Oestrick	2	" 10	" 14	" 7	None.	Few staphylococci.	
Pfefferman..	2	" 14	Sept. 26	" 7	++	++	11
Karpinsky ..	2	" 10	Oct. 3	" 7	None.	None.	
Koszuta	2	" 10	Sept. 30	" 7	Staphylococci.	*Bacilli proteus.*	
Bier.....	2	" 21	Oct. 12	" 15	None.	None.	
Schlange....	2	" 17	" 15	" 18	"	Staphylococci.	
Griechen....	2	" 19	" 7	" 15	"	Few staphylococci.	
Kramer.....	3	" 25	" 14	" 18	"	Colon bacilli.	
Majorowski	2	" 7	" 5	" 7	"	*Bacilli proteus.*	
Gesinski....	2	" 20	" 9	" 15	"	++	6
Kellerman ..	3	" 24	" 13	" 15	"	None.	
Herman	2	" 13	" 9	" 13	"	Few staphylococci.	
Haber.....	1	" 25	" 5	" 15	"	" "	
Poelman....	2	" 25	" 14	" 19	++	++	5
Dich	3	" 21	" 10	" 15	None.	Colon bacilli.	
Sander.....	3	" 25	" 14	" 18	"	Staphylococci.	
Jannssen....	4	" 25	" 14	" 18	"	"	
Miller	2	" 9	" 7	" 7	"	None.	
Mannoff....	3	" 23	" 14	" 23	"	+	9
Hollwege ...	3	Oct. 7	Nov. 3	Nov. 5	"	None.	
Adam	5	" 14	" 2	" 5	"	"	
Gutte.....	3	Sept. 14	" 2	" 5	"	"	

+ indicates typhoid bacilli present; ++, heavy growth of typhoid bacilli; none, sterile.

Accurate Method for Detecting Typhoid Bacilli in Urine.

The objection to using a 24 hour specimen of urine for culturing purposes is the possibility of secondary infection or contamination.

Of the original Endo plates made from the twenty-five 24 hour specimens, 19 were sterile, 3 had typhoid bacteria, and 3 showed staphylococci. Of the broth cultures 8 were sterile, 8 showed staphylococci, 2 showed colon bacilli, 2 showed *Bacillus proteus*, and 5 showed typhoid bacilli. The typhoid bacteria were in pure culture. The liability of contamination should not, therefore, be raised as an objection against culturing 24 hour specimens of urine.

When liquid media are employed, the quantity of urine used for culturing purposes is important. Cultures were made in broth from varying quantities of the same urines and, where the bacteria in the urine were few in number, cultures were positive only when larger quantities of urine were added to the broth. In the cultures of the 24 hour specimens of urine, 15 to 20 cc. of urine were added to 100 cc. of broth. This quantity has a wide margin of safety.

All the observations collected in this study leave no doubt that an accurate result for determining the presence of typhoid bacteria in the urine is possible only from a sample of a 24 hour specimen of urine which should be allowed to grow 24 hours in broth and then plated. The author is certain that were examinations made in this manner, it would be unnecessary to have more than 2 consecutive negative cultures before a patient might be considered free of typhoid bacteria in the urine. Furthermore, such cultures would not require 6 day intervals, but could be made at intervals of only 1 day or even on successive days. If 2 such negative 24 hour specimens are obtained, it is safe to assume that typhoid bacilluria no longer exists; for we know from our observations that if typhoid bacteria are absent from every specimen of urine for several days, they are absent for good and do not recur, except possibly in an isolated specimen which would escape detection anyhow. Certain it is that urines examined at random intervals of 6 days, for example, will not detect all typhoid convalescents who are still carriers. When it is realized that some urines contain as many as 43,000,000 bacteria per cc., the great danger of discharging a patient with bacteria in the urine is evident. The above, accurate, even though more laborious method, is therefore indicated.

B. Character of Urine; Origin and Frequency of Carriers.

Importance of Typhoid Urine Carriers.

While all writers lay sufficient stress upon the feces as a source for the spread of typhoid fever, the importance of the urine is usually underestimated. Even in a very recent and official publication (56) it is stated, "The feces of the patients suffering from typhoid is the most important means of contagion," and little reference is made to the urine. Simon (57) in his excellent book on "Human Infection Carriers" hardly mentions the infections by the urine. At the same time, typhoid epidemics have been traced to urine carriers. Beck and Ohlmüller (58) report such an epidemic which involved 6.7 per cent of the entire population of Detmold in 1904. The epidemic was traced to a water supply of the city. The working men constantly engaged at work at this source were examined and one was found to be a urine carrier. He had had typhoid fever 3 months previously. The bacilli were also found in the soil near the well and were traced to a latrine made of earth which was used by these working men.

The earliest cases of typhoid bacilli in the urine were reported about the same time by Rovsing (59), Houston (60), Young (61), and Brown (62). It is interesting to observe that the carrier problem was not clearly understood, as Brown at that time explained the presence of the numerous typhoid bacilli in the urine by a contamination from the outside, presumably by catheterization, although the woman had had typhoid fever 35 years previously.

From the studies reported by the writer in the previous paper, it seems certain that typhoid patients are very often discharged with typhoid bacilli still in their urine. Because of the frequency with which the urine is voided, the myriads of organisms that it may contain, its relatively inoffensive character, and the consequent indifference to the place of its disposition by ignorant and careless nurses and convalescents, it becomes as potent a factor, if not a more potent one than the feces in the spread of the disease.

The percentage of typhoid *convalescents* who have typhoid bacilluria (49per cent) is greater than the percentage of patients who have bacilli in the feces (39 per cent). Then too, the *actual number of bacteria* is far greater in the urine than in the stool. The greatest

number found in our series of patients was 43 million per cc. Petruschky (63) reports a urine in which there were 170 million bacteria per cc., and Gwyn (64) reports one in which there were 500 million per cc.

The urines containing typhoid bacteria were especially studied with a view to ascertaining whether any relationship could be discovered between the existence or the degree of the bacilluria and any of the chemical properties of the urines.

Chemical Properties of Urines Containing Typhoid Bacilli.

(*a*) It was found that the number of bacteria had no bearing whatever upon the *specific gravity*. In Table XXVII (Streitzel), for example, it is noted that a urine with a specific gravity of 1.014 contained 5,130 bacteria, while a urine with a specific gravity of 1.024 was sterile. Other examples of such lack of relationship are cited in Table XXX. These specimens are all from the same patient passed at different times.

TABLE XXX.

No. of bacteria per cc.	Specific gravity of urine.	No. of bacteria per cc.	Specific gravity of urine.
680,000	1.018	330,000	1.020
1,800,000	1.014	1,060,000	1.022
0	1.014	500,000	1.028
1,050,000	1.020	720,000	1.010
43,000,000	1.022	620,000	1.018

(*b*) The *acidity* of the urines was examined for a possible explanation of the great variations in the number of bacteria. The acidity was roughly estimated by titrating the urines with decinormal sodium hydroxide solution with phenolphthalein as indicator. No relationship between acidity and typhoid bacilluria or the number of bacteria could be determined (Table XXVIII).

(*c*) The *turbidity* of the urine in relation to the number of typhoid bacteria was also studied. If a urine was turbid for no apparent cause other than the bacteriuria, that specimen usually contained more than 4,000,000 bacteria. At the same time it seemed very difficult to attribute the turbidity entirely to the bacilluria because

there were very many absolutely clear urines with even greater numbers of bacteria. One urine had 32,000,000 bacteria per cc. and was clear. Thus, no absolute relationship between turbidity and bacilluria could be offered.

(*d*) The association between *albuminuria* and *bacilluria* was of special interest. The author cannot agree with Besson (65) or with Wood (66) that typhoid bacteria are present only when a considerable quantity of albumin is passing through the kidneys.

While the exact percentage has not been estimated, a great number of specimens that had 10 to 20 million bacteria per cc. evidenced no albumin whatever. There is no doubt that bacilluria can exist without albuminuria. A consideration of the reverse possibility, namely, whether albuminuria necessarily implies the presence of bacilluria, would lead to erroneous conclusions, for there are very few typhoid cases which do not at some time or other during the course of the disease show albuminuria. The synchronous appearance of bacilluria and albuminuria is a coincidence as often as it is a matter of dependence. Sharp lines should be drawn between albuminuria and nephritis. True lasting nephritis (not merely toxic or febrile albuminuria) was found in only 2.5 per cent of our typhoid cases (4 out of 164) and all of them showed typhoid bacilli in the urine. *Vice versa*, of the 49 per cent of patients who presented a typhoid bacilluria, only 5 per cent showed the picture of a true nephritis.

As to the *frequency* of *typhoid bacteria* in the urine, it is interesting to note the wide variations reported. Petruschky (63) found bacilluria in 6 per cent of his 50 cases; Horton-Smith (67), in 28 per cent of his 39 cases; Schüder (68), in 23 per cent of his 22 cases; Cole (69), in 35 per cent of his 49 cases; Richardson (70) in a review of the literature from 1887 to 1903 states that 30 observers had made bacteriologic investigations of the urines in 1,291 cases of typhoid fever and had detected bacilluria in 21.5 per cent of the cases. Osler (71) gives the percentage as 20 to 25.

These differences in statistics are undoubtedly explained by the varied laboratory technique employed, and the frequency of the individual urine cultures.

Of our 164 patients there were 80, or 49 per cent, who showed bacilluria at some time during convalescence. The duration of the bacil-

luria is shown in Table XXXI; the number of weeks after normal temperature that the bacteria continued in the urine is also designated.

Thus, it is seen that 11 cases (13.7 per cent of all positive cases or 6.7 per cent of all typhoid cases) remained positive for longer than 1 month after normal temperature, and 2 cases (2.5 per cent of all positive cases or 1.2 per cent of all typhoid cases) were positive for longer than 2 months. No patients in our series remained carriers for longer than 3 months.

TABLE XXXI.

No. of cases.	Per cent of all positive cases.	Per cent of all typhoid cases.	Duration of positive condition.
			weeks
17	21.2	10.3	1
34	42.5	20.7	2
12	15.0	7.5	3
6	7.5	3.7	4
2	2.5	1.2	5
2	2.5	1.2	6
2	2.5	1.2	7
3	3.7	1.8	8
1	1.2	0.6	9
1	1.2	0.6	11

TABLE XXXII.

No. of cases examined.	Time of examination after normal temperature.	No. of cases positive.	Per cent of cases positive.	No. of cases negative.
	week			
127	1st	80	63	47
145	2nd	63	44	82
162	3rd	29	18	133
164	4th	17	10	147

A tabulation was made of the number of positive and negative cases occurring at various fixed intervals after normal temperature (Table XXXII).

From Table XXXII it may be inferred that one may delay culturing the urine for bacteria until the second week of convalescence, because in almost two-thirds of the cases, typhoid bacilli will be found during the first week after normal temperature. On the other hand, we had 8 patients in whom the urine became permanently nega-

tive starting 3 to 10 days before the temperature had come down to normal.

From Table XXXII it is also noticed that our original figure of 80 cases of bacilluria out of a total of 164, or 49 per cent, would have been as high as 63 per cent had we been able to examine all of our cases during the 1st week after normal temperature. Owing to the stress of work, in 37 patients the first culture of the urine was made only in the 2nd to the 4th week after normal temperature.

In 18 cases during the 2nd week after normal temperature.
" 17 " " " 3rd " " " "
" 2 " " " 4th " " " "

There are some patients in whom the bacilluria disappears with the onset of normal temperature and our investigations covered only the period of convalescence; *i.e.*, after normal temperature had set in.

The author has considered the possibility that practically every patient has typhoid bacteria escaping in the urine at some time or other during the disease or convalescence. This does not seem unreasonable in view of the fact that typhoid bacteria circulate in the blood of every patient, and therefore some of these bacteria may escape through the glomeruli which have been exposed to toxic action for a shorter or longer period. This assumption can readily be investigated at a future occasion.

Source and Origin of Typhoid Bacilluria.

The source and origin of the typhoid bacilluria is not a simple problem for solution. No one explanation can be satisfactorily offered for all instances. Thus, it is erroneous to assume, as does Horton-Smith (67, 75), that in most cases stray bacilli find their way from the blood and through the kidney into the bladder and that it is here that the bacilli grow and multiply. This theory will account for only those cases in which the bladder is not entirely emptied at micturition, as occurs normally in many women or in men with prostatic hypertrophy or diverticula of the bladder. In these patients the retained urine remains to infect subsequent secretions of the kidney. The present writer is of the opinion that the bladder origin for typhoid bacilluria holds true only in a minority of patients and

that in by far the greater majority of cases the bacteria come from the urinary tract above the bladder. The author's studies (see Section V, A) which showed the variations in the number of bacteria of the different specimens and even the complete sterility of some, speak in favor of this view. If the bacteria were present in the bladder alone, one would expect them to be in the urine constantly and in approximately the same quantity, although it cannot be denied that bacteria may multiply in the bladder. The experiments in which hourly specimens were examined eliminated the factor of the bacterial growth in the bladder and proved that the urine as it comes down from the kidney already contains different quantities of bacteria at different times. Furthermore, in two of our cases the ureters were catheterized and typhoid bacilli were found in the urine obtained only from the left side, the urine from the right side was sterile and the washings from the bladder proved sterile also. All these findings convinced the writer that in the usual typhoid bacilluria the urine already contains the typhoid bacilli before it reaches the bladder.

When we attempt to answer the question as to how the bacteria get into the urine, we are confronted with just as difficult a question.

From the standpoint of pathological anatomy, several types of lesions have been found in the kidneys of urine carriers which account for the presence of the typhoid bacilli in the urine.

1. Multiple Focal Abscesses of the Kidney or Hydronephrosis.—Although this condition is not frequent, undoubted examples have been reported (Greaves (72), Meyer and Ahreiner (73)). In the case reported by Lieutenant-Colonel Nichols (41), a urine carrier condition which had continued for 6 years was due to a cystic kidney and was cured by nephrectomy. A pure culture of typhoid bacilli was isolated from the abscesses.

2. Acute Nephritis.—The typhoid bacilli, like any other of the infectious bacteria, may cause a focal localization of the infection in the kidneys, resulting in an acute nephritis and subsequent escape of bacteria into the urine.

Both of these conditions are comparatively rare in proportion to the frequency with which typhoid bacilluria occurs. Such marked anatomical changes probably account for some types of the more chronic urine carriers. The more transient bacillurias, however,

which occur during the acute disease or convalescence cannot be explained on this basis. For these cases, a pathological condition must be found which is of a more general type and one which can proceed more readily to healing and disappearance of the bacilluria. Such a lesion is offered by the areas of focal necroses which take place in the kidneys as they do in the liver and spleen.

3. Areas of Focal Necroses.—When one remembers that bacterial emboli are of frequent pathological occurrence during typhoid fever, the cause of these areas of focal necroses is evident. Cagnetto and Zancan (74) have shown that in most cases of typhoid fever, inflammatory foci (so called "lymphomata") with focal necroses or even minute abscesses are found in the kidneys. These foci may act as vegetation depots for the typhoid bacilli from which the bacteria make their way into the adjacent urinary tubules.

These three hypotheses imply a pathological destructive process of the kidneys which can be made out macroscopically or microscopically. On the other hand, the view may be advanced that the passage of the bacilli into the urine is merely a filtering process.

4. Filtering Process.—Since typhoid organisms invade the blood stream in probably every case of typhoid fever, it is possible that some bacilli may find their way through the glomeruli into the urine. The glomerular structure and function may be rendered of lowered resistance by the bacteria themselves or by the effects of their toxins and thus allow the escape of some bacteria. That the kidney may possess the function of filtration, has not been definitely accepted. Granted that it does, one should expect to find the typhoid organisms in the urine at the time when the bacteremia is at its height. This is contrary to the actual state of affairs, because the bacilluria usually starts at a time when the bacteremia no longer exists. Nevertheless, the writer has several times observed instances in which a former negative urine would begin to show typhoid bacteria with the onset of a relapse.

Thus it is seen that no one cause can be given for all cases of typhoid bacilli in the urine. The combination of the filtration process and the destructive process in the form of the areas of focal necroses will probably explain most of the transient bacillurias occurring during convalescence.

The mechanism whereby the bacteria escape into the urine being granted, this does not, however, by itself account for the marked variations in the number of bacteria in the different specimens and the complete sterility of some. Horton-Smith (67, 75) answers this question by the varying growth of the bacteria in the bladder. For reasons previously quoted we cannot agree with this assumption, but believe that when the urine reaches the bladder, it already contains different quantities of bacteria at different times. Retention of the urine in the bladder with subsequent growth of bacteria therein will only accentuate the already existing differences. The writer is of the opinion that it is an infection in the pelvis of the kidney or its numerous calyces which accounts for these variations in number.

5. *Infection in the Pelvis of the Kidney.*—The pelvis bears the same relationship to the kidney that the gall bladder does to the liver. The bacteria may lodge in some calyces and not in others or some of the calyces may be infected more than others. The difference in the degree of bacilluria at different times will therefore be dependent upon which calyces are flushed out at a particular time and how completely this is done.

Furthermore, it is possible that the pelvis of the kidney does not completely empty at all times. The bacteria may be retained in some dependent portion thereof and grow there until the next occasion when the pelvis is completely flushed out, resulting in a shower of bacteria in the urine.

One cannot designate this condition as pyelitis any more than one can designate the gall bladder condition in some chronic bile carriers as cholecystitis. In neither instance does the microscopical examination of the urine or bile, respectively, necessarily show inflammatory evidences. The pelvis acts as a storehouse for the kidney in the same way that the gall bladder does for the liver. In the pelvis of the kidney, the bacteria do not multiply as readily as in the gall bladder or for as long a time: first, because the outlet into the bladder by means of the ureter is freer than the outlet into the intestines by means of the common bile duct; second, in the case of the urine, there is a more continuous stream and consequently a more frequent flushing than there is with the bile; and third, bile is a better medium

for the growth of typhoid bacilli than urine. These differences probably account for the higher percentage of chronic or persistent gall bladder carriers than urine carriers.

Classification of Urine Carriers.

In the same comparative light one may propose a classification of urine carriers into kidney carriers, pelvic carriers, bladder carriers, or various combinations of these; just as we have classified liver carriers, gall bladder carriers, and intestinal carriers, or their combinations.

While we have offered sufficient clinical and laboratory evidences in proof of the different bile carrier types, the classification of urine carriers is given only as a working basis for further substantiation and complete proof.

That pure *kidney carriers exist,* can be and has been very readily demonstrated by ureteral catheterization where typhoid bacilli are obtained from one side only. Complete removal of the infected kidney clears up the carrier state. In these patients the involved organ usually shows marked destructive lesions, as multiple abscesses or hydronephrosis (case of Lieutenant-Colonel Nichols (41)). Even in the transient bacillurias, the kidney alone may be the organ accountable for the carrier condition. In 2 cases of our series, bladder washings were sterile, while typhoid bacilli were obtained from the left ureter only. These patients remained carriers for 5 and 6 weeks respectively but then cleared up.

That pure *bladder* carriers may exist, with no bacteria in specimens obtained by catheterization of the ureters is also known. Women are more predisposed to this type on account of their incomplete emptying of the bladder. Originally, the bacteria enter the bladder probably by way of the kidneys but continue to grow in the retained bladder urine even after the original process in the kidneys has cleared up. This type of carrier should be readily amenable to local treatment. A bladder carrier of a somewhat different type is represented by the cases of typhoid cystitis, although pure typhoid cystitis is not very frequent. In our entire series of 164 patients, there was only 1 case of true cystitis. The patient persisted with typhoid bacteria in the urine for over 2 months but then cleared up

entirely without any local treatment. Naturally, combinations of the kidney and bladder types are to be readily expected, because of the ease with which extension of the infection either by the descending or ascending route may take place.

Pelvic carriers are as yet entirely problematical. No evidence can be offered as proof of their existence unassociated with either bladder or kidney involvement.

Omission should not be made of the suggestion offered by Pick (76) that in some urinary carriers the bacilli lie in nests situated in recesses of the urinary tract; *e.g.*, the urethral ducts, prostatic ampullæ, or vesiculæ seminales. Pick examined these organs in 32 autopsies of typhoid cases and in 2 instances discovered a suppurative spermatocystitis and prostatitis due only to *Bacillus typhosus*. Such types of chronic carriers are probably exceptional.

Many of the phases of the urine carrier problem have not passed out of the hypothetical or experimental stage. Detailed analyses have been given of some of the facts that are known, and some of the questions must still be investigated in order that we may change our old ideas and stimulate new ones in the consideration of this very important condition.

VI. THE COMPLEMENT FIXATION TEST FOR TYPHOID FEVER. STATISTICAL, CLINICAL, AND EXPERIMENTAL STUDIES.

Complement Fixation Test during Typhoid Convalescence.

Several years ago, the author proposed the complement fixation test as an additional laboratory aid for establishing the diagnosis of typhoid fever (77). It was then shown that practically all patients with typhoid fever develop complement fixation bodies sooner or later during the course of the infection. After further study (78) the writer found that people who had been inoculated against typhoid fever with the usual 3 antityphoid vaccine inoculations (Rawling strain) gave a positive complement fixation test only exceptionally and for a very short time. This reaction therefore becomes of especial importance as a differential diagnostic aid in patients who are suspected of having typhoid fever, and give a positive Widal test due to previous immunization, but in whom no typhoid bacteria can be found in the blood or excretions. Felke (79) from a similar study arrived at a similar conclusion. Hage and Korff-Petersen (80) agreed in principle to this differentiation but found that a small percentage of their inoculated persons gave complement fixation for 2 weeks after inoculation but only exceptionally after 2 months.

The author's report published in 1914 (77) included fixation tests in patients during the active stage of the illness; but no systematic report exists in the literature referable to the value of the complement fixation reaction as a diagnostic test during typhoid convalescence, or months after the infection. The present paper includes such tests made on 160 patients. The stress of other work did not permit of testing the bloods sooner than 5 weeks after normal temperature. Table XXXIII shows the results of the examinations then made.

It is noted that as high as 54 to 57 per cent of patients still give a positive complement fixation test 4 months after the active stage of typhoid fever, and in two-thirds of these the reaction is very strong (++++).

Through the interest of Colonel Russell, samples of blood from these patients (who were then at the Prisoners War Barracks at Fort Oglethorpe), were sent to U. S. A. General Hospital No.12 several months later and the results of the second survey are shown in Table XXXIV.

From a review of these two tabulations, it is evident that in about 30 per cent of typhoid patients the complement fixation bodies disappear from the blood by the 2nd month after the onset of normal

TABLE XXXIII.

Time of examination after normal temperature.	No. of cases examined.	No. of cases positive.	Degree of positive.				Per cent of positive.
			++++	+++	++	+	
2nd month (5, 6, 7, 8 weeks).....	22	16	13	1	1	1	72.7
3rd month (9, 10, 11, 12 weeks)....	114	67	43	5	9	10	54.0
4th month (13, 14, 15 weeks).....	14	8	5	0	0	3	57.0
Total.....	150	91	61	6	10	14	60.0

TABLE XXXIV.

Time of examination after normal temperature.	No. of cases examined.	No. of cases positive.	Degree of positive.				Per cent of positive.
			++++	+++	++	+	
5th month (17, 18, 19, 20 weeks)...	41	24	9	7	4	4	58.0
6th month (21, 22, 23, 24 weeks)...	119	49	20	7	12	10	41.0
Total.....	160	73	29	14	16	14	45.0

temperature. During the 3rd, 4th, and 5th months after convalescence, an additional 10 to 15 per cent of the patients lose this reaction. At the end of a half year 40 per cent of patients still show a positive complement fixation test.

40 cases which were positive at the 2nd examination, were reexamined 2 to 3 months later. Only 2 reactions became negative; 38 continued positive. The detailed results are shown in Table XXXV.

These findings brought up the question why the complement fixation antibodies persisted in some patients long after convalescence, while in others they disappeared soon after or even before the onset of normal temperature.

An analysis of our cases was therefore made from a clinical standpoint in order to determine any factors which might possibly account for these variations.

TABLE XXXV.

No. of cases in each group.	Reaction at 2nd examination.	Reaction at 3rd examination.	3rd examination.	
			No. of cases.	Interval after normal temperature.
				months
10	++++	++++	1	6
			6	7
			2	8
			1	9
5	++++	+++	3	6
			1	7
			1	8
1	++++	—		7
5	+++	+++	3	6
			1	7
			1	8
3	+++	++	1	7
			2	8
9	++	++	4	7
			5	8
1	++	+		7
5	+	+	4	7
			1	8
1	+	—		7

Factors Accounting for the Persistence of a Positive Test.

1. Blood Culture Findings.—It is well known that in animals antibody formation is usually most rapid and marked when the intravenous method of immunization is employed. It was therefore considered probable that patients with a persistent positive blood culture would be more likely to continue with a positive complement fixation test.

It was found that out of 38 cases which had a positive blood culture only 24, or 63 per cent, showed a positive complement fixation test. This lasted in:

3 patients for 3 months after normal temperature.
12 " " 4 " " " "
8 " " 5 " " " "
1 patient " 6 " " " "

Vice versa, of the 83 patients who had a negative complement fixation test during the second or third month of convalescence, 14, or 16 per cent, had shown a positive blood culture during the acute stage of the disease.

It is evident that one cannot consider a positive blood culture as the indicator of a future persisting complement fixation test. Most or even all typhoid cases have a bacteremia during the early days of the infection, but we detect the bacteria in the blood of some and not of others because in some the bacteria are probably in greater numbers in the free circulation or they remain there for a longer time. A negative blood culture only signifies that the blood was examined at an inopportune time.

2. Length of the Fever Course.—It was felt that the longer the fever existed, the more likely was it that live bacteria were in action and consequently antibodies were being stimulated.

Of 59 patients who had a ++ to ++++ positive fixation test 4 to 6 months after normal temperature:

19 ran a fever for less than 4 weeks.
31 " " " " 5 to 8 weeks.
9 " " " " longer than 2 months.

For comparison, of 59 patients who gave a negative fixation test 2 to 3 months after normal temperature:

29 ran a fever for less than 4 weeks.
26 " " " " 5 to 8 weeks.
4 " " " " longer than 2 months.

The patients who ran a longer fever course appeared more prone to develop a lasting complement fixation test. Still, the length of the fever course alone cannot be considered the deciding factor, as there were many patients with a prolonged fever who gave a negative com-

plement fixation test, and some who ran a very short illness who nevertheless showed many fixation antibodies.

3. Severity of Disease.—As has been said before, the severity of the illness of our patients was considered entirely separate from the length of the disease, and was graded on a + + + + scale. The + + + + patients all died.

Of the 59 cases with a positive complement fixation test 4 to 6 months after convalescence:

3	were	graded	−
37	"	"	+
15	"	"	++
4	"	"	+++

or a total of 79 plus.

Of the 59 cases who were negative at the first examination:

5	were	graded	−
42	"	"	+
7	"	"	++
5	"	"	+++

or a total of 71 plus.

From these figures, it seems as if the severer cases evidenced a greater percentage of positive complement fixation tests. On the other hand, severity of disease alone cannot be taken as the criterion, since it is seen from the above statistics that there are many very mild cases which developed lasting complement fixation tests, and *vice versa* many sick cases who are negative early during convalescence.

4. Relapses and Recrudescences.—The relationship which relapses and recrudescences bear to the stimulation of complement fixation antibodies was considered apart from either the length or severity of the illness. It was thought that the patients who have such setbacks usually go through a longer and severer infection and consequently complement fixation tests would be more prone to remain positive for a prolonged period of time. For once, logical assumption found corroboration in statistics. Of 17 patients who suffered *relapses*, 16 were positive for 2 to 3 months, and 14 continued positive for 6 months or longer after normal temperature. It is interesting to note that the only patient who was negative had 3 relapses and finally died with typhoid bacteria still in the gall bladder (possibly a complete lack of

immunizing power). Of 14 patients with *recrudescences*, 9 showed a lasting complement fixation test. Thus, out of 31 patients with either recrudescences or relapses, 25, or 80 per cent, continued with abundant complement fixation antibodies for many months after convalescence.

5. Complications.—It was assumed that patients with true typhoid complications, *i.e.* those caused by *Bacillus typhosus* and not a secondary invader, have a greater number of bacteria or toxins to cope with, and therefore would be more likely to combat the increased infection by the production of a greater number of antibodies. Such conditions as *Staphylococcus furunculosis* or adenitis, nephrolithiasis, chronic bronchitis, etc., were not included in these statistics. Of 31 cases which had a complication due to the typhoid bacillus, 23, or 74 per cent, showed a positive fixation test which continued for many months after complete convalescence (Table XXXVI).

TABLE XXXVI.

	Complement fixation test.			Complement fixation test.	
	No. positive.	No. negative.		No. positive.	No. negative.
Pleuritis	1		Nephritis	2	
Typhoid spine	1	1	Periostitis	1	2
Neuritis	1		Psychosis	1	
Cholecystitis	5	2	Phlebitis	1	2
Parotitis	1		Pneumonia	1	
Hemorrhage	4	1	Perforation	3	
Appendicitis	1				

The Complement Fixation Test in Carriers.

The relationship between the typhoid carrier state and the presence of complement fixation bodies is of extreme importance. It is best to discuss the findings in the temporary and permanent carriers separately.

For the *temporary* carriers, the statistics are based upon patients in whom the bacteria in the urine or stool persisted at repeated examinations. Cases with an occasional presence of the typhoid bacillus in the excreta were almost the rule and were therefore not included in the analysis.

In 10 *urine carriers*, complement fixation persisted strongly positive; in 4 for 2 to 3 months, and in 6 for 4 to 6 months, after convalescence; in 9 of these 10, the test remained positive for months after the carrier state cleared up.

Of 3 *feces carriers*, 2 were positive for 4 months, and 1 negative early during convalescence. Contrary to expectation the latter became a permanent bile carrier.

Of 19 patients with *typhoid bacilli both in the urine and stool*, 15 continued to show strong complement fixation for 6 months and longer after convalescence, while 4 were negative 2 months after normal temperature. The complement fixation tests remained positive after the bacteria had disappeared.

Taking all these classes together, we found that out of 32 typhoid carriers, 27, or 85 per cent, showed a constant and strong fixation reaction, while in 15 per cent the fixation antibodies disappeared early, although the carrier state continued.

Vice versa, in almost all of the temporary carriers with a strongly positive complement fixation, the antibodies remained demonstrable long after the bacteria had disappeared.[7]

As for the *permanent or chronic carriers*, scanty references are found in the literature regarding their complement fixation bodies.

Schöne (81) reports the findings in 3 carriers, only 2 of whom were chronic carriers. The reaction was positive in one of $10\frac{1}{2}$ years duration and negative in the other of 2 years duration. The author (77) reported a urine carrier of 1 year standing with a positive reaction. Henderson-Smith (82) demonstrated these bodies in the sera of 2 carriers both of whom gave negative Widal tests.

Of 4 chronic (probably permanent) bile carriers of 8 to 9 months duration, only 3 continued strongly positive. The fourth (mentioned above) was negative 2 months after normal temperature. In 1 patient who was a very marked bile carrier and who was cured by cholecystectomy 4 months after the acute illness, the complement fixation bodies were still present in great numbers 8 months after operation.

[7] It was difficult to determine how long the test remained positive as all the the patients were transferred to ports of debarkation.

3 chronic carriers were examined by the author for Lieutenant-Colonel Nichols with the following results:

1. Typhoid fever in Jan., 1918; carrier condition results; cholecystectomy in Nov., 1918; cured; complement fixation strongly positive in Feb., 1919.
2. Typhoid fever in 1910; carrier condition results; cholecystectomy in 1918; carrier condition continues; complement fixation strongly positive in Feb., 1919.
3. Typhoid fever in 1905; carrier condition results; cholecystectomy in 1918; carrier condition continues; complement fixation ± in Feb., 1919.

Résumé.—From an analysis of the above discussions, the writer has formed the conclusion that the fixation test is dependent directly upon the number of typhoid bacteria with which the patient is infected, and the length of time that they remain in the system. The greater the number or the longer the time, the stronger and more lasting will the test be. A large number of bacteria may or may not, however, mean a sick patient, a long disease, a relapse, a complication, or a carrier state. Other factors such as the virulence of a particular bacillus, the resistance of the individual, etc., which we cannot estimate, bear additional influence.

Therefore one cannot foretell whether a particular patient will persist with a complement fixation test or not. If from the clinical course of the disease or the laboratory findings, one has reason to feel that the patient is infected with a large number of bacteria and over a prolonged period of time, one may assume that complement fixation will continue positive for a long time, even years after convalescence. This is usually the case in long continued illness, or with relapse or complication or carrier condition. Reversely, the continued positive test may be the first indication of the systemic response to a large number of typhoid bacteria.

With this hypothesis in mind, experiments were undertaken in rabbits in order to reproduce some of the bacteriological factors mentioned above as influencing the production and persistence of the complement fixation test.

Experimental Studies to Prove the Origin of the Complement Fixation Bodies.

Experiment 1.—The first set of experiments attempted to present conditions similar to those existing in man after prophylactic immunization. 2 rabbits

were inoculated subcutaneously with ordinary typhoid vaccine (Rawling strain). The dose of vaccine used (30 million bacteria) bore the same approximate proportion to the dose in man (2,500 million) as did the weight of the rabbit (2 pounds) to that of man (150 pounds).

Result.—Antibodies were not produced in sufficient numbers to be demonstrated by complement fixation tests at intervals of 2, 4, 6, 8, and 12 weeks after inoculation.

The agglutination test was partially positive in the serum dilution of 1 to 50 1 week after inoculation, but negative after that.

These complement fixation results tally with the findings in man after prophylactic inoculation.

Experiment 2.—In the second set of experiments 5,000 million bacteria were given to the rabbits subcutaneously. This was approximately 150 times the dose used in man on the basis of weight comparisons.

Result.—Even this excessive dose did not stimulate sufficient complement fixation bodies to give the test.

Agglutinins were readily demonstrable in serum dilution 1 to 50 1 week after immunization.

Experiments 3 and 4.—The first two experiments were repeated, but instead of the ordinary vaccine, the lipoid vaccine was used both in the small and large dosage employed in Experiments 1 and 2 respectively.

Result.—Complement fixation bodies were not produced in sufficient numbers to give the reaction.

Likewise, 17 nurses who received the triple lipoid vaccine never showed complement fixation bodies.

Experiment 5.—This experiment aimed at producing a bacteremia of a comparatively mild type, and for a short period of time, by means of the repeated intravenous injections of living bacteria. Controls were also made in other rabbits by using the same number of bacteria but killed by heat. The dosage employed in these experiments was similar to the smaller doses used in the former experiments and comparatively the same as are used in human beings for prophylactic immunization. Rabbit 1 received 250 million dead typhoid bacilli intravenously on 3 successive days. Rabbit 2 received 500 million dead typhoid bacilli intravenously on 3 successive days. Rabbit 3 received 250 million living typhoid bacilli intravenously on 3 successive days. Rabbit 4 received 500 million living typhoid bacilli intravenously on 3 successive days (Table XXXVII).

Thus it is noted that complement fixation bodies were readily produced in the serum of rabbits if a sufficient number of bacteria remained in the system long enough. The greater the number of typhoid bacilli, the more marked was the fixation. Living bacteria produced a serum richer in antibodies than did inoculation with dead bacteria.

TABLE XXXVII.

Quantity of serum (heated) used for fixation.	Rabbit 1.			Rabbit 2.			Rabbit 3.			Rabbit 4.		
	1 week after inoculation.	1 month after inoculation.	2 months after inoculation.	1 week after inoculation.	1 month after inoculation.	2 months after inoculation.	1 week after inoculation.	1 month after inoculation.	2 months after inoculation.	1 week after inoculation.	1 month after inoculation.	2 months after inoculation.
cc.												
0.05	++	++	*	+	++	+	++	++	++	++	++	++
0.02	±	+		+	++	+	++	++	++	++	++	++
0.01	±	+		+	+	+	++	+	++	++	++	++
0.005	0	+		±	+	+	+	+	+	++	+	++
0.002	0	±		±	+	±	±	±	0	+	±	+
0.001	0	0		±	±	0	±	±	0	0	±	+
0.0005	0	0		0	±	0	±	0	0	0	±	±

++ = complete fixation; + = partial fixation; ± = slightest fixation; 0 = no fixation.

* Tests were spoiled.

Experiment 6.—This last experiment was similar to Experiment 5, but the dosage of vaccine employed was 6 to 30 times greater than in the latter experiment. Rabbit 1 received 1,500 million living bacteria intravenously on 3 successive days. Rabbit 2 received 1,500 million living bacteria intravenously on 7 successive days. Rabbit 3 received 7,500 million living bacteria intravenously on 3 successive days (Table XXXVIII).

TABLE XXXVIII.

Quantity of serum (heated) used for fixation.	Rabbit 1.				Rabbit 2.				Rabbit 3.			
	3 days after inoculation.	2 weeks after inoculation.	1 month after inoculation.	2 months after inoculation.	3 days after inoculation.	2 weeks after inoculation.	1 month after inoculation.	2 months after inoculation.	3 days after inoculation.	2 weeks after inoculation.	1 month after inoculation.	2 months after inoculation.
cc.												
0.05	++	++	++	++	++	+	++	++	++	+	++	++
0.02	++	*	+	++	++	+	++	++	++	+	++	++
0.01	++	*	+	++	++	+	+	++	++	+	++	++
0.005	+	*	+	+	++	+	+	++	+	+	++	++
0.002	0	+	+	+	+	+	±	±	±	±	+	+
0.001	0	+	±	0	±	+	±	±	0	±	0	+
0.0005	0	+	±	0	0	+	±	0	0	±	0	+

* Tests were spoiled.

Here again it is proved that the greater the number of living bacteria in the circulation, the greater the number of fixation bodies formed. With the same number of bacteria injected, the longer they remain in the blood the more numerous are the complement fixation bodies that are stimulated.

Discussion.—While the author realizes that especially in the study of typhoid fever one should not draw conclusions too readily from experimental results in rabbits, still the problems presented here dealt more with the immunologic reactions to the presence of bacteria than with the anatomical or clinical effects caused by them.

These experiments should be repeated in a larger series of animals in order to be certain that our results were not dependent upon the exceptional reactions of a few animals. As far as we have gone, however, the experiments substantiate our hypothesis based upon clinical analysis of the cases of typhoid fever; namely, that the persistence of the complement fixation test depended upon the actual number of living typhoid bacteria that invaded the system and the length of time they remained there. Already in 1916 (78) we offered this explanation for the almost constant presence of the reaction during typhoid fever and the almost constant absence after prophylactic immunization. During typhoid fever, one is inoculated with live bacteria instead of organisms killed by heat, and with a comparatively much greater number of bacteria than in prophylactic immunization.

Comparison between Widal Test and Complement Fixation Test during Convalescence.

It has already been established (77) that during the acute stage of typhoid fever, the complement fixation test bears no direct relationship to the agglutination test. This also holds true during convalescence. Comparative tests made during the first 2 to 3 months after normal temperature showed:

89	cases	with	positive	complement	fixation	test	and	positive	Widal	test.
11	"	"	"	"	"	"	"	negative	"	"
42	"	"	negative	"	"	"	"	positive	"	"
13	"	"	"	"	"	"	"	negative	"	"

It is important to remember that the complement fixation bodies may persist very much longer than the agglutinins. This is evident from Table XXXIX.

TABLE XXXIX.

Name.	Widal test. Dilution. 1:50	1:100	Complement fixation test.	Time of examinations after normal temperature.
				months
Fricke	—	—	++++	5½
Majorowski	+	—	++++	5
Heinricksen	±	—	+++	5
Gottschalt	±	—	++++	3½
Kreussler	±	—	++	5
Hohlweg	—	—	++++	5½
Muza	±	±	++++	5
Steinhoff	—	—	++++	5

The Complement Fixation Reaction as a Diagnostic Test during Convalescence.

From a *diagnostic standpoint*, the complement fixation test made for the first time during convalescence, was of aid in several classes of cases. They were:

1. Cases That Were Diagnosed as Typhoid Clinically but Had No Other Laboratory Corroboration for That Diagnosis (Table XL).—This included 15 patients in whom: (*a*) *Widal test* was of no help, because of antityphoid inoculations just previous to the onset of the illness. (*b*) *Blood cultures* were negative, probably because the patients were received in the third and fourth week of the disease. (*c*) *The urine and feces* showed no typhoid bacteria. The complement fixation test in all of these patients was strongly positive (in 13 it was ++++; in 1, +++; in 1, ++). A + reaction was not considered sufficiently specific when the complement fixation test alone was to be the support for the diagnosis. These cases comprised *9.2 per cent* of our typhoid patients; in them the positive complement fixation reaction was the only laboratory test which corroborated the definite clinical diagnosis of typhoid fever.

TABLE XL.

Cases Diagnosed Typhoid Clinically with a Positive Complement Fixation Test as the Only Laboratory Evidence of the Disease.

Name.	Age.	Duration of fever course.	No. of typhoid inoculations.	No. of negative blood cultures.	Clinical symptoms.	Blood examination.				Complement fixation test.	
						Total white blood cells.	Per cent of polymorphonuclears.	Per cent of lymphocytes.	Severity of disease.	Degree of reaction.	Interval after normal temperature.
		days									*days*
Schlott.....	29	55	1	3	Abdominal tenderness; toxemia; relapse; diarrhea; headache; enlarged spleen; rose spots.	9,850	63	37	+	++++	49
Sohns.....	32	18	1	1	Diarrhea; headache; enlarged spleen; rose spots; bronchitis; toxemia.	8,600	31	69	+	++++	90
Steinhoff.....	18	27	1	2	Diarrhea; headache; enlarged spleen; rose spots.	6,500	52	48	+	++++	77
Thiel.....	45	16	1	1	Diarrhea; headache; enlarged spleen.	4,600	44	56	+	++++	85
Koenig.....	32	37	1	1	Diarrhea, blood in stools; headache; abdominal tenderness; enlarged spleen; rose spots.	5,950	60	40	+	++++	70
Kertscher.....	30	21	1	1	Headache; enlarged spleen; rose spots; relapse; recrudescence.	7,400	21	79	+	++++	83
Lehman, R.....	31	24	1	2	Headache; toxemia; enlarged spleen; rose spots.	5,800	62	38	+	++++	75
Thiem.....	23	16	2	0	History of 2 weeks of fever before coming to hospital and loss of 18 pounds in weight; regained while there.				—	++++	79

Vilehr.....	19	29	1	1	Enlarged spleen; rose spots.	7,000	41	59	+	+++	75
Canzler.....	52	78	3	4	Headache; toxemia; enlarged spleen; rose spots; relapse.	3,850	69	31	++	++++	39
Hausen.....	34	24	3	1	History of 3 weeks of fever before coming to hospital; no temperature while there.				−	++++	68
Hohling.....	38	22	1	2	Enlarged spleen; intestinal hemorrhage.	6,500	61	39	+	++++	78
Roessler.....	22	14	2	0	History of 2 weeks of fever before coming to hospital with loss of 26 pounds in weight; regained; enlarged spleen.	9,200	46	54	−	++++	76
Schumacher.....	28	40	1	1	Enlarged spleen; blood in stools.				+	++	
Knoblauch.....	62	40	1	2	Headache; enlarged spleen.	3,500	56	40	+	++++	67

It is interesting that most of these patients suffered from comparatively mild typhoid infections. On the basis of a ++++ scale for estimating the severity of the disease, 3 of these patients were classed as negative, *i.e.* were only very slightly sick; 11 were +; and only 1 was ++. It must be noted from the table that the test was first done as late as 39 to 90 days (1 to 3 months) after the beginning of normal temperature. If the tests had been done earlier, the positive diagnosis could have probably been established in 10 other cases (Table XLI) which were diagnosed typhoid clinically but had no laboratory corroboration whatever. Even the complement fixation reaction was negative; but, as is seen by the table, the tests in these cases were not started before the 2nd month of convalescence. In 1 case it was done on the 31st day after normal temperature, in 4 during the 10th or 11th week, and in 5 during the 12th or 13th week after the absence of fever. This group of cases is collected to show that while we have a great many laboratory methods at our disposal for establishing the existence of typhoid fever, 6 per cent of the typhoid cases remained unconfirmed by the usual methods. Had it been possible in our epidemic to perform the complement fixation reaction earlier during convalescence, or during the acute stage of the disease, it is certain that the clinical diagnosis could have been confirmed in most of the cases. This would have made about 15 per cent of typhoid cases where the complement fixation test would have been the only laboratory means of diagnosis.

2. Cases That Were Diagnosed as Typhoid Clinically and Gave a Widal Reaction as the Only Positive Laboratory Test (Table XLII).—This class included 6 patients and all of them had a ++++ positive complement fixation test. While a positive Widal test, properly performed and controlled, is sufficient for the diagnosis of typhoid fever, one is usually more satisfied when another corroborative test is at hand. This is especially so in epidemics similar to this one, where the history of previous inoculation is obtained from a great percentage of the patients, and where it is perfectly possible that some patients deny inoculation while others forget about it at the time of questioning. If one adds the number of patients in this series to the number in Class 1, we get 13 per cent of typhoid cases where the complement fixation test was of direct help in establishing the laboratory diagnosis.

TABLE XLI.

Cases Diagnosed Typhoid Clinically; No Laboratory Evidence; Complement Fixation Test of No Aid; Probably Done Too Late.

Name.	Age.	Duration of fever course.	No. of inoculations.	No. of negative blood cultures.	Clinical symptoms.	Blood examination.				Complement fixation test.	
						Total white blood cells.	Per cent of polymorphonuclears.	Per cent of lymphocytes.	Severity of disease.	Degree of reaction.	Interval after normal temperature.
		days									*days*
Gutsch	27	13	2	0	Enlarged spleen; rose spots.				+	—	86
Seiling	45	34	1	2	Enlarged spleen; rose spots.				+	±	74
Konkel	48	47	1	1	Diarrhea; headache; rose spots; relapse.				+	—	31
Lecker	23	26	1	1	Diarrhea; headache; enlarged spleen; rose spots.				+	—	72
Mensching	30	28	1	1	Headache; enlarged spleen; toxemia.				+	+	74
Johns	24	13	1	1	Headache; enlarged spleen; loss of weight.	6,600	37	63	+	±	82
Schneider	34	16	1	1	Enlarged spleen; rose spots.	4,300	52	48	+	—	83
Fitz	31	28	1	2	Headache; toxemia; enlarged spleen; blood in stools.	9,050	44	56	+	—	85
Gromisch	31	23	1	1	Enlarged spleen; headache; rose spots; abdominal tenderness.	6,950	39	61	+	+	86
Holtz	40	41	1	2	Enlarged spleen; rose spots; headache; abdominal tenderness; blood in stools; toxemia.	11,950	51	49	+	+	76

TABLE XLII.

Cases Diagnosed Typhoid Clinically; Positive Widal Test the Only Laboratory Evidence besides a Positive Complement Fixation Test.

Name.	Age.	Duration of fever course.	No. of typhoid inoculations.	No. of negative blood cultures.	Widal test in dilution 1:50.	Clinical symptoms.	Blood examination.				Complement fixation test.	
							Total white blood cells.	Per cent of polymorphonuclears.	Per cent of lymphocytes.	Severity of disease.	Degree of reaction.	Interval after normal temperature.
		days										*days*
Schoon.....	24	29	0	1	+	Epistaxis; blood in stools; headache; enlarged spleen; rose spots; periostitis.	7,150	48	52	+	++++	87
Coert.....	25	46	0	1	+	Enlarged spleen; loss in weight, 19 lbs.; regained.	13,200	57	43		++++	72
Grief.....	30	46	0	1	+	Diarrhea; headache; enlarged spleen; rose spots.	5,900	39	61	+	++++	67
Hoera.....	25	58	0	1	+	Diarrhea, blood in stools; headache; enlarged spleen; rose spots.	8,100	44	56	+	++++	82
Heitman.....	40	34	0	1	+	Blood in stools; toxemia; delirium; incontinence; enlarged spleen; rose spots.	4,000	62	38	+++	++++	64
Hogencamp.....	30	56	0	2	+	Diarrhea, blood in stools; delirium; toxemia; enlarged spleen; rose spots; phlebitis; hemorrhage.	3,150	56	44	+++	++++	51

TABLE XLIII.

Clinically No Typhoid; Diagnosis Corroborated by a Negative Complement Fixation Test.

Name.	Age.	No. of typhoid inoculations.	Widal test.	Feces examination.	Urine examination.	Clinical symptoms.	Diagnosis.	Blood examination.				Complement fixation test.
								Total white blood cells.	Per cent of polymorphonuclears.	Per cent of lymphocytes.	Severity of disease.	
Meyer.....	50	2 {Aug. 7 " 15	+	—	—	Came to U.S.A.G.H. 12 on Sept. 3 with normal temperature; had reported sick for several days on Aug. 15; no clinical signs of typhoid.	Reaction following inoculation.	7,400	54	46	—	—
Gardewischiki....	32	3 {Aug. 4 " 12 " 22	+	—	—	Was sick before reaching U.S.A.G.H. 12; no spleen enlargement; never any symptoms of typhoid.	Inoculation.	7,100	57	43	0	—
Bendhake.....	56	3*				Hemiplegia.	Hemiplegia.	9,750	72	28		—
Gasser.....	35	3† 2*				No clinical symptoms; came to hospital with normal temperature Aug. 14.	Pleuritis; pneumonia.	7,950	77	22		—
Kelber.....						Died of meningitis soon after admission; no examination.		4,800	68	32		—

— indicates negative.

* After admission to hospital.

† Before " " "

3. Cases in Which There Was a Possible Doubt but Which Clinically Did Not Give the Impression of Typhoid Fever, and in Which the Complement Fixation Test Was Negative.—The Widal test was of no aid in this group because of previous inoculations; the blood cultures were either negative or were not made because the patients first came under observation when the acute stage of the disease was over and the temperature was normal. There were 5 such instances; the diagnoses were, meningitis, hemiplegia, pleuropneumonia, reactions following inoculation (2 cases) (Table XLIII). All of these had negative fixation tests.

Summary.—Adding the number of patients in these 3 groups, 36, one finds that the complement fixation test gave valuable information in 21.5 per cent of the cases diagnosed as typhoid fever during a typhoid epidemic amongst patients only partly, entirely, or not at all immunized by previous inoculation.

Especially with the more generalized use of antityphoid inoculations, some test is needed to replace the agglutination reaction when the blood culture and other methods are negative. The fixation reaction aims to supply this demand. The Dreyer agglutination method has also been advocated with this object in view (83).

Technique of the Complement Fixation Tests.

The technique employed in performing the complement fixation tests is the same as that reported in the author's original publication (77). It will be reviewed in part in order to discuss several important additional observations.

Antigen.—The preparation of the antigen is the most important element in the reaction. The author's method followed the directions of Wasserman and Citron (84) for preparing artificial aqueous aggressins. Cultures are grown for 24 hours upon agar slants or plates (Kolle's flasks are preferable). The growth is washed off with sterile distilled water, about 1 cc. to an agar slant. The total emulsion is kept in a hot-water bath at a temperature of 60–65°C. for 24 hours, then transferred to a strong bottle and shaken vigorously with the aid of glass beads for 24 hours. The bacteria are thus thoroughly broken up. The mixture is next centrifugalized for a long time (4 to 8 hours, depending upon the total quantity) until all the bacteria have sunk to the bottom and the supernatant fluid is absolutely clear. The latter is carefully pipetted off, and can be preserved for about 6 to 8 months in sealed tubes, not exposed to sunlight or room tempera-

ture. Sterile precautions must be taken throughout the preparation. The object of using a total small quantity of distilled water is to eliminate the hemolysis of red blood cells, which may be occasioned by employing large doses of antigen in the test.

It is best to titrate the strength of the antigen about 1 week after its preparation as at the end of this period its titer usually remains unchanged. Varying quantities of antigen are mixed with the constant units of complement, hemolysin, and red cells (sheep system), and those amounts are determined which do not of their own accord inhibit hemolysis (Table XLIV).

TABLE XLIV.

Titration of Antigen.

Typhoid antigen.	Complement.	Hemolysin.	Red blood cells.	Saline.	Result.
cc.	*cc.*	*cc.*	*cc.*	*cc.*	
0.2	0.5 (1:10)	0.5 (1:1,000)	0.5 (1:20)	Up to 2.5.	No hemolysis.
0.1	0.5 (1:10)	0.5 (1:1,000)	0.5 (1:20)	....	" "
0.5	0.5 (1:10)	0.5 (1:1,000)	0.5 (1:20)	....	Complete hemolysis.
0.025	0.5 (1:10)	0.5 (1:1,000)	0.5 (1:20)	....	" "
0.0125	0.5 (1:10)	0.5 (1:1,000)	0.5 (1:20)	....	" "
0.00625	0.5 (1:10)	0.5 (1:1,000)	0.5 (1:20)	....	" "

In accordance with the summation law of Weil and Nakajama (85), the dose of antigen to be employed in complement fixation experiments is represented by one-half of the maximum quantity of antigen that does not of itself bind complement; that is, in the above instance one-half of 0.05 cc., or 0.025 cc., or 0.5 cc. of a dilution 1 to 20. This unit of antigen is then tested with a known typhoid serum to prove that complement fixation is possible.

In a recent article (86), studying the comparative antigenic sensitiveness of nine typhoid antigens each prepared in a different way, Matsumoto showed that the method of preparation has a decided effect upon the occurrence and degree of complement fixation tests. In his experiments the antigen similar to the author's, prepared from the filtrate of typhoid bacilli autolyzed in distilled water aided by heating at a high temperature, proved least antigenic. Mainly immune sera from rabbits were used for this differentiation and no series of typhoid patients or inoculated persons were studied. At first sight this would speak against the use of such distilled water extract antigens. We do not, however, consider this lessened sensitiveness a disadvantage if the test is to differentiate between a serum from a patient with

an active typhoid fever and a serum from a person inoculated with typhoid vaccine. In the former individual, the antibodies stimulated by the great excess of typhoid bacilli in the system have been produced in such great numbers that they readily give complement fixation even with a less sensitive antigen. On the other hand, in inoculated persons the number of complement fixation bodies are so few that they do not react with an antigen which is not highly sensitive. In other words, for this purpose it is better to have the antigen not too sensitive.

The distilled water extract antigen described by Matsumoto is probably less sensitive than the similar antigen of the author's (Garbat), for Matsumoto places the suspension of bacilli in a water bath at 80°C., while the writer does not allow a temperature over 65°C. Higher temperatures interfere too much with antigenic properties.

Matsumoto also noticed that inactivating the human serum at 56°C. for 30 minutes causes a marked reduction in the degree of complement fixation. In the writer's studies, all the sera examined were previously heated thus. Just as with the reduced sensitiveness of the antigen, the writer believes that the reduction in sensitiveness of the serum by its being heated is not a disadvantage when one wishes to differentiate between a serum from an inoculated person and the serum from a typhoid patient.

As has been said before, a typhoid fever serum usually has an excess of antibodies and even a slight diminution in their number by heating will still leave sufficient for the reaction. Done in this less sensitive way, as recommended by the author, the complement fixation test when positive is all the more specific for active typhoid fever.

If it should be determined by the study of a large number of cases that the more sensitive antigens of Matsumoto react not only with the sera from cases of typhoid fever but also with the sera from inoculated persons, then sensitive antigens combined with unheated serum will have a limited use only in patients who are suffering from a suspicious typhoid illness but who have with certainty not been inoculated, while our less sensitive antigen combined with heated serum will be employed in other suspicious typhoid cases which may have received typhoid inoculations. Then, too, it will have to be

shown that the more sensitive antigens do not react with sera from patients with high fever suffering from acute infections other than those of typhoid origin. The less sensitive typhoid antigen has proved specific.

As many different typhoid strains should be employed in the preparation of the antigen as possible. A highly polyvalent antigen is an element which accounts for a higher percentage of positive complement fixation tests (77). Antigens prepared from autogenous strains give the best fixation. The antigen used in the present study contained 15 strains out of a total of 45 strains isolated. It is probable that all the typhoid bacilli were the same, since they were derived from the same epidemic. We were able to employ an autogenous antigen because the fixation tests were started only during convalescence.

Comparative fixation tests done in 17 patients with 2 different antigens, 1 from the autogenous strain and 1 from the Rawling strain, showed 10 positive with the autogenous antigen and only 4 positive with the Rawling antigen. There was no cross fixation with a paratyphoid antigen.

Complement.—In the original publication (77), a fixed dose of complement, that is 0.05 cc. of the mixed serum from several guinea pigs, was employed for the $\frac{1}{2}$ quantity system. This technique has not been changed. Titration of complement was intentionally omitted because it has not as yet been worked out whether titration of complement so that no excess is used, may not make the reaction too delicate and thus non-specific. A patient with typhoid fever usually produces so many antibodies that the fixation test is not overshadowed by a slight excess of complement. Water bath incubation for 1 hour was used.

Hemolytic System.—The sheep corpuscles system was continued for the same reason. Similarly, the natural antisheep amboceptors in human serum were not taken into account as is done in the Wassermann test. 2 units of hemolysin were used. After the system was added, the tests were read in 20 minutes.

CONCLUSIONS.

I.

1. This report is based upon the study of a typhoid epidemic including 183 patients, and probably arising from polluted drinking water. Laboratory tests were performed in only 164 cases.

2. The typhoid illness was divided into "the acute fever stage" and "convalescence," the first day of the persisting normal temperature being used to separate the two periods.

3. The Endo medium, properly prepared, is very satisfactory for distinguishing the typhoid and the colon bacilli.

II.

4. Feces cultures employed for detecting the presence of typhoid bacteria in the intestines, fail in 15 per cent of typhoid carriers. Direct examination of the bile by duodenal cultures is the only accurate means for the detection of the carriers. In 15 per cent of typhoid convalescents, typhoid bacilli may be discovered in the bile after 3 consecutive stool cultures have been negative and they are not destroyed in the intestines but merely escape detection by stool examination.

5. Feces cultures should not, however, be entirely discarded because of the existence (although very rare) of pure intestinal carriers, in which typhoid bacilli occur in the stool and not in the bile. One such definite intestinal carrier is reported.

6. An absolutely safe indication of the complete absence of typhoid bacteria in the intestinal tract would be offered by 2 consecutive negative bile cultures and 2 consecutive negative feces cultures. No special interval of days between these examinations is required.

III.

7. 32 per cent of typhoid patients become *feces* carriers. Of these, 28 to 29 per cent are temporary carriers while 3 to 4 per cent become permanent carriers.

8. Of the temporary carriers,

17.5 per cent are carriers for 1 month.
8.0 " " " " " 2 months.
3.0 " " " " " 3 "

9. Three distinct types of feces carriers have been found, according to the original source of the infection: (*a*) liver, (*b*) gall bladder, (*c*) intestinal. In the first two only are the bacilli transmitted by the bile. Combinations of these types may result, if the carrier state continues.

10. During early convalescence, one may attempt by stool cultures to differentiate between bile carriers (liver and gall bladder) and intestinal carriers. In the intestinal carriers, the bile cultures show no typhoid organisms, while the stool cultures show almost a pure growth of the bacilli; the bacilli appear in the stool at the beginning of convalescence and continue there persistently.

In the bile carriers, the feces cultures may show only few typhoid colonies, or even none at all, while the bile shows typhoid bacilli usually in pure culture.

11. As an explanation for the origin of bile carriers, ascending infection of the gall bladder or liver from the intestines is possible, although the usual path is by way of the blood and a descending route.

12. Cholecystectomy is curative in pure gall bladder carriers only. Liver and intestinal carriers are uninfluenced by cholecystectomy.

13. Instead of cholecystectomy as the routine operation, in carriers cholecystectomy should be combined with hepatic drainage both for diagnostic and therapeutic purposes. Long continued drainage of the hepatic duct should be carried out in liver carriers.

IV.

14. No factors can be cited as definitely predisposing to the carrier condition.

15. Patients who present symptoms of cholecystitis or cholelithiasis during the course of the typhoid illness, usually become carriers, but not all the carriers necessarily present symptoms referable to the gall bladder during the acute illness.

16. Surgical measures to remove the carrier state should be undertaken very much earlier in those carriers who have had a complicating cholecystitis.

17. Cholelithiasis following typhoid fever may only be associated with and not necessarily induced by the typhoid bacillus. On the other hand, gall stones of unusually large size may be formed during the typhoid illness and originate from the typhoid bacillus.

V, Part A.

18. The use of solid media alone (Endo plates) for detecting typhoid bacilli in urine is inadequate. A 24 hour growth in broth is first necessary before plating.

19. The excretion of typhoid bacteria in the urine is of an intermittent character. The urine may change from a specimen with no bacteria to one with very many bacteria within several hours.

20. It is misleading to consider the urine of patients free from typhoid bacteria on the basis of 3 consecutive negative examinations at 6 day intervals; such a rule fails in 20 to 25 per cent of urine carriers.

21. A negative culture in broth from a 24 hour specimen of urine sterilely collected is the only safe guide.

V, Part B.

22. The urine is as important a factor as the feces in the spread of typhoid fever.

23. 49 per cent of patients present typhoid bacilluria during convalescence.

24. There is no relationship between typhoid bacilluria and the specific gravity, acidity, or albumin content of the urine.

25. Bacilluria is most frequent during the first week after normal temperature, when as many as 63 per cent of cases show typhoid bacilli in the urine.

26. 6.7 per cent of all typhoid cases, or 13.6 per cent of all cases of bacilluria, remain positive for 1 to 2 months after the absence of fever. In only 1.2 per cent of all typhoid cases, or 2.5 per cent of all positive cases, does the bacilluria continue for 2 to 3 months.

27. No definite explanation can be given for the intermittency with which the typhoid bacilli are excreted. It was suggested that an infection in the pelvis of the kidney or its calyces might account for the condition. Under these circumstances, the pelvis of the kidney bears the same relation to the kidney as does the gall bladder to the liver.

VI.

28. In 30 per cent of typhoid patients, the complement fixation bodies disappear from the blood by the 2nd month of convalescence, while 40 per cent of patients still show a positive fixation at the end of 6 to 8 months after the temperature has become normal.

29. A persisting complement fixation test is dependent directly upon the number of bacteria which have invaded the body and the length of time they remained there.

30. In 80 per cent of patients with recurrences or relapses, and 85 per cent of carriers, a strong complement fixation test persists. However, not all carriers, not even all permanent ones, necessarily continue to give the fixation test.

31. In 9.2 per cent of our cases of typhoid fever the complement fixation examination was the only laboratory test which confirmed the clinical diagnosis of typhoid fever; the reaction was performed for the first time as late as 1 to 3 months after the onset of normal temperature. The test was of valuable assistance in 21.5 per cent of our cases.

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