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FILTERABLE VIRUSES WITH PARTICULAR REFERENCE TO PSITTACOSIS[1]

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INTRODUCTION

WITHIN recent years the filterable viruses have been discussed before the Harvey Society by d'Herelle (1), Goodpasture (2), and Kunkel (3). A review (4, 5) at this time would be repetitious. Consequently, I shall make only a few remarks concerning the nature of viruses and virus diseases and then present several groups of experiments planned and conducted in the effort to solve some of the problems confronting workers in the virus field. The development of our knowledge of psittacosis, a disease recently placed in the virus group, will be described in addition in order to illustrate how information about virus maladies is obtained.

NATURE OF VIRUSES AND VIRUS DISEASES

In this discussion the terms *filterable viruses* and *viruses* will be used interchangeably. For workers intimate with the active agents under consideration these terms have taken on a significance similar to that carried by such words as *bacteria*, *fungi*, *spirochetes*, and *protozoa*, and designate the etiological agents of a large group of diseases attacking all forms of life, e.g., bacteriophagy, mosaic diseases of plants, wilt diseases of caterpillars, fowlpox, Rous' sarcoma of chickens, cow-pox, foot-and-mouth disease of cattle, rabies, canine distemper, infectious anemia of horses, warts, yellow fever, smallpox, measles, poliomyelitis, etc. For reasons that need not be enumerated here the Rickettsia diseases, e.g., typhus fever and Rocky Mountain spotted fever, are not included in the virus group. Since pleuropneumonia of cattle and agalactia of goats are caused by filterable organisms capable of

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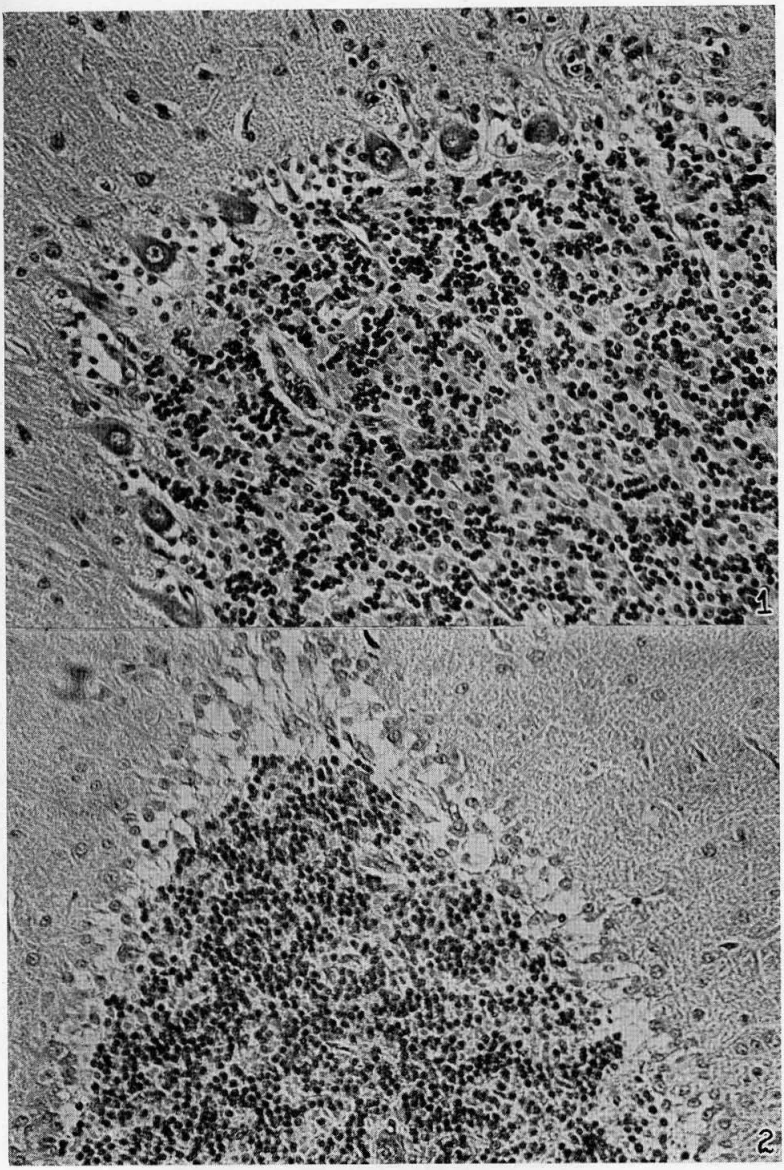


FIG. 1. Section of the cerebellum of a normal monkey. Note the well-stained Purkinje cells. Compare with figure 2. E.M.B. × 215.

FIG. 2. Section of the cerebellum of a monkey with louping ill sacrificed on the first day of symptoms. Note absence of Purkinje cells and lack of evidences of inflammation. Compare with figure 1. E.M.B. × 215.

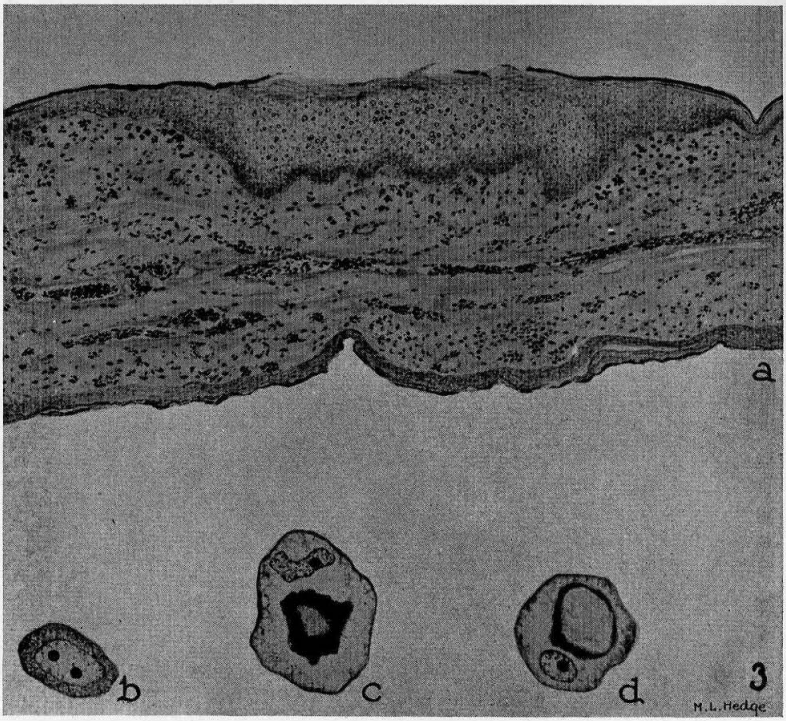


FIG. 3. *a* represents a section of a young fowl-pox lesion induced in the wattle of a chicken by the bite of an infected Culex mosquito. Note the hyperplasia of the epithelial cells and the absence of inflammation in the corium. × 50. *b* represents a normal epithelial cell. × 1200. *c*, *d* represent epithelial cells with Bollinger bodies in their cytoplasm which in turn are composed of a mass of Borrel bodies similar to the Paschen bodies shown in figure 9. × 1200.

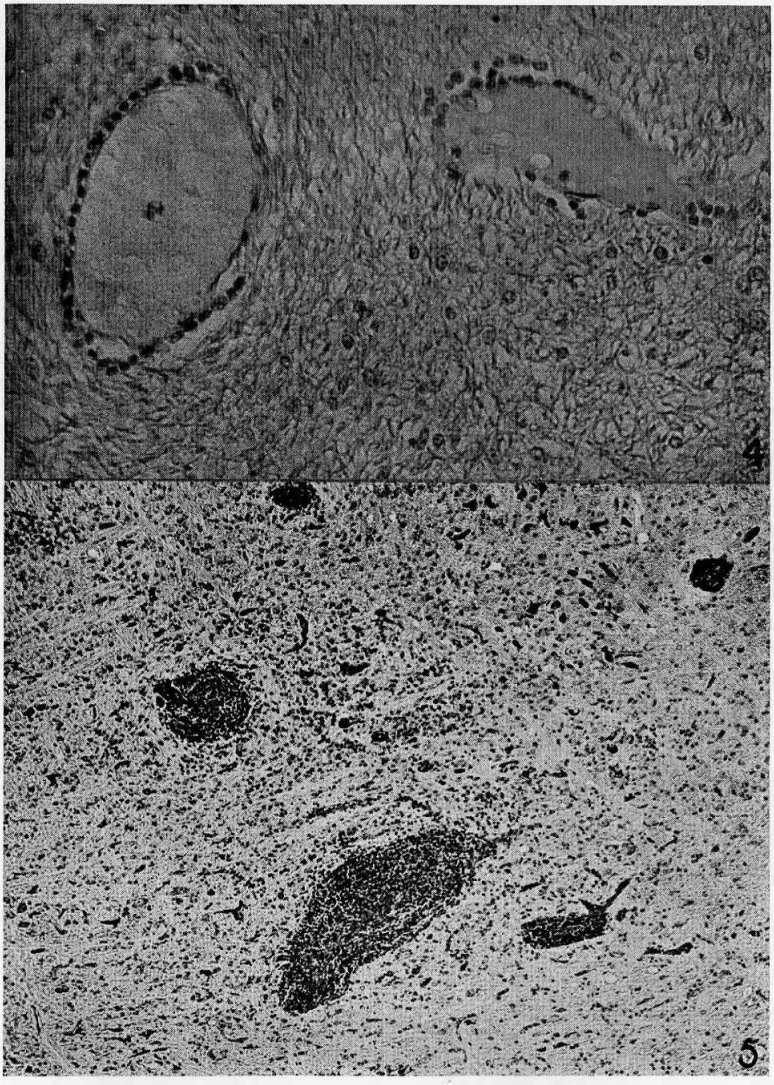
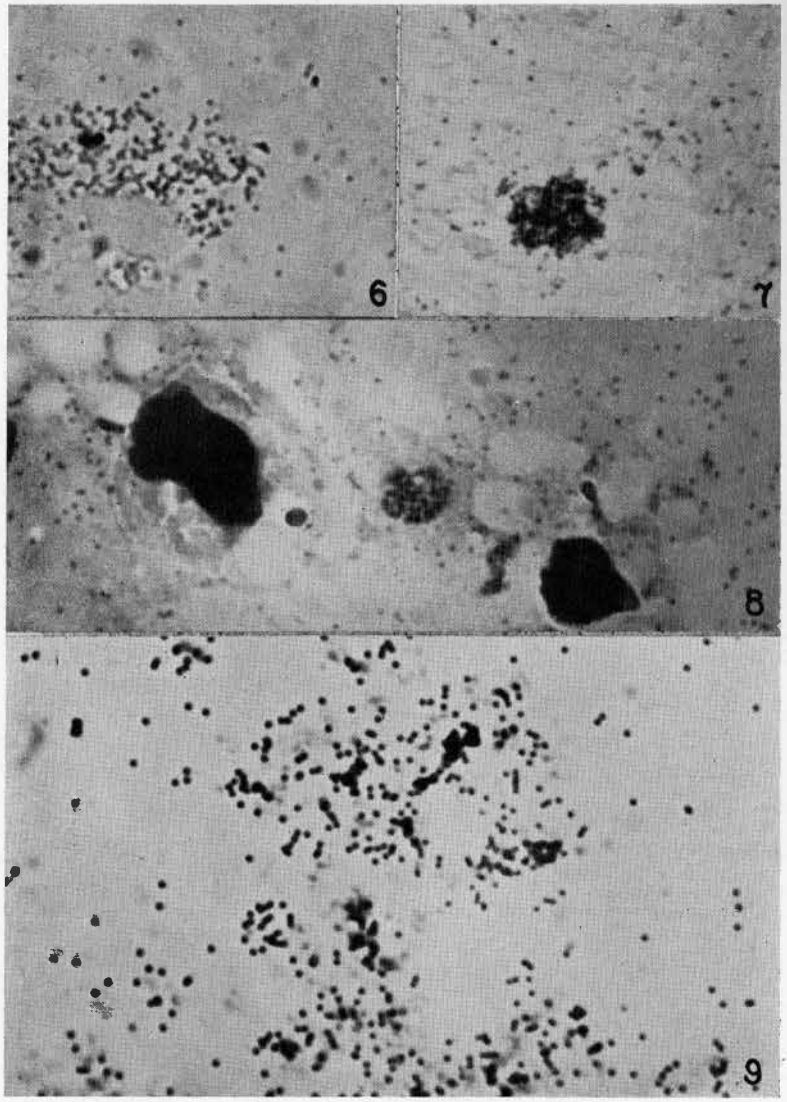


FIG. 4. Section of the pons of a monkey with louping ill sacrificed on the first day of symptoms. Although the Purkinje cells (fig. 2) have been destroyed the minimum amount of perivascular infiltration is present. E.M.B. × 250.

FIG. 5. Section of the pons of a partially immune monkey that recovered from a louping ill infection. Animal was sacrificed (23 days after onset of disease) at a time when all symptoms had practically disappeared. Note the intense inflammatory reaction. E.M.B. × 63.



FIGS. 6, 7, AND 8. Psittacosis bodies; some are still clumped in the manner that they appear in cells; others are disposed as minute discrete bodies. Modified Castaneda's stain. × 1200.

FIG. 9. Washed Paschen bodies obtained from dermal vaccine virus. Morosow's stain. × 1200.

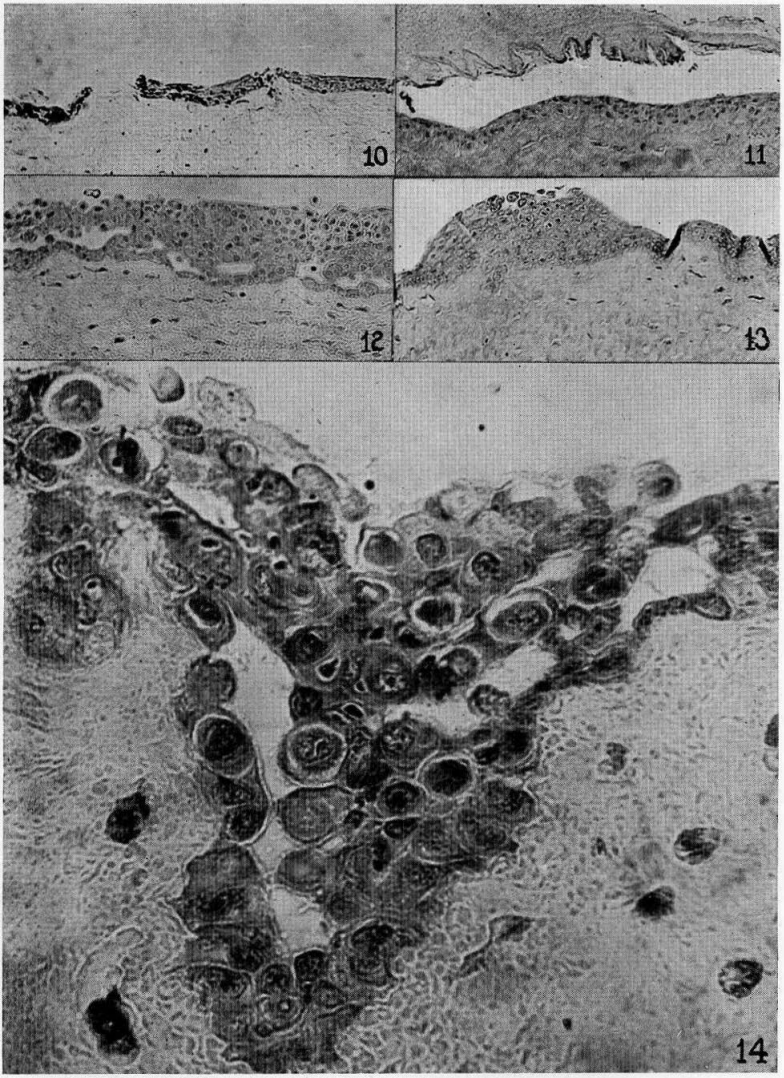


FIG. 10. Normal rabbit cornea showing defects in epithelium due to scarification. Giemsa. × 155.

FIG. 11. Normal rabbit cornea cultivated *in vitro* 48 hours. E.M.B. × 155.

FIG. 12. Rabbit cornea infected with vaccine virus and cultivated *in vitro* 48 hours. Note hyperplasia and beginning destruction of cells. Compare with figures 10 and 11. E.M.B. × 155.

FIG. 13. Rabbit cornea infected with herpetic virus and cultivated *in vitro* 48 hours. Note marked hyperplasia of cells. Compare with figures 10 and 11. Giemsa. × 155.

FIG. 14. Rabbit cornea infected with vaccine virus and cultivated *in vitro* 48 hours. The defect in the layer of epithelial cells has been filled in with new cells, many of which contain Guarnieri bodies. Giemsa. × 700.

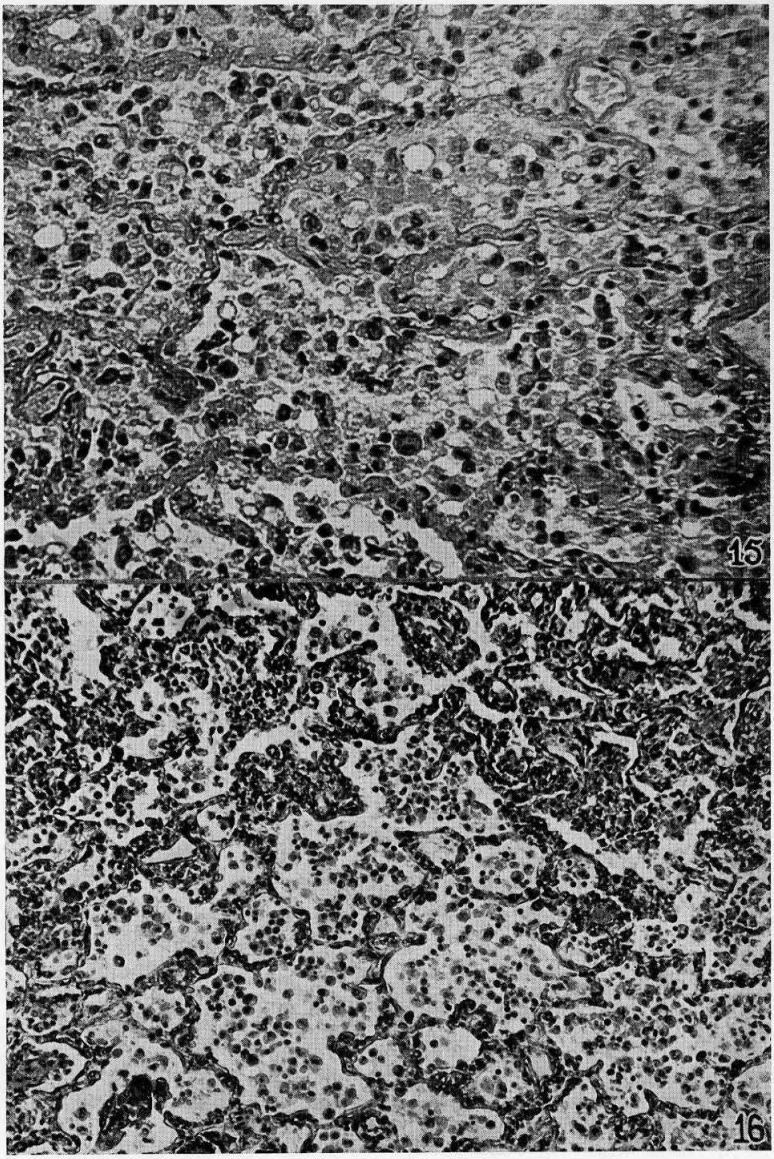


FIG. 15. Section from the lungs of a human being who died of psittacosis. Compare with figure 16. E.M.B. × 125.

FIG. 16. Section from the lungs of a monkey with psittacosis pneumonia. Compare with figure 15. E.M.B. × 125.

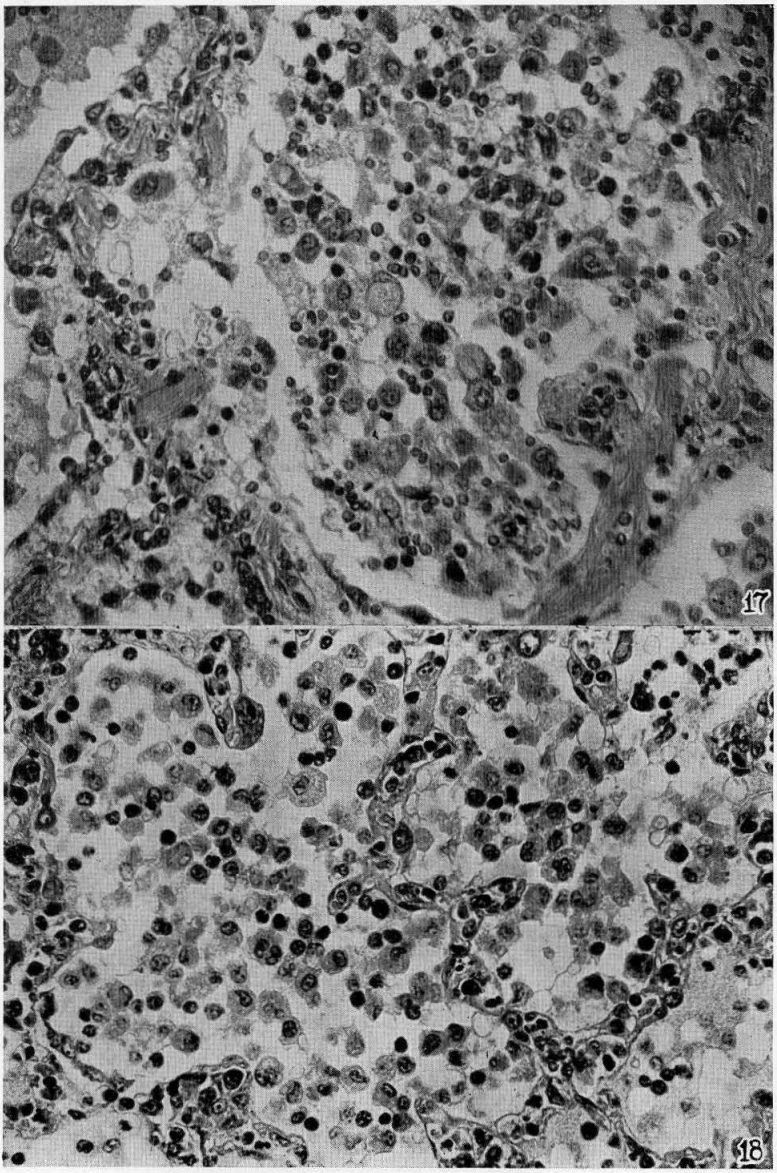


FIG. 17. Section illustrating the alveolar exudate in human psittacosis pneumonia. Compare with figure 18. E.M.B. × 370.

FIG. 18. Section illustrating the alveolar exudate in the lungs of a monkey with psittacosis pneumonia. Compare with figure 17. E.M.B. × 370.

cultivation on ordinary lifeless media, and since Borrel (6), Ledingham (7), and others (8) believe that their etiological agents are closely related to the fungi, these two maladies also are not grouped with the virus diseases.

A casual consideration of the virus diseases frequently leads one to look upon them as a group of heterogenous maladies of unknown etiology. A careful examination of these diseases, however, discloses the fact that they possess many features in common. The intimate association of the viruses with the cells of their hosts (4) gives rise to a stimulation of affected cells as is seen, for example, in the Rous sarcoma of chickens, or to a necrosis or lysis of cells as is observed in the case of Purkinje (figs. 1 and 2) cells infected with louping ill virus, or to a stimulation of cells followed by necrosis as in fowl-pox (fig. 3). Inflammation (figs. 4 and 5) is a secondary phenomenon in virus maladies (4). In the light of these facts one readily understands why bacteriophagy, smallpox, fever blisters, warts, and certain kinds of tumors due to viruses, in spite of their gross clinical differences, are grouped together. The activities of viruses frequently lead to the appearance of inclusion bodies in affected cells but not in all instances. Most, but not all, of the sicknesses due to viruses are followed by a lasting immunity in recovered hosts. So far as is known drugs and antisera do not act specifically upon the virus maladies after signs and symptoms of infection have become evident. The etiological agents of this group of diseases also have certain characteristics in common. They are filterable and are smaller than ordinary bacteria. They cannot be resolved by the best microscopes when ordinary light is used. None of them has been grown on ordinary lifeless media, while many have been induced to multiply *in vitro* in the presence of living susceptible cells. Thus it seems that the viruses are minute obligate parasites.

Although the viruses are small it does not follow that they are all of one size. In fact it appears that some of them can be photographed by means of ultra-violet light or can be made visible with ordinary light by means of special mordants and stains (figs. 6 to 9). Such viruses, e.g., vaccine virus, have an estimated diameter of 175 to 200$\mu\mu$. Others, however, e.g., some of the bacterio-

phages and the virus of foot-and-mouth disease, have an estimated diameter of 8 to 12 $\mu\mu$. Indeed, the latter viruses are very small being only slightly larger than molecules of hemoglobin which have an estimated diameter of 5.5$\mu\mu$.

The fact that the viruses are extremely small does not warrant the conclusion that they represent agents of the same nature any more than it warrants a decision that they are of one size. Some of them may be minute living organisms representing the midgets of the microbic world, others may be primitive forms of life unfamiliar to us, still others may be inanimate transmissible incitants of disease. If it be assumed that the viruses differ in natures, one comes up against the question of why the diseases caused by them manifest many striking features in common. To find an answer to such a question may not be exceedingly difficult. At least a partial solution might well lie in the phenomenon of the intimate association of the viruses, animate or inanimate, with the susceptible host cells. Furthermore, the viruses may be situated near the line that separates inanimate transmissible incitants from minute living organisms. The transition from one side of the line to the other may be so gradual that no great difference in the types of disease caused by agents near the line is perceptible. Such a statement is strongly reminiscent of a remark by Aristotle that "nature makes so gradual a transition from the inanimate to the animate kingdom that the boundary lines which separate them are indistinct and doubtful."

In concluding these general remarks I wish to emphasize the fact that the virus group of diseases does not represent a "catch-all" for maladies of unknown etiology. It is true that the exact nature of the viruses is unknown, but to say that the agents themselves are unknown is somewhat of an exaggeration. Individuals who believe them to be unknown tacitly admit that they are incapable of knowing infectious agents that are invisible or unable to grow on lifeless media. Without going into a detailed philosophical discussion of what can and can not be known, one is justified in saying that the viruses of vaccinia, yellow fever, rabies, poliomyelitis, herpes simplex, foot-and-mouth disease, fowl-pox, Rous' sarcoma of chickens, canine distemper and psittacosis are not

entirely unfamiliar to the initiate. They can be separated one from another and from ordinary bacteria. Furthermore, in many instances their presence in a variety of materials can be ascertained with much more ease and precision than is the case with certain bacteria. The old admonition regarding human beings, "Ye shall know them by their fruits," is applicable to the viruses, because they are known by their activities, that is, by the type of host or hosts attacked, by the clinical and pathological pictures induced, and by the immunological responses excited. In order to know an infectious agent it is not essential to see it or to grow it on lifeless media any more than it is imperative to see electricity in order to recognize it and to control it for our daily needs.

CULTIVATION OF VACCINE VIRUS

In spite of numerous claims to the contrary, none of the viruses has as yet been cultivated *in vitro* in the absence of living cells (9). However, in 1913, Steinhardt, Israeli, and Lambert demonstrated that vaccine virus is capable of multiplication in the presence of bits of viable tissue embedded in plasma. Although this work has been confirmed by a number of investigators, cultures of vaccine virus made by means of the cover-slip technique have proved of no value in the preparation of an active agent for Jennerian prophylaxis, and have been of no great assistance in studies of the nature of the virus or in attempts to find adequate explanations for certain immunological phenomena. It seemed to us that modern tissue culture technique modified to suit the various problems in the virus field might yield excellent material for vaccination purposes as well as act as an aid in obtaining certain kinds of information. With this idea in mind we set out in 1927 to cultivate vaccine virus *in vitro*. Without going into details of the various methods employed, I shall describe briefly the one that has proved most satisfactory and give the results obtained in human beings when the culture virus was used for Jennerian prophylaxis (10–12).

Chick embryo tissue was obtained from eggs, incubated 9 to 12 days, that had been opened aseptically. The eyes of the embryos were removed because the pigment granules contained in them

often led to confusion when smears were made to determine the sterility of the cultures. The embryos were placed in a sterile watch glass contained in a Petri dish, finely minced with scissors, and then distributed in amounts of approximately 0.1 gram into "collar flasks" or into 50 cc. Erlenmeyer flasks containing 5 cc. each of sterile Tyrode's solution. The medium was inoculated with 0.25 cc. of a virus emulsion, the mouth of each flask containing its cotton plug was securely covered with several layers of tinfoil, and the cultures were incubated at 37°C. for 5 days. New cultures were made by direct transfer of 0.25 cc. of an old culture into flasks of fresh medium. All cultures were tested for the presence of ordinary bacteria. In this simple manner, vaccine virus after it has been adapted to cultivation can be propagated through an indefinite number of culture generations. One should be careful not to use too much tissue for a given amount of Tyrode's solution, inasmuch as it appears that an optimum ratio between tissue and fluid exists for the maximal multiplication of virus. Although the medium is simple and easily prepared, one must remember, however, that living cells are essential and that any manipulation which leads to the death of all the cells renders the medium valueless.

Vaccine virus prepared in the manner described can be preserved with or without glycerol. In the undiluted state, the virus applied to scarified areas of human skin results in typical vaccinal reactions which are followed by an immunity to calf lymph virus. The virus diluted 1:10 with sterile saline solution and administered intradermally in 0.1 cc. amounts produces a red induration which disappears leaving no scar but a refractory state to calf lymph virus. Furthermore, the culture virus, mixed with an equal amount of sterile 50 per cent egg albumen, frozen, and desiccated, retains its potency for long periods of time. To obtain typical vaccinal lesions in human beings with the dried virus, one opens a tube, breaks up the contained material into a fine powder by means of a sterile wooden applicator, and then places a bit of the powder on a small area of scarified skin. It is not advisable to use the virus preparations containing albumen for intradermal vaccinations because certain individuals are sensitive to the white of egg. With

different preparations of culture virus we have vaccinated 127 children of which 109 responded with typical vaccinal reactions.

PRODUCTION OF VIRUS LESIONS IN VITRO

Inasmuch as vaccine virus and the virus of fever blisters produce characteristic intracellular changes, we decided to ascertain whether these changes occur in tissue infected by the viruses after removal from the body and then cultivated in sterile plasma clots in flasks or in test tubes (13). To this end experiments were performed in the following manner.

Rabbits, under light ether anesthesia, were exsanguinated from the heart by means of syringes into which 1 cc. of a 1:1000 heparin solution for each 10 cc. of blood had been previously drawn. Then, to obtain a tissue extract, the spleens were excised, minced, added to a small amount of Ringer's solution, and centrifuged. Finally, the eyes were enucleated intact after stout silk ligatures had been placed around the nerves and large vessels. The eyes, fixed in the grip of special forceps and suspended over a large Petri dish, were thoroughly washed with Ringer's solution to remove as many contaminating bacteria as was possible. With a sterile cataract knife the eyes to be used as controls were cross-hatched with closely spaced scarifications just deep enough to penetrate the layer of epithelial cells. Test eyes were treated in a similar manner with the exception that prior to each scarifying stroke the knife was dipped in a virus emulsion. The corneas were removed from the eyes and each one was divided into 4 to 8 pieces. The pieces from test eyes were further inoculated by immersion in an emulsion of virus for 1 or 2 hours at 37°C.

With plasma, spleen extract, and bits of cornea, cultures were set up in duplicate or triplicate. Into a sterile 50 cc. Pyrex centrifuge tube 2 or 4 cc. of plasma were introduced, to which a few drops of tissue extract were added to induce clotting. On top of each clot was placed a piece of normal or infected cornea which was then covered with another thin clot of plasma and spleen extract. The tubes, sealed with sterile corks, were incubated at 37°C. for 24, 48, and 72 hours. After incubation half the cultures were studied histologically and half were tested in rabbits for the presence of active virus.

From experiments performed in the manner just described the following results were obtained. In spite of the fact that corneal tissue from adult rabbits was used, many of the cultures showed no evidence of bacterial contamination, while others contained only a few colonies of organisms that did not disturb the activity of the viruses. A few of the cultures, however, were sufficiently infected with bacteria to be valueless. Control tissue (fig. 11) always revealed definite evidence of growth of epithelial cells in the form of new epithelium filling in the defects caused by scarification, but did not contain significant inclusions. Corneas inoculated with vaccine virus (figs. 12 and 14) showed either discrete lesions separated by normal tissue or a diffuse infection involving all the epithelium. At the sites of infection the epithelium was thickened because of an increase both in the number of cells and in the size of individual cells. Numerous typical Guarnieri bodies (fig. 14) situated in clear spaces within the cytoplasm of epithelial cells were found. These intracellular changes occurred both in the cells present at the time of inoculation and in the new cells growing in to fill the defects produced by scarification in the corneal epithelium. On several occasions cells undergoing mitosis were observed to contain vaccine bodies. The end result of the vaccinal infection as studied under the conditions of these experiments was a complete dissolution of the majority of the involved cells. By tests of the material on the skin of rabbits, active vaccine virus was demonstrated in the cultures exhibiting characteristic lesions.

Herpetic virus also produced striking changes (fig. 13) in infected bits of cornea embedded in plasma. In certain areas the increase in the thickness of the epithelium produced hummocks. The intracellular bridges disappeared, the cells were swollen, many amitotic giant cells appeared, and numerous typical acidophilic nuclear inclusions were present.

The method of regularly producing virus lesions *in vitro* offered an opportunity to investigate certain immunological phenomena associated with virus infections (14). At this time, however, only the results of one set of experiments which seem to throw some light on a clinical problem will be given. Normal cornea, inoculated with vaccine virus and then cultivated in antivaccinal

plasma, developed typical vaccinal lesions with Guarnieri bodies. In such bits of cornea, although they had been cultivated in the presence of plasma containing immune bodies against vaccine virus, an abundance of active vaccine virus was demonstrated. The results of these experiments do not seem unusual provided one assumes that the activity of virus situated intracellularly could not be influenced by the antibodies in the immune plasma. This assumption at least agrees with the experimental observations of Rous and Jones (15) who found that intracellularly situated typhoid bacilli and red blood cells were not susceptible to such injurious agents as potassium cyanide and antisera. Furthermore, such an assumption—the assumption seems to be gradually becoming a fact—offers a rational explanation of why virus maladies, once signs and symptoms have evidenced themselves in infected hosts, are not appreciably affected by the administration of immune sera.

PSITTACOSIS

In 1880 Ritter (16) described the clinical and pathological picture of psittacosis in human beings and designated the malady as "pneumotyphus." Furthermore, he recognized the fact that his patients probably contracted the disease from a sick parrot in their home. Since Ritter's description of the disease appeared, cases of psittacosis in man have been reported from time to time and several epidemics of the malady have been recorded. Claims of etiological significance have been made for a variety of bacteria, e.g., streptococci and Nocard's bacillus, but none of them has been substantiated. Indeed, many physicians refused to believe that psittacosis occurs in man.

Such was the state of affairs in 1929–30 when psittacosis appeared in many places throughout the world. The number of cases at that time created considerable interest in the malady and numerous workers immediately began to seek for the etiological agent. Very quickly Bedson and his coworkers (17) demonstrated that the causative agent is in the blood of human beings suffering from the disease. This was accomplished through the injection of blood from patients into the peritoneal cavity of budgerigars

or love birds. Furthermore, they showed that the etiological agent is capable of traversing filters impervious to ordinary bacteria. Shortly after Bedson's report appeared, Levinthal (18) described in infectious material minute bodies (figs. 6 to 8) which he considered to represent the etiological agent of psittacosis. Bedson's and Levinthal's findings were immediately confirmed by a number of investigators. In addition to this, Dr. Krumwiede (19), while ill of a fatal malady, demonstrated in association with coworkers that white mice are susceptible to the virus of psittacosis and that the disease is capable of transmission from mouse to mouse by means of intraperitoneal injections of emulsions of infected livers and spleens.

At this time it became evident that laboratory investigators are likely to contract psittacosis, and work on the malady in all the laboratories in the United States was discontinued. Krumwiede turned over his material to us in February of 1930 and since then we have continued the investigation begun by him. Bedson (21, 22) in England, however, continued his studies and recently Meyer (23, 24) in California has investigated the infected breeding aviaries in that state. The remaining portion of the lecture will be devoted (*a*) to a description of what has been done in my laboratory to obtain further knowledge of psittacosis in lower animals and in man, particular emphasis being placed on the vaccination of human beings against the malady, and (*b*) to a discussion of the relation that the causative agent of psittacosis bears to other infectious agents, such as fungi, bacteria, Rickettsia, and viruses.

Early in the course of our investigations we found that psittacosis in parrots (25) attacks the liver and spleen-producing areas of extensive focal necrosis in which the minute bodies of Levinthal are readily found. We were also able to demonstrate the virus in the nasal discharges and uncontaminated cloacal contents of infected birds. Furthermore, we showed that parrots are susceptible to the virus instilled intranasally or injected intramuscularly. Regardless of the portal of entry of the virus, psittacosis in the parrot is essentially an infection of the liver and spleen with an infrequent involvement of the lungs. Parrots that have recovered from the disease are refractory to reinfection.

Inasmuch as parrots and parrakeets are the natural hosts of psittacosis, it seemed advisable to see if small mammals other than the mouse are susceptible. It was found that rabbits and guinea pigs (26) develop a meningoencephalitis when the virus is injected into them intracerebrally. From this infection the animals usually recover and are refractory to reinfection. In no instance after intracerebral inoculation of the virus into rabbits and guinea pigs did an involvement of the lungs occur.

We experienced no difficulty in demonstrating that mice (27) are susceptible to the virus of psittacosis. The active agent in minute amounts kills these animals when it is introduced intracranially or intraperitoneally. Upon intracranial inoculation a severe meningoencephalitis unaccompanied by an involvement of the liver and spleen occurs. Upon intraperitoneal inoculation an extensive focal necrosis of the liver and spleen is found. The lungs of mice inoculated in the manner described are rarely if ever involved. Mice that have recovered are refractory to reinfection.

After working with the virus of psittacosis for several months we became impressed with the fact that none of the experimentally infected animals showed an involvement of the lungs. This fact interested us particularly because psittacosis in man exhibits itself as a peculiar type of pneumonia. Consequently, we attempted to produce in monkeys (28) a pneumonia similar to that seen in human beings. Psittacosis virus injected intracranially or intraperitoneally into monkeys induced respectively a meningoencephalitis or a moderate degree of illness but no pneumonia. Virus instilled intranasally or introduced intratracheally, however, gave rise to a pneumonic process free from bacteria which was characterized by necrosis of the alveolar walls accompanied by hemorrhage, inflammation, and thickening of the interstitial tissues, hyperplasia and desquamation of the alveolar epithelium, and exudation into the alveolar spaces of mononuclear elements, fluid and fibrin. This picture closely resembles that seen in psittacosis pneumonia of man (compare figs. 15 and 17 with figs. 16 and 18).

From the results of the work reported it is now easy to understand how psittacosis spreads from birds to man. The virus is in the nasal discharges and feces of infected birds which, particularly

in the dry state, contaminate the air in the neighborhood of the cages and reach the upper respiratory tracts of human beings who come within the contaminated zone. Indeed, three patients in whom the diagnosis of psittacosis was confirmed in our laboratory did not possess birds and contracted the disease by visiting bird stores harboring infected parrots and parrakeets.

In order to diagnose psittacosis in human beings Bedson injected blood from suspected cases into budgerigars or love birds. Early in our investigations we realized the dangers of this method because budgerigars are one of the natural hosts of the disease and may carry the virus. Since pneumonia is such a prominent feature of psittacosis in man, it occurred to us that the infectious agent might be in the sputum and that its presence might be demonstrated by means of the intraperitoneal injection into white mice of large amounts of filtered sputum or of small amounts of unfiltered material coughed up from the lungs (29).

Five weeks after the initiation of our investigations one of the physicians (30) in the laboratory developed psittacosis and bits of his unfiltered sputum were injected intraperitoneally into mice. The ordinary bacteria were killed off in the mice, but the virus of psittacosis multiplied and proved fatal for some of the animals. Since that time 26 specimens of sputum either in the unfiltered or filtered state collected from suspected cases of psittacosis have been tested in mice. Of these, 13 contained psittacosis virus. Careful examinations, clinically and at necropsy when possible, of the cases from which the specimens of sputum were collected, efforts to obtain a history of contact of the patients with sick birds, and examinations in the laboratory of the birds associated with the cases make it evident that a very high percentage of the cases of psittacosis in man can be correctly diagnosed by properly trained individuals by means of the intraperitoneal injection of sputum into white mice.

Cases of psittacosis are still occurring in birds and in human beings and it is obvious that the disease has become endemic in the breeding aviaries of this country, and that cases among human beings will continue to occur until the breeders are forced by the government to close the aviaries or to get rid of their infected

stock. In view of the fact that laboratory workers exposed to infection frequently contract psittacosis, it seemed advisable to ascertain whether individuals who have had the disease are refractory to reinfection and to devise a method for the vaccination of man[2] against the disease.

In approaching the problem we decided that it would be advisable to ascertain what could be accomplished in monkeys before anything was attempted in human beings. Consequently, we set out to determine (*a*) whether monkeys that have recovered from psittacosis pneumonia are refractory to reinfection, (*b*) whether such monkeys have neutralizing antibodies in their sera, (*c*) whether large doses of psittacosis virus administered intramuscularly and intravenously to monkeys produce a serious infection, e.g., psittacosis pneumonia, (*d*) whether monkeys that have received repeated small doses of active psittacosis virus intramuscularly, that is vaccinated monkeys, are refractory to intratracheal inoculations of the virus and possess neutralizing antibodies in their sera.

Very quickly it was found that monkeys after recovery from psittacosis are more refractory to infection by the virus introduced intratracheally than are normal control monkeys. However, when we attempted to demonstrate neutralizing antibodies in their sera against psittacosis virus it was evident at once that the ordinary neutralization test was not adequate. We then devised the following test which seems to yield satisfactory results.

A 20 per cent liver and spleen emulsion from infected mice was prepared and thoroughly centrifuged. The supernatant fluid was collected and decimal dilutions of it were made with Locke's solution. Definite amounts of at least 4 of the dilutions of the virus were mixed with equal amounts of the sera being tested. A positive and a negative control serum were run in each experiment. The mixtures were incubated at 37°C. for 2 hours. Then 5 mice were inoculated intraperitoneally, each receiving 0.5 cc., with each mixture. The mice were observed for 18 days. The percentage

[2] The details of this work in which Dr. Schwentker was co-author will appear in The Journal of Experimental Medicine.

of mice that died and the average time of their death were noted. See summary of an experiment in table 1.

TABLE 1

Summary of an experiment illustrating the manner in which neutralization tests were performed

DILUTION OF VIRUS	NUMBER OF MICE INOCULATED	NUMBER OF DEATHS	PERCENTAGE OF DEATHS	DAY OF DEATHS	AVERAGE TIME OF DEATH	NEUTRALIZATION INDEX
Known normal serum + virus dilutions						
10^{-4}	5	5		5, 6, 6, 6, 6		
10^{-5}	5	5		5, 6, 6, 6, 7		
10^{-6}	5	5		7, 7, 8, 8, 8		
10^{-7}	5	5		8, 8, 8, 9, 9		
	20	20	100		6.9	14.5
Serum being tested + virus dilutions						
10^{-4}	5	5		6, 7, 7, 8, 8		
10^{-5}	4	4		6, 7, 8, 9		
10^{-6}	5	5		8, 8, 8, 12, 12		
10^{-7}	5	1		10		
	19	15	79		8.3	9.5
Known immune serum + virus dilutions						
10^{-4}	5	5		6, 7, 7, 9, 9,		
10^{-5}	5	5		5, 8, 10, 10, 10		
10^{-6}	5	4		9, 10, 12, 12		
10^{-7}	4	1		10		
	19	15	79		8.9	8.9

Neutralization index = percentage of deaths divided by average time of death.

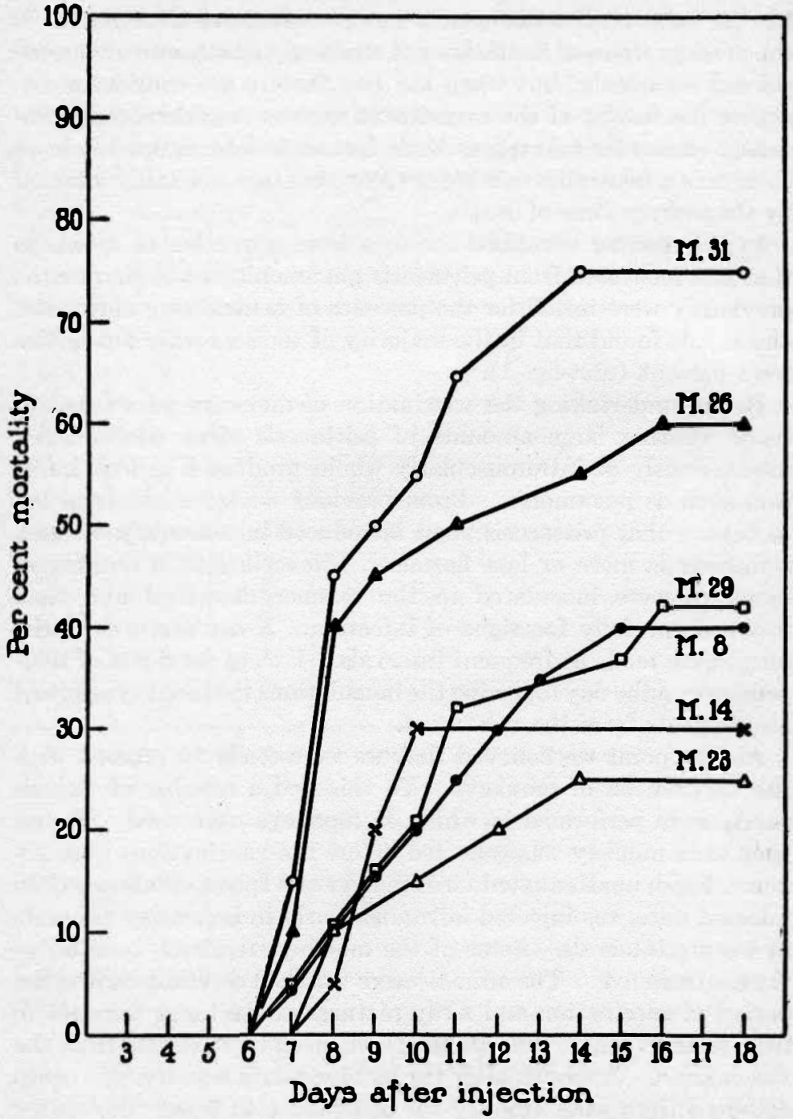
An examination of the results reveals that more of the mice receiving mixtures of normal serum and virus died than did those receiving virus mixed with the other sera. Furthermore, the average time of death for the former set of mice was shorter than that

for the latter. The differences in percentages of deaths and in the average times of death are not strikingly significant when considered separately, but when the two factors are considered together the results of the experiment assume considerable significance. In order to express both factors in one figure we have chosen as a neutralization index the percentage mortality divided by the average time of death.

In the manner described the sera from a number of monkeys that had recovered from psittacosis pneumonia one to six months previously were tested for the presence of neutralizing antibodies and it was found that in the majority of the sera such antibodies were present (text-fig. 1).

Before undertaking the vaccination of monkeys we wanted to know whether large amounts of psittacosis virus administered intravenously or intramuscularly would produce a serious infection such as pneumonia. From previous work we had been led to believe that psittacosis virus introduced into monkeys in such a manner is more or less harmless. Nevertheless, a number of monkeys were inoculated in the manner described and then watched carefully for signs of infection. X-ray pictures of the lungs were made at frequent intervals. Except for a rise of temperature on the day following the inoculations the monkeys showed no ill effects from the treatment.

At this point we believed that we were ready to proceed with the vaccination of monkeys. To this end a number of experiments were performed in which 31 monkeys were used. Serum from each monkey was collected before the vaccinations were begun. Fresh unattenuated virus in liver and spleen emulsions from infected mice was injected intramuscularly in increasing amounts at weekly intervals. Some of the monkeys received three injections, others five. The animals were watched carefully during the period of vaccination and x-ray pictures of the lungs were taken from time to time. The animals evidenced no ill effects from the vaccination. A month after the last inoculation serum was again collected from each monkey for neutralization tests. Following this, the vaccinated animals and control unvaccinated animals received intratracheal inoculations of active psittacosis virus. The



TEXT-FIG. 1. Graphic portrayal of the evidence that neutralizing antibodies are present in the sera of monkeys that have recovered from psittacosis pneumonia. M. 31 was a normal control monkey; the other animals had had psittacosis. Tests made with mice as described in text.

results of the experiments (details of one experiment are shown in chart 1) clearly indicate that vaccinated monkeys were more refractory to psittacosis virus than were the unvaccinated animals. Furthermore, vaccinated monkeys possessed in their sera neutralizing antibodies for psittacosis virus (text-fig. 2).

When we began to consider the vaccination of human beings it was obvious that we could not test the refractory state of the subjects by means of intranasal or intratracheal inoculations of active virus. Consequently, the neutralization test was the only one at our disposal for the determination of a refractory state. In previous work of ourselves (20) and others (21) it was found that the ordinary neutralization test showed little or no neutralizing antibodies in the sera of human beings who had recovered from psittacosis. With our improved test, however, we decided to see whether we could obtain better results. Sera from fourteen people without a history of psittacosis were tested. Twelve of these were negative and two were positive. Then sera from twelve individuals who had recovered from psittacosis one month to three and a half years previously were tested. Of these, six were positive and six were negative. From the results it is evident that the neutralization test is not a very reliable one for the diagnosis of psittacosis in retrospect. However, inasmuch as many of the individuals had recovered from the disease several years before the tests were made and since it is a recognized fact that seasoned bird handlers are more resistant to psittacosis than are novices in the trade, we were not discouraged and decided to proceed with the vaccination of human beings.

In approaching the question of vaccination of man against psittacosis we decided that the first volunteer must be a physician who had not had the disease and who had no neutralizing antibodies in his serum. Furthermore, it was decided that, inasmuch as the period of vaccination is five or six weeks, the procedure must be safe in the presence of a common cold. Therefore, such a volunteer was found and we waited until he developed a common cold accompanied by a bronchitis. As a control on this individual another volunteer, a physician, who had had psittacosis one and a half years previously and who had neutralizing antibodies in his serum was also subjected to vaccination.

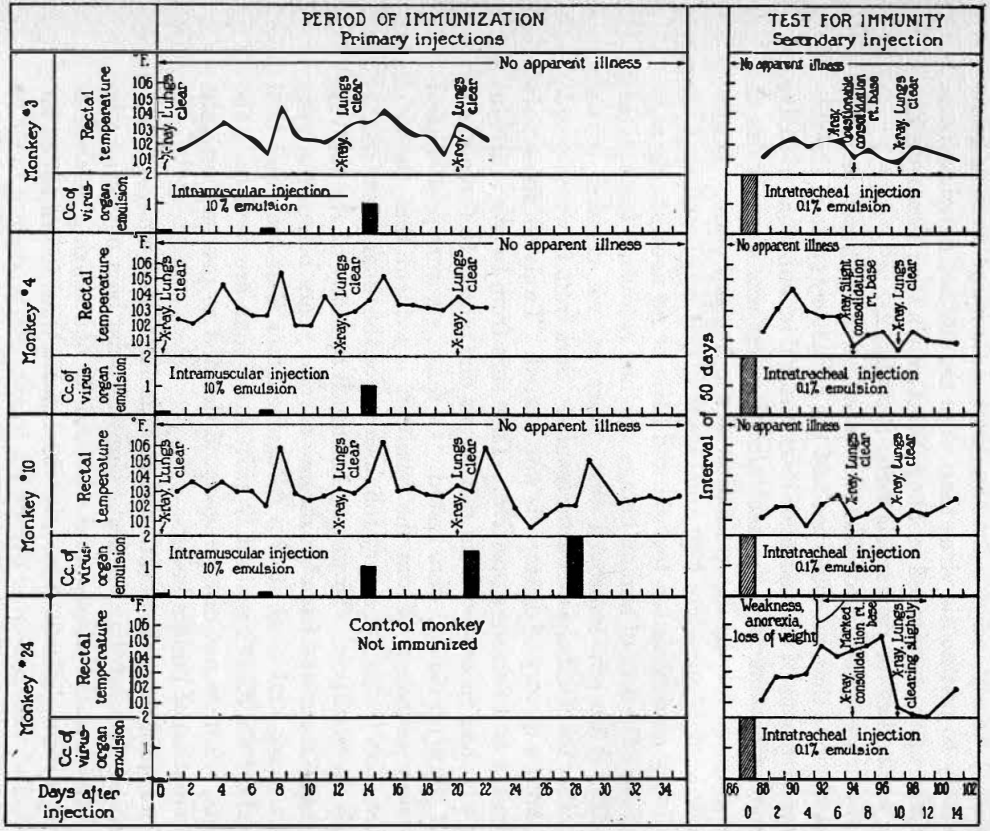
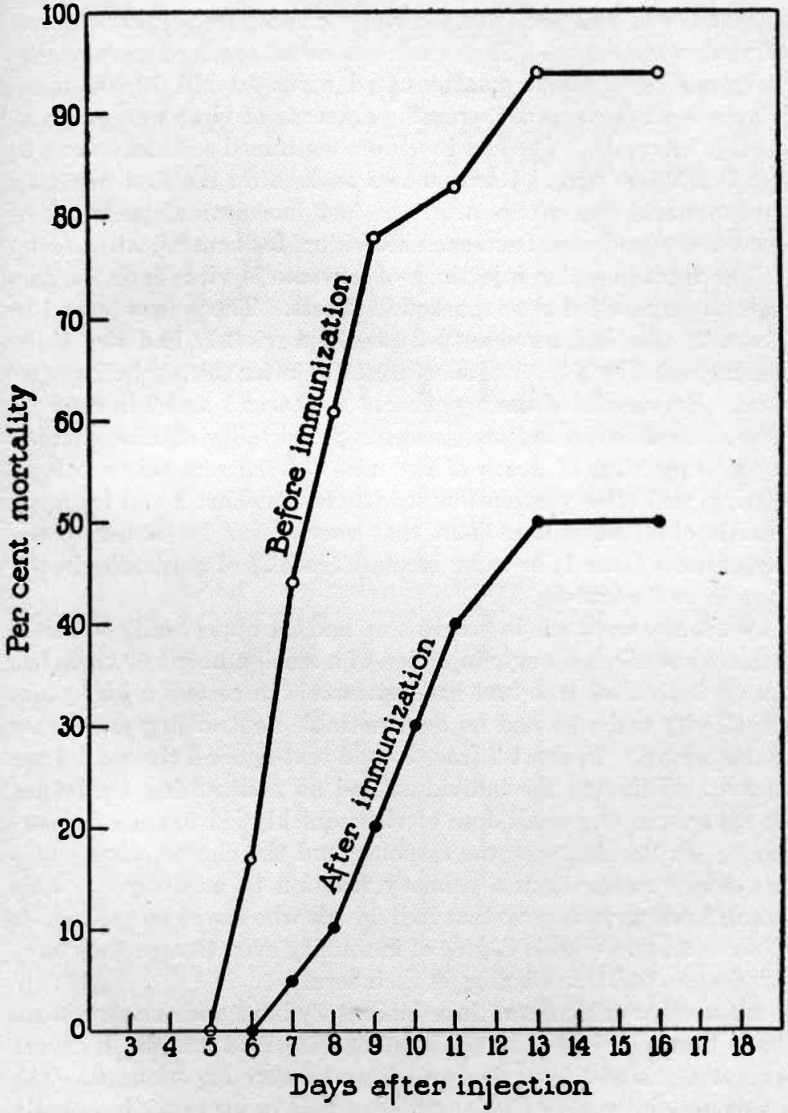


CHART 1. Summary of the results obtained in one experiment which shows that vaccination of monkeys against psittacosis by means of intramuscular injections of active virus is a safe procedure and leads to an increased resistance in the animals to virus introduced intratracheally.



TEXT-FIG. 2. Graphic representation of the evidence that vaccination of monkeys against psittacosis leads to the appearance of neutralizing antibodies in their sera. Tests made with mice as described in text.

Serum was collected from the volunteers before the inoculations of virus were begun. Then each individual received intramuscularly sufficient active unattenuated virus to kill 10,000 mice. Five other injections of increasing amounts of virus were given at weekly intervals. The last inoculum contained sufficient virus to kill 10,000,000 mice. One and two weeks after the first injection and two and four weeks after the last inoculation specimens of serum were collected from each individual for neutralization tests.

The intramuscular injections of psittacosis virus into the two volunteers resulted in no marked ill effects. The person (case 1 in chart 2) who had not had psittacosis previously had two chills accompanied by a fever of short duration after the second inoculation. For details of the experiment see cases 1 and 2 in chart 2. The neutralization indices (percentage mortality of mice divided by average time of death of the mice) of the sera taken before, during, and after vaccination are plotted in chart 2 and from the results obtained it is evident that vaccination led either to the appearance (case 1) or to an increase (case 2) of antibodies in the sera (also see text-fig. 3).

While the work was in progress we had the opportunity to determine what effect a single injection of a small amount of virus has on an individual who had had psittacosis three and a half years previously and who had no demonstrable neutralizing antibodies in his serum. In chart 2 (case 3) and text-figure 4 the results are shown. Although the individual had no neutralizing antibodies in his serum, one small dose of virus quickly led to their appearance. In this instance the response had the characteristics of a secondary rather than a primary reaction to an antigen. This result leads us to believe that individuals who have had psittacosis possess an appreciable degree of immunity even though they have no demonstrable antibodies in their sera.

Four other volunteers, three laboratory boys and one physician, have been vaccinated in the manner described and no ill effects except chills and fever in two instances were experienced. The chills and fever were similar to those that occur not infrequently after vaccination against typhoid fever. All of these individuals also developed neutralizing antibodies as the result of vaccination.

The results of our work on psittacosis point to the fact that when the virus produces a pneumonia in man its portal of entry has been through the upper respiratory tract. At least it has been clearly demonstrated that man is very unlikely to contract psittacosis

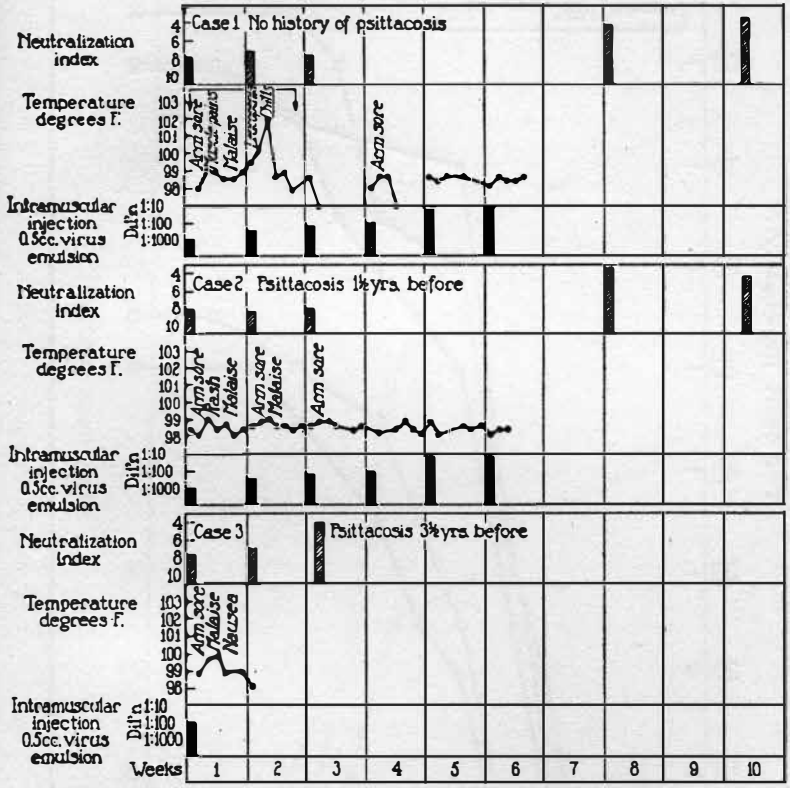
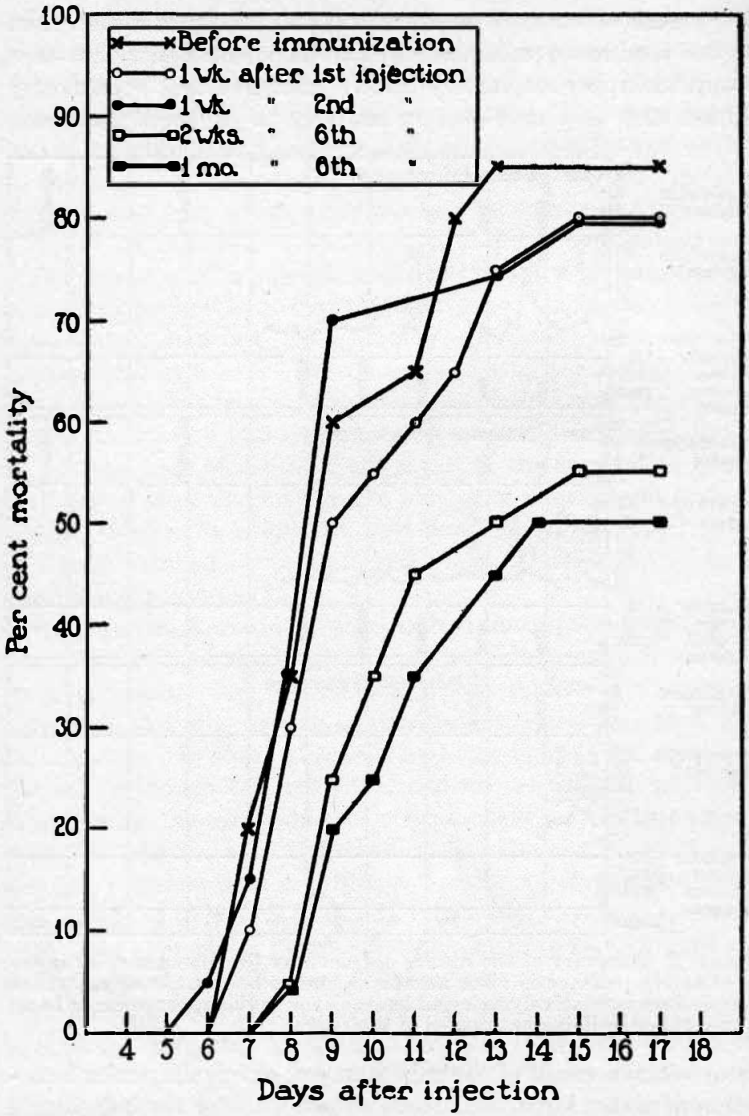
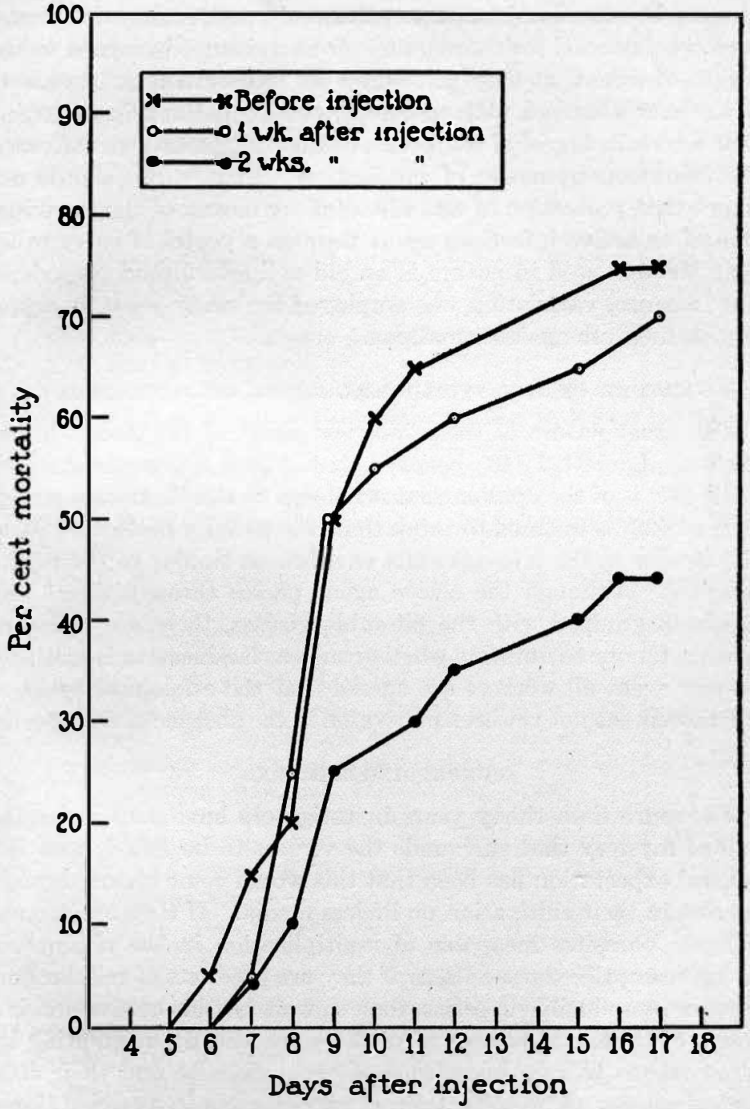


CHART 2. Summary of the results obtained by the intramuscular injection of active psittacosis virus into three human beings. It appears that the procedure is relatively safe and leads either to the appearance or to an increase of neutralizing antibodies in their sera.

pneumonia as a result of the subcutaneous or intramuscular introduction of active virus. It is not known whether the individuals who have received repeated intramuscular injections of active virus possess, in addition to the neutralizing antibodies in their



TEXT-FIG. 3. Graphic portrayal of the evidence that vaccination of human beings (case 1, chart 2) leads to the appearance of neutralizing antibodies in their sera. Tests made with mice as described in text.



TEXT-FIG. 4. Graphic portrayal of the evidence that a single small intramuscular injection of active virus into a human being who has had psittacosis and does not possess neutralizing antibodies in his serum (three and a half years after recovery) leads to the rapid appearance of such antibodies. Tests made with mice as described in text.

sera, an increased resistance to psittacosis, and, if they do possess such a heightened resistance, there is no evidence in regard to the length of time that they will retain it. Nevertheless, in view of the results obtained with monkeys, one is justified in assuming that a certain degree of protection against psittacosis was afforded the volunteers by means of vaccination. Finally, one should not forget that protection of an individual by means of the introduction of an active infectious agent through a portal of entry other than the one used in nature is an old and established procedure. For instance, variolation was employed for many years as a protective measure against smallpox.

NATURE OF THE ETIOLOGICAL AGENT OF PSITTACOSIS

The exact nature of the etiological agent of psittacosis is not known. Levinthal (18) believes that it is a minute bacterium. Lillie (31) is of the opinion that it belongs to the Rickettsia group. Bedson (22) is inclined to think that it is either a protozoan parasite similar to the microsporidia or a fungus similar to the myxomycetes. Although the active agent passes through filters and has been grouped with the filterable viruses, there are sufficient reasons for one to question whether such a classification is justified. In any event all workers are agreed that the etiological agent of psittacosis has not yet been cultivated in the absence of living cells.

CONCLUDING REMARKS

For more than thirty years investigators have waited for the veil of mystery that surrounds the viruses to be lifted, and the general expectation has been that this would come about through success in their cultivation on lifeless media. If they are minute obligate parasites incapable of multiplication in the absence of living susceptible host cells, or if they are products of cellular perversion reproducible in series, then to wait for this cultivation is a waste of time. A number of workers are already attempting by other means to gain knowledge of virus diseases and their etiological agents. The cultivation of vaccine virus in modified tissue cultures, the production of virus lesions *in vitro*, and the development of our knowledge of psittacosis have been described in more

or less detail in order to illustrate the manner in which the identity, modes of spread, pathogenesis, immunological phenomena, and measures for the control of certain infectious diseases can be investigated in spite of the fact that the nature of their etiological agents is not definitely known.

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THE NERVOUS MECHANISM OF CARDIOVASCULAR CONTROL[1]

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THE coördinated control of the heart and blood vessels is one of the most important and remarkable reflexes in the organism. Because the circulation supplies the essential and varying needs of the complex body, it can best fulfill its functions under the integrated regulation of the nervous system. The way in which this is accomplished is a striking example of precise and efficient nervous action.

In speaking before your Society, which honors William Harvey, I would choose one of his observations as an appropriate introduction to my remarks. But I find in his writings little mention of possible nervous influences on the circulation. The capacity of the heart for independent action he recognized, as in his admonition: "Nor must we disagree from Aristotle concerning the principality of the heart, that it does not receive motion and sense from the brain" (1). The control of the heart's independent contractility by the nervous system was not discovered until more than two hundred years later.

In 1845 the brothers Weber (2) described the regulation of the heart as being due to the balanced action of vagus and sympathetic nerve fibers. They showed that impulses coming to the heart over the vagal fibers have primarily an inhibitory function, slowing the rate and decreasing the strength of the beat. For sympathetic fibers they postulated an accelerator and augmentor action. The proof of this suggestion was given by many succeeding investigators and finally completed by Schmiedeberg and Ludwig (3) in 1871. Such a nervous control of the heart makes possible an adjustment of the circulation to the varying demands of the body.

[1] Lecture delivered May 17, 1934.

This adjustment is likewise dependent on the caliber of the vessels, variations in their size altering the distribution of blood to the various parts and organs in accordance with their several needs. As early as 1733 the notion that the small arteries change their caliber was put forward by Stephen Hales. From that time on the conception gradually developed that these changes were somehow due to a nervous influence, until in 1831 E. H. Weber (4) proposed for the first time, I believe: "that pallor and blushing could be explained if it were taken for granted that the caliber of the peripheral arteries could under nervous influence become smaller or larger." The means by which this is accomplished were soon discovered. It was found that arteries are supplied by nerves, and about the same time Henle (5) demonstrated involuntary muscle cells in the middle coat of the small arteries.

Thus was the stage set for the famous discovery by Claude Bernard (6) which definitely proved the existence of a vasomotor mechanism. It is so familiar that it needs little recounting. The vessels in a rabbit's ear were seen to dilate when the cervical sympathetic was cut, and it was therefore reasonable to assume that certain of the blood vessels were under the influence of vasoconstrictor nerves. This conclusion was soon substantiated by Brown-Sequard (7), Bernard (8) and Waller (9), who showed that electrical stimulation of the peripheral stump of the cut nerve causes the vessels to constrict again. And then finally Schiff (10) and more especially Bernard (11) established the existence of a second type of vasomotor nerve with a dilator function. By this dual mechanism the distribution of blood in the body is effectively controlled.

A feature of great importance in this controlling and regulating mechanism was subsequently discovered by Ludwig and Cyon (12). Stimulation of the central ends of a certain pair of nerves which end in the aortic arch caused a decreased heart rate and a marked depression of blood pressure. These so called depressor—or aortic—nerves were soon found to play an important part in the reflex regulation of the circulation. More recently Hering (13), following Pagano and Siciliano (14), has discovered a nerve with similar functions coming from each carotid sinus—or bulbous

enlargement at the bifurcation of the common carotid artery. Stimulation of the central ends of these carotid sinus nerves likewise gives a reflex slowing of the heart and lowering of the blood pressure. There is a further similarity. The fibers of both these nerves end in receptors located in the walls of the vessels and normally they are stimulated by a distention of the vessel wall. Eyster and Hooker (15), for instance, have shown that stretching the aorta causes a reflex inhibition of the heart and a fall in blood pressure, and the many beautifully executed experiments of Heymans and his collaborators (16) have given abundant evidence of such reflex effects when the carotid sinus is distended.

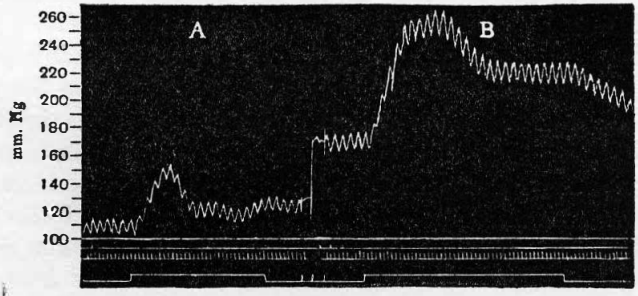

FIG. 1. Left splanchnic nerve cut and peripheral end stimulated at *A* and *B*. Between *A* and *B* both common carotids closed. (Izquerido (17).)

These effects have their origin in the receptors which, lying in the walls of the vessels, are sensitive to variations in pressure. It is their function to signal changes in blood pressure, and it is the function of their afferent fibers to provide a means for communication between these receptors and the central nervous system. Thus the centers are kept informed regarding the pressure within the blood vessels.

The reflex action of the afferent and efferent nerves we have been describing is admirably illustrated by a simple experiment of Izquerido (17). In figure 1 at *A* the peripheral end of the left splanchnic nerve was stimulated with the usual rise in blood pressure due to constriction of the vascular bed in this area. Sub-

sequently both carotid arteries were clamped so that the receptors in the carotid sinuses were non-functional. This produced a rise in blood pressure which may be explained on the assumption that this afferent mechanism had been reflexly holding down the pressure until it was taken out of action, whereupon the blood pressure rose. Stimulation of the splanchnic nerve, as at *B*, now produced a much greater rise in pressure due to the fact that the rise was not held in check by the carotid sinuses.

Such then is the mere outline of a system which provides for the nervous regulation of the heart and blood vessels. It is of profound importance in controlling the blood pressure and holding it within certain limits against factors which would otherwise produce too great fluctuations. It effects a balance and distribution of blood among the various parts and organs in accordance with their several needs. It is of great significance in many pathological conditions. Without stopping longer to discuss the vast literature pertaining to the subject I wish to go on to a consideration of the nervous mechanism whereby this regulation is achieved.

The most direct method of attacking the problem is to intercept and record the nerve messages which accomplish the control. Thus shall we see how the receptors in the vessel walls signal arterial pressure, what is the character of the nervous discharge from the centers which govern the heart and regulate the caliber of the vessels and how the activity of these cardio-vascular centers is reflexly modified by impulses coming over the afferent pathways.

It is possible to record these messages going to and from the central nervous system because the impulses of which they are composed are accompanied by electrical pulses in the nerve. This physical evidence of nervous activity is very small and very brief but it can be accurately measured and recorded with the aid of vacuum tube amplifiers and rapid electrical recording devices. The method is as follows. An afferent or efferent nerve is freed from the surrounding tissues, and electrical connection is made between two points on the nerve and the input of an amplifier. The exceedingly small potential changes in the nerve appear in the output stage greatly amplified. These potential fluctuations, or amplified nerve action potentials, are applied to an oscillograph

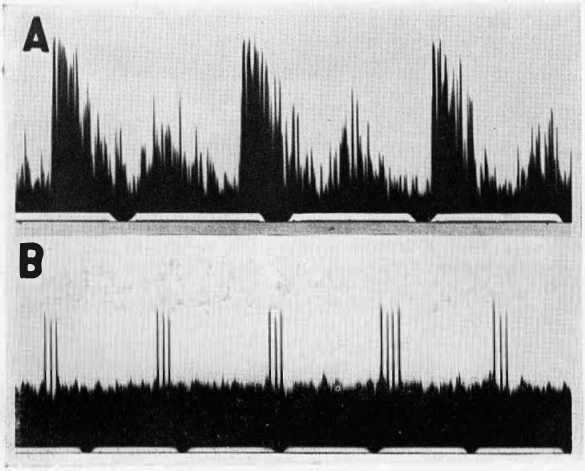

FIG. 2. *A*. Action-potential record of carotid sinus nerve, all fibers intact. *B*. Same after cutting the fibers from all but one end organ. Time marker gives $\frac{1}{5}$-second intervals. (Bronk and Stella (19).)

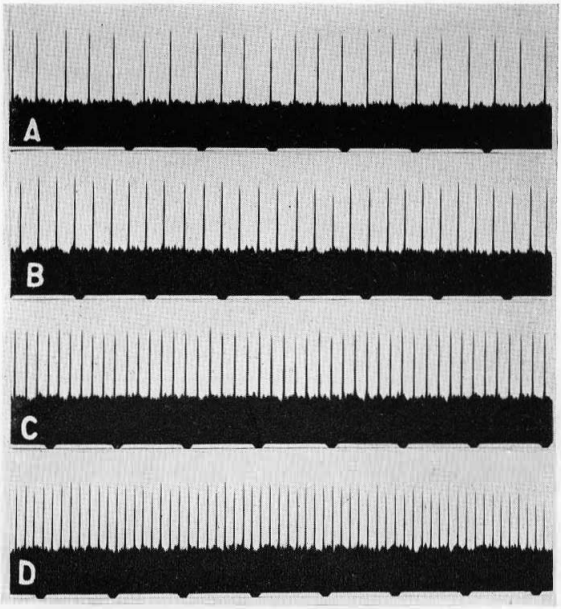

FIG. 3. Afferent impulses from a single end organ in the carotid sinus stimulated by constant pressures within the sinus. In *A* 40 mm. Hg.; *B*, 80 mm. Hg.; *C*, 140 mm. Hg.; *D*, 200 mm. Hg. Time marker gives $\frac{1}{5}$-second intervals. (Bronk and Stella (23).)

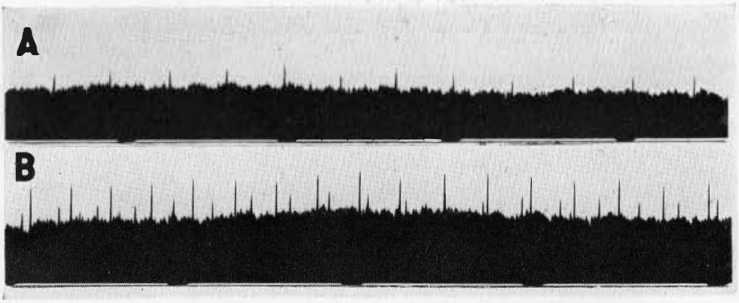

FIG. 4. Discharge of impulses from two end organs in carotid sinus, *A*, when stimulated by a pressure of 70 mm. Hg. and *B*, 100 mm. Hg. The second end organ comes into action in *B*. Time marker gives $\frac{1}{5}$-second intervals. (Bronk and Stella (23).)

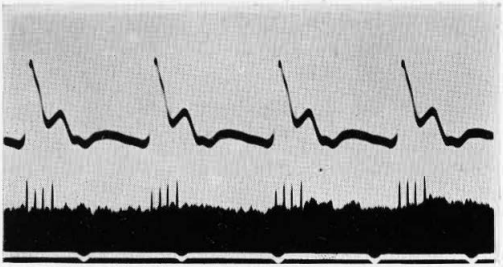

FIG. 5. Nerve impulses from a single end organ photographed simultaneously with optical record of arterial blood pressure. (Bronk and Stella (19).)

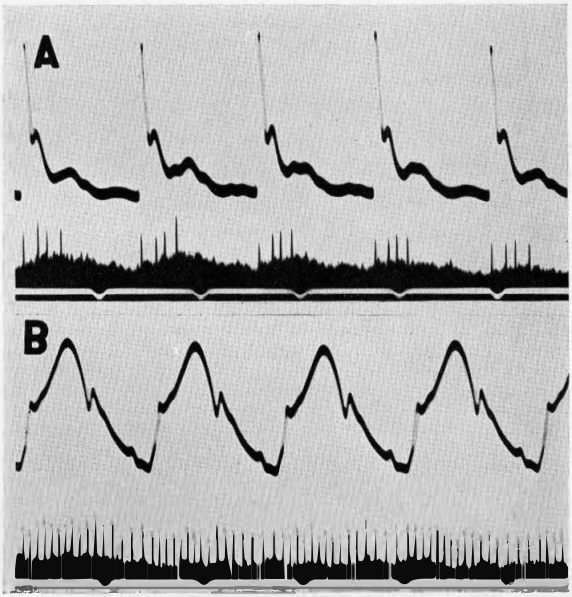

FIG. 6. The effect of increased mean blood pressure on the discharge from a single receptor in the carotid sinus. *A*. Mean blood pressure, 55 mm. Hg. *B*. Mean blood pressure, 135 mm. Hg. (Bronk and Stella (19).)

which transforms them into movements of a beam of light. Thus we obtain a photographic record of the activity in the nerve. Precise physical methods of analysis have in the past played a major rôle in the study of the circulation—through the use of the electrocardiograph, the measurement of blood pressure, the determination of blood flow. Here again the use of a new physical tool serves well in the investigation of an important circulatory mechanism.

A typical message (18, 19) from a group of vascular sense organs is given in figure 2A. It consists of periodic bursts of impulses and a less active continuous discharge. The analysis cannot be carried much further because of a difficulty which is common in physiological investigation in general and neurological investigation in particular. It is frequently responsible for the variability of biological data and the impediment one meets in their analysis. I refer to the fact that when we study the activity of a large number of cellular units we gain little information regarding the behavior of any one unit and often as little about the behavior of the aggregate. This is because the activity of the individuals is so varied that the integrated result is a jumbled confusion. If, by analogy, we wished to investigate the social activity of a community we would not intercept and record the simultaneous conversations of a thousand citizens by connecting a receiver to a telephone trunk cable. On the contrary we would eliminate all but one or a few of the circuits at a time. Only then could we understand any one of the messages. And only then could we attempt an integrated picture of the activity of the community as a whole out of the information gained from the several individuals. Similarly, in order to understand the nerve messages which keep the central nervous system informed as to the level of arterial pressure we must put all but one or a few of the nerve fibers or receptors out of action. Means for doing this are available in a well-known method for recording impulses from single sensory and motor nerve cells. (Adrian and Zotterman (20), Adrian and Bronk (21) and Bronk (22).)

It is readily possible, for instance, to cut all but one of the active fibers in the carotid sinus nerve and thus make physiological con-

nection with a single pressure receptor in the walls of the sinus (19). The record, figure 2B, of the impulses from the single receptor represents a much simpler message. It consists of short bursts of impulses, the impulses in each burst following one another with remarkable regularity. The simplification is striking, and the individual receptors apparently behave in a very orderly fashion. It should now be possible to consider how they signal differences in vascular pressure.

In order to do this Stella and I (23) have investigated certain of their properties under carefully controlled conditions. The sinus was isolated and all of the associated arteries ligated excepting the common and external carotids. Into these two, cannulae were inserted through which the sinus could be perfused at any desired, constant pressure. The distention of the vessel wall by such a steady pressure stimulates the receptors, and from each of them goes out a continuous train of impulses. Such a series of impulses, regularly spaced and of a definite frequency, is the characteristic form of all sensory nerve messages. And indeed the properties of nerve fibers are such that we could expect nothing else.

This is because when a receptor system has discharged an impulse it must go through a refractory period before it can again come into action. How soon it again discharges depends on the strength of the stimulus: the stronger the stimulus, the more rapidly do the impulses follow one another. In this way the sensory message from the arterial wall is modified by variations in vascular pressure. This is well illustrated by figure 3 where four records represent the discharge from a pressure receptor in the carotid sinus in response to pressures in the sinus ranging from 40 to 200 mm. Hg. It is obvious that the frequency of impulses in the afferent train varies with the pressure.

There is another important effect of pressure variations on the afferent discharge. Every sense cell requires a certain intensity of stimulus in order that it may respond; some have low, others higher thresholds. And so, as the pressure within the carotid sinus or aorta is raised, more and more receptors should come into action. In order to observe this it is necessary to deal with several receptors instead of just one. This is the situation in

figure 4 where, with a pressure of 70 mm. Hg, there is a discharge of impulses from one receptor at a frequency of 15 a second. When the pressure was increased to 100 mm. Hg the frequency of response from that receptor went up to 25 a second, and a second receptor came into action. By means of these two mechanisms—by variations in the frequency of impulses from the individual unit and by variations in the number of active units—these receptors should be capable of keeping the central nervous system informed of the pressure in the vessels.

We may now consider how they do so under normal conditions of arterial stimulation. In a lightly anesthetized cat or rabbit, one of the carotid sinus or aortic nerves is freed from the surrounding tissues, and electrical connection made between a single nerve fiber and an amplifier-recording system. In this way we find that the characteristic discharge from a receptor (fig. 5) consists of a volley of impulses synchronous with each systolic rise in pressure, the impulses dropping off in frequency and finally stopping completely during diastole. Because it is the function of the receptors to signal variations in blood pressure, the character of the discharge will presumably be different at different pressures. How it varies is shown by altering the mean blood pressure with injections of adrenalin, by occlusion of the descending aorta, by inhalation of amyl nitrite, etc. Typical results are shown in figure 6. With a rise in mean blood pressure the average frequency of impulses increases, as would be expected on the basis of the preceding paragraph. And furthermore the duration of the discharge increases from a short burst during systole to a train of impulses which lasts into diastole and, at still higher pressures, throughout the cardiac cycle. If several or more end organs are under observation we also find that the number in action increases with the rise in blood pressure.

The variable then in the sensory message which indicates the level of pressure in the vascular system is the total number of afferent impulses going to the centers in a given time. This depends on (*a*) the frequency of discharge from any one end organ, (*b*) the number of end organs that are active, and (*c*) the portion of each cardiac cycle they are in action. Such a triple mechanism greatly

increases the range of the signalling system. Of the three, the variation in frequency is presumably most useful for giving sensitive indications of the finer fluctuations in pressure, as illustrated in figure 7. The frequency speeds up with the systolic pressure rise, the small drop in pressure at the incisura is definitely reflected in the decreased impulse frequency, which again increases and falls with the pressure during diastole. The parallelism is remarkably faithful.

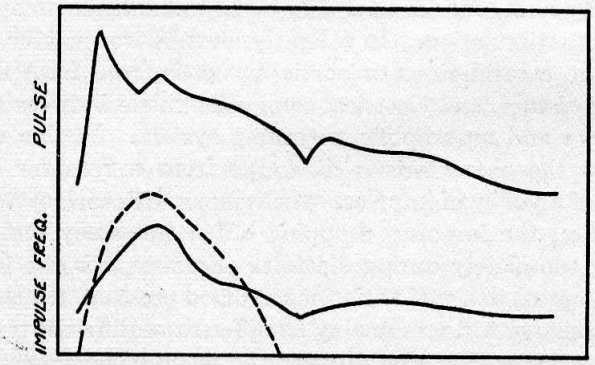

FIG. 7. Above: Tracing of arterial pressure pulse. Below: Frequency of impulses from two end organs in carotid sinus throughout the cycle, plotted as reciprocals of the intervals between successive impulses. (Bronk and Stella (19).)

This fidelity would be lost if the receptors were extremely sensitive to variations in the chemical composition of their environment as is true of certain other sense organs. This consideration and the findings of Heymans (24) and of Schmidt (25) that the carotid sinus has an important rôle in the chemical regulation of respiration led us to inquire whether the response of the pressure receptors in the carotid sinus is modified by variations in the chemical composition of the blood. If this were so, their report of blood pressure would be false because it would be modified by other variables than variations in pressure. And so we have perfused the sinus with normal arterial blood and recorded the frequency of impulses elicited by various steady pressures. The

blood was then flowed back into the general circulation and the animal was asphyxiated or caused to rebreathe a mixture low in oxygen until the blood was highly venous, when it was again perfused into the sinus. The pressure level was now varied as before. In the case of every individual receptor studied, the frequencies at each pressure in the two series (fig. 8) were practically identical. From such experiments we conclude that the pressure receptors are insensitive to variations in the composition of the blood. This is a property which increases their capacity

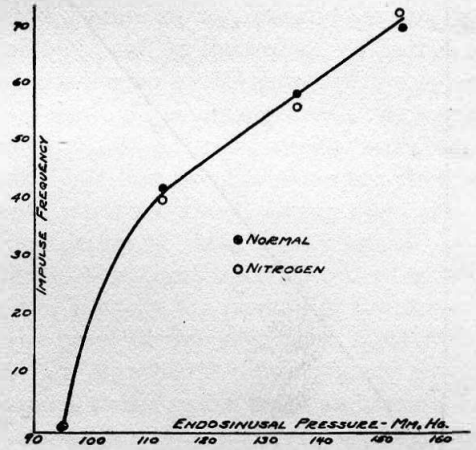

FIG. 8. The response of a pressure receptor at various pressures, *a* (full circles), with the sinus perfused with normal arterial blood and, *b* (open circles), with blood from the animal after a prolonged period of rebreathing N_2. (Bronk and Stella (23).)

for signalling without ambiguity the level of pressure at every instant.

There are obvious teleological considerations which make the aorta and the carotid sinuses ideal regions for the location of pressure receptors. There are equally important considerations, however, which indicate that there would be advantages to the organism in having such receptors in other parts of the vascular system as well. At present little is known about additional zones of vascular sensibility, but the method of recording nerve messages

which I have been describing should readily reveal their location and functional character—if they exist.

Thus Gammon and I (26) have found that there are periodic volleys of afferent impulses synchronous with the pulse in the splanchnic nerve which, of course, come from an area of great importance in circulatory adjustment and regulation. We traced the impulses down the mesenteric nerves, out their terminal twigs

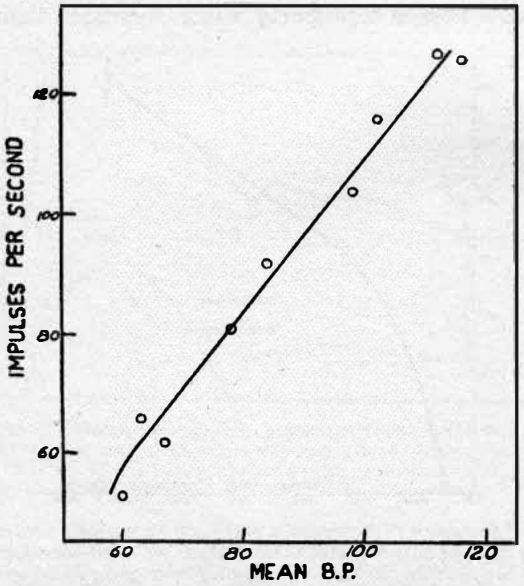

FIG. 9. Number of afferent impulses per second at different blood pressures. Several end organs. (Bronk and Stella (19).)

and so to Pacinian corpuscles in the mesentery. Here large numbers of these sense organs are massed about the vessels where they would be readily susceptible to mechanical deformation resulting from arterial distention. It had already been shown by Adrian and Umrath (27) that similar end organs located in the joints are readily stimulated by mechanical pressure. That they are here under the influence of the vascular system we have shown by isolating a portion of the mesentery, perfusing the vessels and

recording the impulses in a nerve twig coming from one or a few Pacinian corpuscles. Under such conditions there is a continuous discharge of impulses, the frequency of which increases as the pressure in the vessels is increased. Here is a rôle for these structures whose function has been so long debated.

Let us consider how they respond to varying circulatory conditions. For instance, the impulse discharge has been recorded, and the blood volume then reduced by bleeding the animal. As a result the frequency of impulses from the single end organs has invariably fallen, and the number of end organs in action has decreased. Reinjection of the blood promptly increased again the number of impulses from the region. This then is a mechanism for signalling the degree of distention of the splanchnic vessels.

Thus far we have not established with any certainty its reflex rôle. Certain preliminary experiments, however, indicate that as the vessels dilate and the Pacinians are stimulated they discharge afferent impulses which, acting upon the centers, cause reflex vasoconstriction of these same vessels. It would be the function of the reflex to check too great distention of the splanchnic vessels and thus prevent the pooling of too much blood in that area. But the proof of this suggestion must wait upon further experiments. On the other hand, the afferent mechanism is clear-cut and, inasmuch as this region plays an important part in determining the distribution of blood in the organism and the general blood pressure, the mechanism may have great functional importance. I believe it is reasonable to assume that there are still other portions of the vascular system which give rise to afferent impulses signalling blood pressure and the degree of distention of the vessels.

The number of these impulses playing upon the cardio-vascular centers in a given interval of time varies with the pressure and the degree of arterial distention. (See fig. 9.) It has been pointed out that this variation in number of impulses is due to three factors. The reflex effect on the blood pressure of those three variables in the afferent discharge has been determined by introducing these same variables into the electrical stimulus applied to the central ends of either the carotid sinus or aortic nerves.

The frequency of stimulation was varied, corresponding to a variation in the frequency of discharge from the individual receptors; the strength of stimulus was varied, and that is the equivalent of a variation in the number of end organs in action; the stimulus was altered from a series of short bursts interspersed with periods of no stimulation to continuous excitation, which

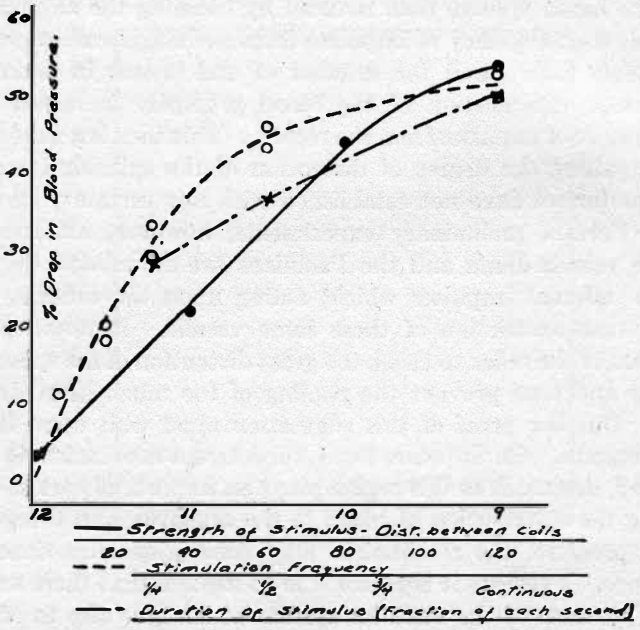

FIG. 10. Drop in blood pressure resulting from variations in the character of carotid sinus nerve stimulation.

simulates the change from the volley type of discharge to a continuous train of impulses. Each one of these variations accomplished a reflex modification of the blood pressure as shown in figure 10. The three variations in the normal sensory message are therefore effective in regulating the activity of the cardio-vascular centers and constitute the quantitative basis for the afferent side of this reflex.

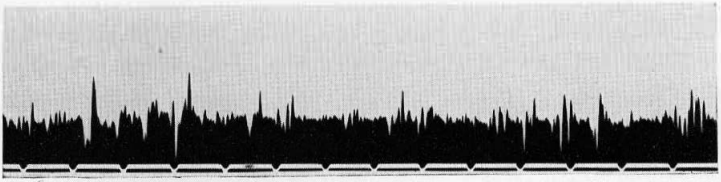

FIG. 11. Vaso-constrictor impulses in a sympathetic nerve. Time marker gives $\frac{1}{3}$-second intervals.

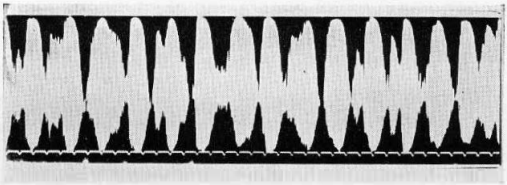

FIG. 12. Above: Volleys of accelerator impulses in right cardiac sympathetic nerve. Below: Simultaneous record of impulses in left cardiac sympathetic nerve. (Bronk, Ferguson and Margaria (29).)

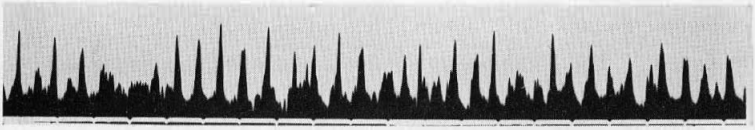

FIG. 13. Rhythmic groups of sympathetic impulses in a few fibers of a cardiac nerve. (Bronk, Ferguson and Margaria (29).)

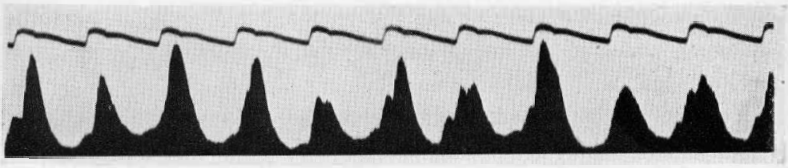

FIG. 14. Volleys of cardiac-accelerator impulses synchronous with pulse. (Bronk, Ferguson and Margaria (29).)

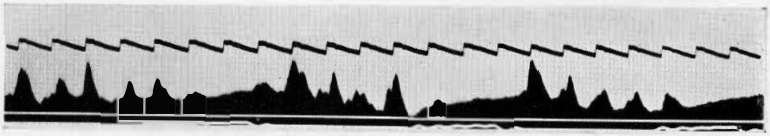

FIG. 15. Cardiac-accelerator impulses inhibited with each inflation of the lungs. Upward movement of lowest line indicates inflation. (Bronk, Ferguson and Margaria (29).)

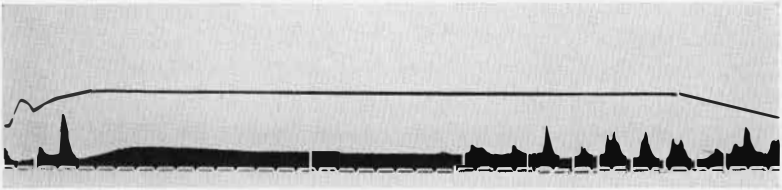

FIG. 16. Inhibition of efferent sympathetic impulses to the heart by distention of the carotid sinus. Upper record: Pressure in the sinus. Lower record: Sympathetic impulses. (Bronk, Solandt and Ferguson (30).)

Before going on to a discussion of how the reflex effects are accomplished it will be profitable to consider the nature of the efferent nerve messages transmitted from the central nervous system to the heart and blood vessels. For it is these messages that are modified by the afferent impulses in effecting the regulation of the cardio-vascular system.

The character of the vasomotor discharge has been determined (Adrian, Bronk and Phillips (28)) by recording the impulses in sympathetic nerve twigs going to blood vessels. Under fairly normal conditions of blood pressure there is a succession of potential waves passing down the nerves, and the function of the impulses which they represent is presumably to maintain the tone of the vessels. In any event, conditions which reflexly or centrally induce vasodilatation cause a cessation of these impulses.

Similar persistent discharges are found (Bronk, Ferguson and Margaria (29)) in the cardiac sympathetic nerves from the stellate ganglia which confirms previous evidence that the heart is normally under a tonic accelerator influence. Thus far we have not investigated very thoroughly the nature of the efferent discharge over the vagi but many experiments have shown that these nerves also conduct to the heart a steady succession of impulses. The normal activity of the heart is carried out under the balanced control of vagal and sympathetic influences.

The activity in the vasomotor and cardiac sympathetic nerves is characterized by two types of impulse groupings. The first results because each pre-ganglionic fiber synapses with a considerable number of post-ganglionic fibers, and consequently a whole group of the latter act synchronously and as a unit. By thus synchronizing the activity of a considerable number of these small post-ganglionic fibers, the ganglion functions as a natural amplifier and makes readily possible the determination of the activity of the individual units. This unitary activity is characteristically less regular than the sequence of impulses from a sense organ or a somatic motor nerve cell. It is quite true that rhythmic activity is frequently observed for short periods, but the rhythm is soon interrupted and lost, due presumably to the suceptibility of the sympathetic centers to many influences. The degree to which

their activity is modified by various factors is shown in the consideration of a second type of impulse grouping.

This second grouping is a more or less synchronized volley of impulses in a much larger number of fibers and is revealed by large potential waves in the nerve. That this is due to a fairly well synchronized beating of the cells in the centers has been shown by recording simultaneously the activity in a cardiac sympathetic nerve from the right stellate ganglion and in one from the left. It will be seen in figure 12 that the general time relations of the waves in the nerves on the two sides are the same. This certainly can not be due to coincidence and must be interpreted as the result of a synchronized rise and fall in the activity of the cells in the centers.

Often, as in figure 13, these bursts of impulses have a fairly regular rhythm which is related to no other obvious rhythm of the organism. We can only say that they represent groups of impulses which, when they arrive at the heart, produce increased cardiac activity. At other times, however, the waves are very definitely related to the pulse rhythm, sometimes to the respiratory rhythm and often to both. It will probably have occurred to the reader that certain of these periodic variations in sympathetic activity play some rôle in periodically modifying the frequency of the heart beat. They might for instance provide an explanation of certain of the arhythmias. In general, however, the inertia of the effector mechanism in both the heart and the blood vessels is sufficiently great to smooth out such variations in the frequency of efferent impulses, so that the results of sympathetic activity are due to the total number of impulses arriving in a fairly long period of time.

In connection with these centrally determined rhythms I would like to call attention to the extremely labile character of the cells in the sympathetic centers and the extent to which their activity is modified by variations in their environment. The inhibition of sympathetic impulses, synchronous with respiration for instance, is due to some influence exerted by the respiratory center directly, for the effect persists after all afferent pathways are interrupted. Asphyxia invariably and promptly produces a marked increase in the discharge of impulses to the heart and blood vessels. Drugs

such as acetyl-chlorine, which acting peripherally produce vasodilatation, aid that dilatation when acting centrally by inhibiting the activity of the sympathetic cells with a consequent decrease in the discharge of constrictor impulses. Veratrine not only has its well-known stimulating action on the vagal centers but also inhibits the discharge of accelerator and constrictor impulses from the sympathetic centers, thus giving a remarkable picture of direct reciprocal action on two groups of nerve cells.

But we are primarily concerned with the nervous mechanism of cardio-vascular control as determined by normal reflex influences. How, to be specific, are the efferent messages to the heart and blood vessels modified by the afferent messages coming in from the outlying pressure receptors in the carotid sinus or aorta? To answer this question we have arranged to vary the pressure in the carotid sinus by means of a perfusion system while recording the discharge of sympathetic impulses (30). The effect is dramatic and striking. As the pressure is increased the number of efferent impulses decreases, and if the pressure be raised to 120 to 140 mm. Hg the impulses stop completely. Thus the accelerator and constrictor impulses are checked by the arrival at the centers of afferent messages signalling a rise in blood pressure.

It must be emphasized, however, that the afferent control of the sympathetic centers is not quite so simple and straightforward as the preceding paragraph indicates. If, for instance, the pressure within the sinus be elevated and maintained at a given high level, there is frequently an escape from the complete inhibition after some seconds. Or, on the other hand, we have often found quite the opposite situation in which the inhibition outlasts the afferent impulses by many seconds. In the latter case the afferent impulses have developed a condition in the centers which persists long after the inflow of impulses ceases. This is a phenomenon that may be related to modern theories which explain central inhibition or excitation by assuming the formation of some humoral substance at the site of the cellular processes. In the former case the centers quickly adapt to the afferent impulses and partly escape from their influence. Such variable effects emphasize again the extreme lability of the cardio-vascular centers and the im-

possibility of quantitatively predicting the effect of a given afferent stimulus unless all of the many factors by which the centers are influenced can be controlled and maintained constant.

In any event the degree and duration of sympathetic inhibition or vagal excitation increase with an increase in the total number of impulses sent into the central nervous system over the vasomotor afferents, either as a result of increased vascular pressure or increased duration of the stimulus. Similarly the degree and duration of the inhibition increase with an increase in the total number of afferent impulses when that increase is the result of additional afferent pathways coming into action. In one case, for instance, distention of the right carotid sinus by 160 mm. Hg produced a sympathetic inhibition for 13 seconds; distention of the left carotid sinus, an inhibition for 25 seconds; while the simultaneous distention of the two gave an inhibition for 40 seconds. Distention of either sinus alone at a pressure of 120 mm. Hg did not cause a complete inhibition, but when they were distended simultaneously at this pressure they did so. Here we have definite summation of the central effects produced by the impulses from each carotid sinus. And in general the effect is determined by the total number of afferent impulses flowing into the cardio-vascular centers from the several sources.

We may now gather together our evidence into a brief, integrated summary of how the nervous system regulates the heart and blood vessels. Many details are still lacking but the essential features are clear enough. Located in the walls of the aorta, of the carotid sinuses, in the mesentery and perhaps more generally throughout the vascular system are receptors sensitive to mechanical deformation. These sense organs respond to variations in pressure within the vessels by discharging a train of nerve impulses whose frequency closely follows fluctuations of arterial pressure. The fidelity with which they do so is strikingly illustrated in figure 17 where even the smallest variation in pressure is reflected in the impulse frequency. A rise in pressure also calls more receptors into action and increases the duration of response in each cardiac cycle. Thus, as the blood pressure rises, more impulses are transmitted to the cardio-vascular centers each unit of time.

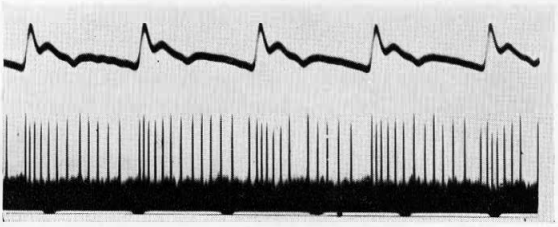

FIG. 17. Frequency of afferent impulses from a carotid sinus receptor closely following pulse pressure. (Bronk and Stella (19).)

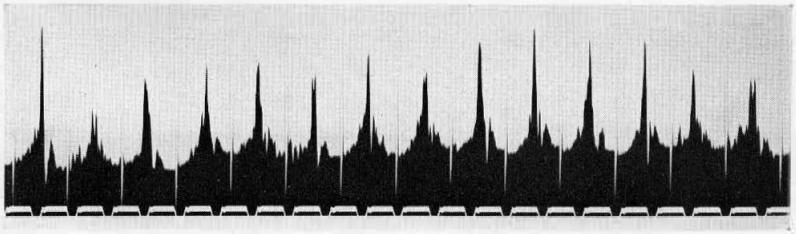

FIG. 18. Rhythmic discharge of sympathetic impulses to the heart produced by rhythmic stimulation of central end of carotid sinus nerve. Stimulus frequency shown as vertical white lines. The afferent stimulus rhythm is reflected in the efferent discharge rhythm, due, presumably to the recurring periods of sympathetic inhibition and escape. (Bronk, Solandt and Ferguson (30).)

Coming out from these centers over the sympathetic nerves is a steady stream of impulses which maintains the vessels in a state of tonic contraction and exerts a continuous accelerator influence on the heart. This is balanced by inhibitory impulses over the vagus to the heart and presumably by dilator impulses to the vessels. By such a reciprocal or balanced innervation, positive and effective control is possible.

This control is initiated by the nerve cells in the cardio-vascular centers whose activity is constantly varying in response to changes in the chemical composition of the blood, the activity of other groups of nerve cells, and especially in response to those sensory messages from the arterial walls which indicate so precisely the level of blood pressure. As these impulses signal a rise in pressure they decrease the activity of the sympathetic cells and stimulate vagus action with a resulting vasodilatation, cardiac inhibition and consequent drop in pressure. Thus the circulation is under a self-initiated control. The importance of the afferent messages in regulating the activity of the centers is shown by the last figure in which the sympathetic centers are being rhythmically driven by volleys of afferent impulses in a carotid sinus nerve.

Such are the mechanisms involved in one of the most essential regulatory systems in the body. The circulation may continue to function without it, but a delicacy of control is then lost. And such controls are characteristic of the normal organism.

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