ALLERGIC IRRITABILITY.

THE FORMATION OF ANTI-SHEEP HEMOLYTIC AMBOCEPTOR IN THE NORMAL AND TUBERCULOUS GUINEA PIG.

BY PAUL A. LEWIS, M.D., AND DOROTHY LOOMIS.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

(Received for publication, June 12, 1924.)

GENERAL INTRODUCTION.

Many special cases are now known in which some measure of acquired immunity is transmitted by a mother to her offspring. This transmission is generally believed to be "passive" in its nature, antibodies being transferred either through the placenta or with the milk. The resistance of the young is temporary as would be expected if the immunity depended on a passive transfer of antibodies. The anaphylactic or hypersensitive condition is similarly transmitted by the mother in some instances, appearing as a temporary quality in the young. This sensitization is likewise supposed to be passive, depending on the transfer of a special class of antibodies.

In the course of a discussion of one of the clear cases of the passive transmission of immunity, that against diphtheria toxin, Theobald Smith (1) pointed out that the amount of antibody formed by a given animal was doubtless influenced by the male parent as well as the female: "Though he (*i.e.* the male parent) does not transmit directly any passive immunity, yet there is no evidence to show that he does not equally, with the mother, transmit the capacity for producing antibodies, which capacity . . . . varies much from family to family, quite independently of the treatment."

Later Hagedoorn-La Brand and Hagedoorn (2) described an instance in mice in which resistance to an infection (supposedly by a staphylococcus) is influenced by heredity, control apparently being exercised through one Mendelian character.

Wright and Lewis (3) determined the resistance of pure lines of guinea pigs, developed by continuous brother and sister matings, against infection with the tubercle bacillus and found demonstrable differences, evidently inherited. The

authors consider that several factors are involved. The resistance is under the influence of the male parent equally with the female. The factors seem to be, at least in part, of Mendelian character.

Webster (4) has shown that if mice surviving a controlled epidemic of mouse typhoid infection are interbred their offspring is more resistant to the same and similar infections than is the general stock from which it is derived. Repetition produced a third generation (second selected generation) which was even more resistant. Resistance to mouse typhoid was associated with an increased resistance to poisoning with bichloride of mercury. These experiments make it plain that the resistance dealt with is not wholly specific. They do not show how far, if at all, specifically directed factors are concerned.

In these instances no suggestions appear as to the functional nature of the significant characters,—obviously a question of the utmost interest and one the consideration of which has furnished the background for the experiments presented in this paper.

It is generally believed on the basis of much evidence that the major defenses of the animal body against bacterial invasion involve the activities of the phagocytic cells on the one hand; on the other, the ability to produce and throw into action specific antibodies. We have therefore given precedence to these functions in our consideration of the nature of the factors in question and our ultimate purpose is to see whether variations in antibody production or action, or in the activities of body cells, both as related to the tubercle bacillus, can be correlated with the observed differences in the resistance of the guinea pigs to infection.

Those familiar with the general state of our knowledge of immunity toward tuberculosis will recognize that the possibilities of direct experimental approach to this problem are decidedly restricted. Antibodies against the tubercle bacillus are formed in relatively small amounts and with difficulty by any species. In the case of the guinea pig they have been scarcely more than occasionally demonstrated. It is probably true that as a rule it has not so far been possible to produce in this species any artificial immunity against the tubercle bacillus of comparable degree to the natural variation found by Wright and Lewis (3). The usual procedure of studying the phenomena of the state of acquired immunity with subsequent application to the problem of natural resistance is thus scarcely available to us. For the time being, therefore, our approach has been through

the study of the general cellular and humoral reactions of the animals, in the hope that a fuller knowledge of the peculiarities of these might show correlations with family and that, with the resistance known, suggestions for crucial experiments might appear.

We thought, for example, that it might be possible to determine the capacity of a family of guinea pigs to produce antibodies as a general proposition; that if this were accomplished it might be fair to assume that in so far as antibodies may be influential in tuberculosis they would be produced in accordance with the general scheme, and that then even rather unsatisfactory direct determinations of antibodies against the tubercle bacillus or its products might assume significance according to their relation to the more general result. A considerable number of experiments on this basis made it plain that we had entered upon quite another field to which the classical immunization experiments furnish the general method, but in which our previous knowledge of immunity reactions is insufficient to enable us to foresee results with any degree of accuracy. Our conception of immunity is dominated by the idea of specificity, and properly so, where judged only by the effect produced, either the actual protection attained by passing through a disease, or the recognized reaction products as they become available for examination. But looking at the matter from the view-point of the animal's capacity to produce antibodies, or, more generally, its capacity to be immunized, it becomes a pertinent question whether the mechanisms concerned are specific, wholly, in part, or not at all. Assuming that the mechanisms are highly specialized, can a number of them operate simultaneously at highest efficiency? Or if, on the contrary, the mechanism is common to all, can it be occupied by the first comer to the temporary exclusion of all others? It is to these questions that the experiments here reported relate directly. It has also developed that our knowledge of the reactions of the guinea pig to foreign cells has been incomplete and the observations reported add something to it.

Our title requires a brief explanation. In use, the phrase "capacity to be immunized" has proved cumbersome, nor does it quite accurately reflect the situation, since it leaves the phenomena of anaphylaxis and hypersensitization out of account or represented only by inference. This has been recognized previously and it has been custom-

ary to say that an animal once subjected to a foreign protein, or once vaccinated against a particular microorganism, is rendered allergic, or put into a state of allergy with reference to that protein or that microorganism. The word, it is true, is not so often used because we have most frequently been dealing definitely with either immunity or hypersensitiveness as particular conditions. Here we are trying to define an underlying mechanism and an accurate terminology may prove useful. Academically considered, as a matter of physiology, the production of immunity or hypersensitiveness would appear to involve the conception of stimulation, and the capacity to be stimulated involves the conception of irritability. We are thus led to propose the term "allergic irritability" as a general characteristic of the animal on the basis of which it reacts to stimuli of the antigenic class, whether they be helpful, injurious, or indifferent to bodily health.

EXPERIMENTAL.

Observational Part.—While using the outline somewhat as above presented as a guide we were led by an accidental occurrence to carry through an experiment designed to show whether a preexisting tuberculosis affected the production of antibody for an antigen unrelated to the tubercle bacillus. The result was definite, showing a decided increase in anti-sheep amboceptor production by tuberculous guinea pigs. Certain other experiments were then carried through to control and amplify the first. The procedures and results are herewith presented.

Methods.—Twenty guinea pigs of Family 13 of the inbred stock as developed at the Bureau of Animal Industry and used by Wright and Lewis[1] were inoculated intraperitoneally with $\frac{1}{100}$ mg. of tubercle bacillus Bovine C_2. The same number of animals, of the same family and in general of the same age, were put aside as controls. At the end of 3 weeks all were injected with washed red blood corpuscles of the sheep. They were given 10 cc. of a 20 per cent suspension, one-half the amount intraperitoneally, one-half subcutaneously. On the 33rd day succeeding, the surviving animals were again injected with the same amount of sheep corpus-

[1] The cooperation with Dr. Wright of the Bureau of Animal Industry has continued since the writers became associated with The Rockefeller Institute. We are gratefully indebted to him for breeding stock and for continuous increments of animals belonging to the inbred families.

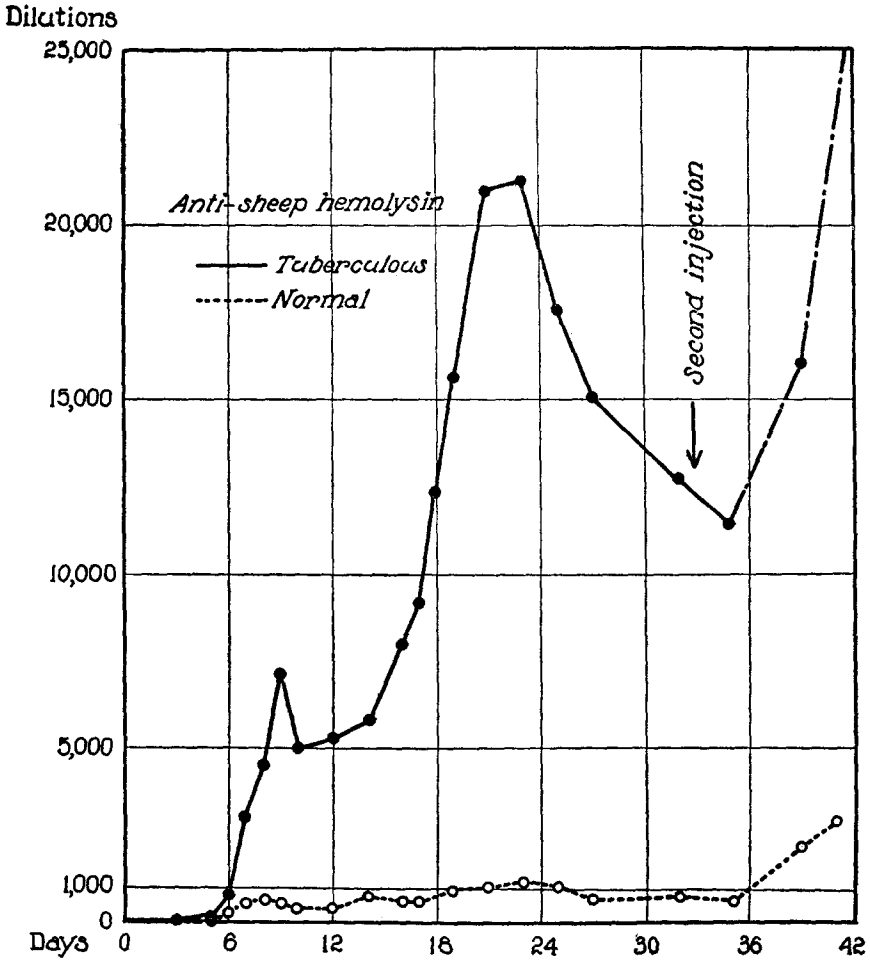

CHART 1. Average amboceptor production of tuberculous guinea pigs (solid line) contrasted with normal guinea pigs (broken line). Tuberculous pigs infected 21 days previous to the zero point of the chart. At zero days all were given 5 cc. of 20 per cent sheep red blood corpuscles intraperitoneally and the same amount subcutaneously. All bled 1 cc. from heart on days 3, 5, 6, 7, 8, 9, 10, 12, 14, 16, 17, 19, 21, 23, 25, 27, 31, 35, 39, 41. A second blood cell injection on the 33rd day.

cles in the same localities. This was successfully accomplished by giving the intraperitoneal portion first in divided doses over a period of several hours, thus avoiding anaphylactoid complications. The animals were bled on the 3rd day following the first injection of corpuscles and at intervals until the 41st day when the experiment was closed.

Bleeding was from the heart. 1 cc. was taken and immediately added to 4 cc. of normal salt solution in small test-tubes. These when clotted were stirred with a wooden applicator and the clot separated, usually by the centrifuge but sometimes by standing in the refrigerator.

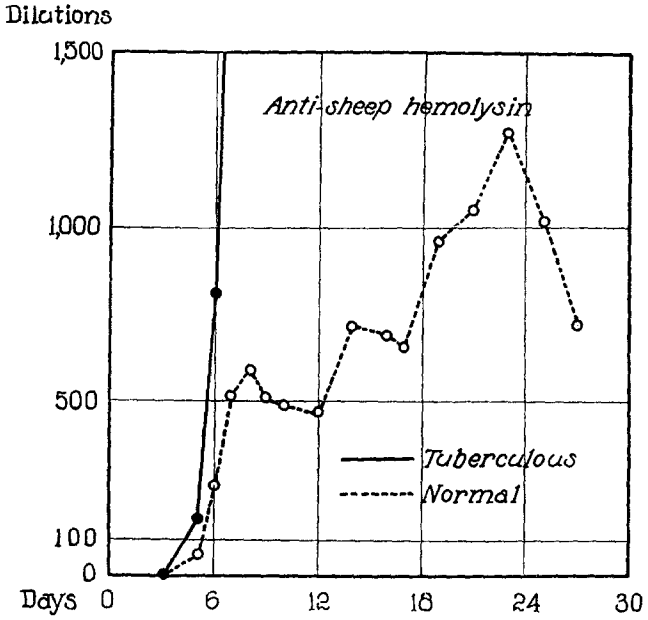

CHART 2. Same observations as Chart 1 but plotted on a larger scale to show that there are significant fluctuations in the curve for the normal animals and that the apical points are coincident with similar points in the tuberculous.

The amboceptor content was determined with these samples in much the usual way. Four units of fresh guinea pig complement (a definite excess) were used. The total volume was 1.1 cc. The amount of red corpuscles was 0.1 cc. of a 10 per cent suspension. The time of incubation in the water bath, at 35–37°C., was ½ hour, and the end-point that of complete hemolysis.

Determinations were made more closely in the case of the less active sera. The original dilution was 1:5 of blood and this was regarded as a dilution of 1:10 of serum. 1:20 was the first dilution tested. Progression was by 10's to 1:100, by 100's to 1:1,000, by 250's to 1:2,000, by 500's to 1:5,000, and by 1,000's beyond that point.

The variable factor in the test itself is the suspension of red corpuscles. This was made to 10 per cent of the whole defibrinated blood, with adjustment to the density of the preceding suspension when necessary. Fresh suspensions were prepared weekly, and it was found that at the end of the week the superficially intact corpuscles had become definitely more susceptible to the hemolytic effect of the serum. Tests of controls and the tubercular series of the same day of bleeding were therefore carried out so far as possible on 1 day. Thus, read vertically, the charts give an accurate comparison between the two groups. The progression of the curves is less accurate, but sufficient checks were done to make certain the day to day changes as observed were essentially correct.

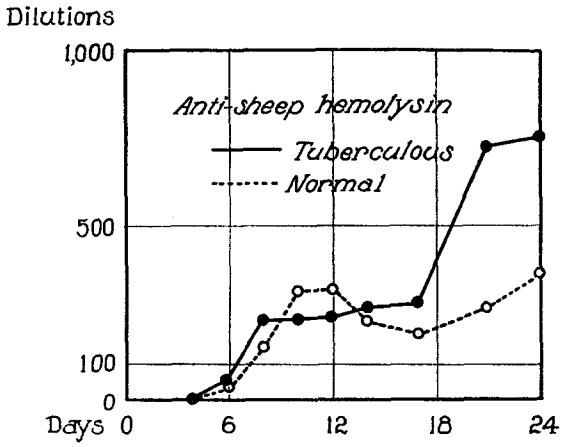

CHART 3. Average amboceptor production of tuberculous guinea pigs contrasted with normal guinea pigs. The infection was made 2 days before the injection of the blood cells, the total amount being the same as in the first experiment. The blood cells were given only subcutaneously. Bled 0.5 cc. from heart on days 4, 6. 8. 10. 12, 14, 17, 21, 24.

Bleeding from the heart involves considerable mortality, at best. The numbers included in the averages are progressively smaller as the experiment proceeds. As a check the curves for the surviving individuals have been developed and found to agree in essential characters with those of the averages.

The results of the experiments are shown in three charts and the legends which accompany them.

DISCUSSION.

Amboceptors Produced by Normal Guinea Pigs.—The curve of normal antibody production has been determined many times in the past and

something approaching a general law has seemed to be apparent. Rabbits, goats, sheep, and the horse have been the animals commonly used for these observations. The results have fallen into two classes.

When living bacteria capable of establishing disease processes in the animal have been used the curve has been irregular and prolonged. Fluctuations in the production of antibodies under these circumstances undoubtedly bear a relationship to the changes of balance between host and parasite as these develop and alter with time.

When the antigen used has been dead material, whether indifferent cells or proteins on the one hand or strong toxins on the other, the curves have shown a sharp rise following the last injection, the peak of the rise occurring from the 6th to the 23rd day. A sharp fall succeeds in the next few days followed by a more gradual decline over a period often very prolonged. The differences in detail among the curves as heretofore determined seem to be related to the antigen used. Thus the maximum for diphtheria antitoxin falls on about the 10th day (Salomonsen and Madsen (5)), that for tetanus antitoxin (Brieger and Ehrlich (6)) about the 20th day whether goats or horses be used for production. In the case of hemolytic amboceptor it is commonly accepted that the peak is on the 9th or 10th day, and that this may be advanced to the 7th or 8th day by injecting the antigen intravenously (Sachs (7)). We find no reference to the guinea pig's having been made the basis for observations on this point.

Our determinations agree among themselves in showing a departure from the accepted type. There is always a peak at about the 9th day followed by a sharp fall. Then instead of a steady decline there is a second rise beginning about the 12th day and continuing to a second maximum about the 22nd day. This again has been followed by a sharp decline. Our observations have not been carried beyond this point. It will be noted that Chart 2 shows a small peak at the 14th day also. This has frequently been seen but is somewhat less regular in its occurrence than the apices of the 9th and 22nd days.

Consideration of the experimental details makes it possible to state that this form of curve is the response to a single injection of sheep red corpuscles in the normal guinea pig (Chart 3), and that the simul-

taneous administration of the cells intraperitoneally and subcutaneously has the same effect as a single injection with respect to the form of the curve (Chart 2; *cf.* Chart 3). The amount of antibody formed is apparently somewhat greater when the cells are so administered (Charts 2 and 3 compared).

It is also plain from our experiments, although not shown in the charts, that heredity, in so far as our material permits its consideration, does not influence the form of the curves. Stock crossbred animals and two inbred families have given similar reactions.

Influence of Infection with the Tubercle Bacillus.—As shown by Chart 1 preexisting tuberculosis greatly increases the amount of amboceptor formed. At the point of maximum production and on the average this difference may be as much as twentyfold. A study of the individual animals in the series showed that no animal in the control series produced as much antibody as the least active of the series with tuberculosis. The differences at these extremes were so much less marked, however, that we would expect occasional overlapping if the experiments were often repeated.

It is to be noted that the form of the curves is not altered (compare Charts 1 and 2). This would seem to suggest that the effect of the tuberculosis is one of stimulation to a normal mechanism, or possibly, that the cellular basis of such a mechanism is hypertrophied, rather than that any wholly new factor has been introduced by the disease.

It is evident that the condition underlying increased amboceptor production in the first experiment was fully established when the blood corpuscles were injected. At this time the omentum was thickened moderately and in some animals was definitely the subject of caseous degeneration. Invasion of the liver and spleen was present, early gray tubercles in moderate numbers being just visible to the naked eye. Toward the close of the experiment the liver and spleen showed advanced tuberculosis. The second experiment (Chart 3) was designed to develop the point in the tubercular infection at which the influence in question becomes manifest. It is seen in this chart at the 14th day; in others, essentially the same and not published, the difference is evident on the 15th to the 17th day. With the

dosage and culture used it appears that the effect on amboceptor production will be invariably present and sustained after the 17th day.

In the first experiment (Chart 1) a second injection of blood cells was given 33 days after the first. This has point in the case of the tubercular animals only. There were but two of these left at the time and the graph is continued with a broken line to indicate that in the late range it has not the same finality as the preceding portion based on larger numbers. It does definitely show that the exaggerated amboceptor production of the 1st month did not exhaust the stimulating effect of the disease. In relation to a second injection the tubercular animals reacted similarly to the normal but on an abnormally high level.

Our experiments thus show that the curve of anti-sheep amboceptor production in the guinea pig is of an unusual type, and that in tuberculous guinea pigs, the form of the curve being essentially unaltered, there is a very marked increase in the amount of antibody produced in response to a given injection of antigen from the time the disease is well established.

We have done some complement fixation reactions with the serum of these animals, using tuberculosis antigens, with the idea that the stimulating action on antibody production might be more or less mutual. The results have been entirely negative. This aspect of the subject is not, however, exhausted thereby, as the conditions are far from optimal for the production of tuberculosis antibodies.

There is a considerable literature relating to the ways and means of influencing antibody production. We find no exact analogy to our experiment, no instance in which preexisting disease is clearly shown to influence the production of reaction products toward another antigen.

Directly applicable is the observation of Thompson (8) that old tuberculin or tuberculin B.E. injected into rabbits 1 or 2 days before the administration of sheep red blood corpuscles increased amboceptor production greatly. Quantitatively his results are entirely comparable to ours, the difference being that his effect was more nearly immediate. Thompson bases his interpretation on the previous work of Kyes (9), who connected the phagocytic activity of the fixed endothelium of liver and spleen with the destruction of red blood cells and bacteria, and of Cary (10), who assigned to the same cells the rôle of antibody production. Showing that tuberculin alone causes "a profound modification of the endothelium of the

liver and spleen leading to the formation of multinucleated phagocytic giant cells," Thompson believes that this change is responsible for increased production of amboceptor.

McJunkin (11) has shown that an increase in circulating endothelial leucocytes is a feature of tuberculosis in the guinea pig, and that it may also be induced by suitable administration of non-pathogenic acid-fast bacilli. Aside from any such demonstration there is every reason to believe that the epithelioid cell so prominent in tuberculous lesions is closely related to the cells which the authors just mentioned are concerned with.

Accepting and assigning full value to such evidence as to the localization of the reaction, we feel that some measure of additional interest attaches to our experiments as showing that exaggerated antibody-producing capacity is a feature of experimental tuberculosis considered as a disease complex. Moreover, knowledge of the locus of antibody formation, granted that it were complete, would in no way satisfy our need for a better understanding of the interrelations of reactions toward different antigens.

There are very many observations showing that antibody production may be increased or diminished by the administration of substances not in themselves antigenic, and there is some evidence that the process is influenced by physiologic state and by measures which alter this. This literature was well reviewed by Hektoen (12) in 1910, and most striking results have recently been reported by Madsen (13). The immediate bearing on our problem of observations along these lines is not at present apparent.

Friedberger (14) states that in his own experiments the simultaneous administration of two different antigens diminished the response to each. It is a well known fact that guinea pigs treated with diphtheria toxin-antitoxin mixtures are more highly sensitized than are those treated with equal amounts of antitoxin or normal horse serum. In the light of our experiments it would seem possible to offer a hypothetical stimulating effect of diphtheria toxin on the production of the anaphylactic antibodies as a tentative explanation of this obscure phenomenon.

So much discussion doubtless suffices to show how very much involved is the field which must be covered in an endeavor to arrive at a just interpretation of these relatively simple experiments, and

especially in trying to develop our knowledge of the nature of the inheritance of disease resistance.

The experiments themselves agree with those of Webster (4) in showing that where we are dealing with the "capacities" which must underlie the phenomena of immunity, whether natural or acquired, the rules of specificity are no longer a sure guide to either experiment or interpretation.

With relation to the problems outlined in our introduction the experiments show definitely that the body may be much occupied with one microorganism (*i.e.* one antigenic complex) without losing its capacity to react to an unrelated one. This statement does not, of course, commit us to any view with regard to the source of the antibodies, or the nature of the stimulus evidenced by increased antibody production, particularly as to whether that stimulus is exerted by an antigenic or a non-antigenic substance.

The experiments also show that in tuberculosis that character which we have designated as allergic irritability is high with respect to an antigen not related by origin to the tubercle bacillus, and thus form the background for further study in the general direction contemplated.

SUMMARY.

The guinea pig infected with virulent tubercle bacilli develops much more anti-sheep amboceptor than do controls when given like amounts of sheep red blood corpuscles.

The curve of antibody production in the guinea pig when treated with sheep red blood corpuscles shows a departure from curves previously determined in other animals.

These facts were ascertained as part of an effort to learn more of the functional nature of the inheritable factors controlling natural resistance to disease. The nature of some of the problems involved is outlined, and the limited bearing of the experiments on these is discussed.

BIBLIOGRAPHY.

1. Smith, T., *J. Med. Research*, 1907, xvi, 359.
2. Hagedoorn-La Brand, A. C., and Hagedoorn, A. L., *Am. Naturalist*, 1920, liv, 368.

3. Wright, S., and Lewis, P. A., *Am. Naturalist*, 1921, lv, 20.
4. Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 129, 879.
5. Salomonsen, C. J., and Madsen, T., *Ann. Inst. Pasteur*, 1897, xi, 315.
6. Brieger and Ehrlich, *Z. Hyg. u. Infectionskrankh.*, 1893, xiii, 336.
7. Sachs, H., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, ii, 818.
8. Thompson, H. L., *J. Med. Research*, 1922, xliii, 37.
9. Kyes, P., *J. Infect. Dis.*, 1916, xviii, 277.
10. Cary, W. E., *J. Med. Research*, 1922, xliii, 399.
11. McJunkin, F. A., *J. Med. Research*, 1920–21, xlii, 201.
12. Hektoen, L., *The Harvey Lectures*, 1909–10, v, 150.
13. Madsen, T., *Communications Inst. sérothérap. Etat Danois*, 1923, xiv, 1.
14. Friedberger, E., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, ii, 335.