THE INDUCED SUSCEPTIBILITY OF THE GUINEA-PIG TO THE TOXIC ACTION OF THE BLOOD SERUM OF THE HORSE.[1]

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The tests and experiments on which this paper is based were commenced in April, 1903, in connection with the routine of diphtheria antitoxin production. It was customary at that time to inject samples of the serum into animals before putting it on the market in order to guard against any possible contamination with pathogenic bacteria. It was found that when guinea-pigs which had survived an injection with a mixture of diphtheria toxin and antitoxin were used for this purpose, they frequently died within a few hours. For guinea-pigs that had not been used before, the injection was nearly always harmless. During the past year there has been available a considerable material which could be used to extend these observations. This material, together with the data of the earlier tests, was put at my disposal by the director of the laboratory, Dr. Theobald Smith. The preliminary observations were made by him, and I gratefully acknowledge my indebtedness to him for valuable advice throughout the course of my own work in this field.

The phenomena with which this paper concerns itself belong to a class some members of which have long been known, and to which a number of examples have been added within recent years and months. Known to the French as "Anaphylaxis," to the Germans as "Ueber-emfindlichkeit" and to the English as "Hypersensitiveness," or more recently "Supersensitiveness," the class has certain reactions in common. These depend on the fact that certain substances acting on animals continuously or repeatedly, and in suitable dose, render the animal after a time, not immune, but more than normally susceptible to their further toxic action. The substances are unknown in the chemical sense. They are constantly associated with certain mixtures of albuminous substances, bacteria, or bacterial products and are recognized biologically by their specific reaction. An example generally known is the abnormal reaction developed

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when an animal infected with tubercle bacilli is injected with the products of growth of the tubercle bacillus. Blood serum contains a substance, or substances, which under suitable conditions develop a reaction of this character. A number of years ago it was known that the blood serum of one animal was frequently toxic for animals of another species when administered by injection directly into the circulation, and the pathological effects of such toxic action were studied. Flexner(1) (1894) in the report of such a study says, "On the contrary, I found that animals that had withstood one dose of dog's serum would succumb to a second dose given after the lapse of some days, or weeks, even when this dose was sublethal for a control animal." This isolated observation was not developed further.

Richet is credited with an observation of similar import on eel serum, at about the same time. The blood serum of the horse is not toxic for the guinea-pig in the accepted sense of the word, and this may be the reason that the reaction with which we have to deal was not noticed earlier.

Acknowledging a verbal communication from Professor Smith to Professor Ehrlich in regard to the phenomenon, Otto(2), working under the latter, formally drew attention to the reaction which we are now particularly concerned with. He easily repeated the fundamental observation that while horse serum is not a poison for normal guinea-pigs, it causes sudden death or severe illness in animals treated previously with the toxin-antitoxin mixture. He showed that a period of ten days or over must elapse after the injection of the mixture before the serum becomes an active poison. He showed further that the phenomenon could be developed for the blood serum of animals other than the horse, but that it was essentially a specific reaction. That is, horse serum did not become a poison for a guinea pig previously treated with a mixture of toxin and an antitoxin derived from the goat. He developed the fact that normal serum is as effectual in killing animals as is antitoxic serum. He was able to demonstrate a reaction subsequent to a single small dose of normal serum (1/500 c.c.–1/200 c.c.) as a sensitizing treatment, but this reaction was never so severe as in the case of animals treated with the toxin-antitoxin mixture. That is, the animals when tested became very sick, but none died. He states in a footnote, however, that he was able to develop the maximum reaction by giving repeated small doses of horse serum without diphtheria toxin. That is, the toxin was eliminated as an essential factor in the development of the reaction. The animals that survived the reaction, Otto discovered, were immune to subsequent injections of serum. From this he concluded that the substance which killed was a "haptin" in the Ehrlich sense. The more recently developed facts in regard to the immunity indicate that it depends on a combination of reactions which is without well-known analogy.

Rosenau and Anderson(3) and later Anderson(4) by work begun independently confirmed many of these results. They extended their observations in various directions by testing the influence of heat, antiseptics, precipitants, etc., on the toxic substance, and by showing that the offispring of hypersensitive female guinea-pigs are hypersensitive also.

Both Otto and Rosenau and Anderson state positively that death in this manner is unaccompanied by pathological lesions.

Currie (5) writing of the antitoxin rashes in human beings includes the guinea-

pig reaction in his discussion. He reported no experiments with the guinea-pig, and his explanation of the mechanism of the reaction is more complicated than is necessary to explain the facts known in regard to the course of the reaction in the lower animal. Nicolle(6) working with the closely allied "Phenomenon of Arthus" obtained experimental results comparable to my own, and they will be discussed later. Besredka and Steinhardt(7) have experimented with the immunity which is developed when the hypersensitive animal is treated with a large but not fatal dose of serum. Their results touch my work at one point only, and will be referred to later.

Gay and Southard(8) were the first to point out in writing the fact that the intoxication of the hypersensitive animal is accompanied by very obvious anatomical lesions. These lesions in their gross aspects were clearly described in the earliest notes in our laboratory, but were completely overlooked by the earlier experimenters elsewhere. Gay and Southard have worked them out in great detail and the pathological histology of this intoxication is the sound advance made by their work. These writers also formulated a theory to explain the development of the reaction which I will discuss in some detail after reporting on my own work.

Rosenau and Anderson(9) have more recently published results of observations along the lines previously laid down. The advance which concerns us at present is the fact that the transmission of the hypersensitive condition from mother to offspring is ante-natal in its accomplishment. The milk of the mother as tested by experiments in which the nurses were changed is not an essential factor in the transmission. They are now led to the opinion, opposite to that which they formerly held and to that of Otto, that the toxin injected with the sensitizing dose does not affect the degree of the resulting sensitization. It is probable that their earlier opinion is better supported by experiment.

Vaughan and Wheeler(10) have worked on the hypersensitive reaction to egg-white. They hold that the sensitizing dose induces the formation of an entirely new ferment which first gradually disposes of the original proteid injected and then remains for a long time stored in the cells as a zymogen. This zymogen is capable of being activated by the second injection of proteid and of splitting it into a toxic and a non-toxic portion more rapidly than the toxic portion can be safely disposed of. They are able to separate by a process of chemical mutilation a toxic portion which cannot sensitize the animal, and a non-toxic residue which sensitizes against the whole egg-white. Their case differs in important respects from that of the hypersensitiveness against serum. For instance they are unable to demonstrate any newly-formed substance in the blood of the hypersensitive animal which is capable of passively sensitizing a fresh animal.

Otto(11) in a recent paper, which came to hand after the completion of my work, has demonstrated by passive transfer, a newly-formed antibody in the blood serum of the hypersensitive animal. He believes, as I do, that this antibody is distinct from the horse serum "rest" present in the same serum—the "anaphylactin" of Gay and Southard. He does not, however, distinguish the hypersensitive reaction developed in the fresh animal by the transfer of a small amount (.1 c.c. to 2.5 c.c.) of hypersensitive guinea-pig serum after the two weeks' incubation period, from the immediate reaction (twenty-four hours) that can be developed by the transfer of 10 c.c. to 15 c.c. of the same blood or blood

serum. He was able to demonstrate the antibody in the blood serum of the immune or refractory animal, and on this ground doubts the opinion of Bedreka and Steinhardt, that the immunization in this instance is really a desensitization or exhaustion of the antibody. If he had made the more decisive intracirculatory test to determine the full development of the refractory state, or had injected a considerable surplus of serum over the amount needed to enable the animal to withstand the further subcutaneous or intraperitoneal injection of horse serum, he would probably have been willing to agree to their view in its essential features. Otto further shows that the combination of hypersensitive antibody with horse serum has no power to divert to itself guinea-pig complement when tested by the method of Bordet and Gengou. He holds, on general grounds, that this reaction is the manifestation in the guinea-pig of the "phenomenon of Arthus."

It is my purpose to present as briefly as possible our early and more recent experiments, in so far as they show the early, general observations on serum anaphylaxis. In extending these observations my work has been carried on in large part with a view to answering certain specific questions. These questions may be briefly stated, and for purpose of presentation my report of results will be, in part, grouped around them under the following heads:

I. Are the various methods of inducing the hypersensitive state and of detecting it of equal value in the determination of the nature of the reaction and of the factors involved?

II. Synopsis of early experiments.

III. What are the facts in regard to the transmission of anaphylaxis from mother to offspring, and what light do these facts shed on the problem of direct or active sensitization?

IV. What is the mechanism of the acute hypersensitive reaction? More particularly, is it possible to transfer the hypersensitive condition from animal to animal with the blood or blood serum?

V. What are the facts in regard to the immunity which is developed when a hypersensitive animal is treated with a sublethal dose of horse serum and what is the nature of this immunity? Included with the discussion of this question is the rather detailed description of a type of serum hypersensitiveness unusual in the guinea-pig. The reaction is largely localized in the subcutaneous tissues, and results in necrosis.

VI. A more general discussion of the problem as a whole.

VII. Summary.

I. METHODS OF SENSITIZATION AND TEST.

The quantity of serum given at the second or test injection and the method of its administration are of primary importance in the study of the reaction. The results obtained by different workers and especially the interpretation of these results have been influenced in no small degree by the particular method of test chosen by them. In our early work and in that of Otto, the guinea-pigs were sensitized by treatment with non-fatal toxin-antitoxin mixtures. They were tested after four weeks or later with from three to six cubic centimeters of serum injected subcutaneously. Under the circumstances all of the animals proved hypersensitive and about fifty per cent. of the cases were fatal. The incubation period found to be necessary for a positive result was about two weeks, but very few of the animals were used so soon. As other workers came into the field they felt it desirable to push the work faster, and using this method of injection after incubation of from ten to fourteen days they could not get such consistent results. Rosenau and Anderson adopted the intraperitoneal method; Besredka and Steinhardt developed the intracranial injection. Gay and Southard used injections of serum alone to sensitize, and found that they were unable to kill their animal when testing by the subcutaneous method They adopted the intraperitoneal injection for routine work, and called attention to the very great sensitiveness of the animals to a test injection made directly into the circulation. I have not used the intracranial and intraperitoneal methods, and my statements in regard to them are based on the reports of others. I have recently used the direct injection into the circulation for special purposes. I find that the injection directly into the heart is the simplest procedure. After some practice it is quite certain to succeed, and it can be carried out rapidly. About two cubic centimeters of horse serum can be introduced into a normal guinea-pig of 230 grams weight without causing any symptoms. The method, the accidents incident to it, and the controls necessary, are fully described by Morgenroth(12). My technique differs from his in that I work without an assistant, tying the animal out firmly on a suitable board. I use a smoothly-working glass hypodermic syringe of capacity of two and a half cubic centimeters instead of his canula and detached syringe barrel.

Tested by this method after an incubation period of two weeks the certainly fatal dose of serum for animals sensitized by the toxin-antitoxin mixture is probably about 1/100 c.c.; 1/200 c.c. of serum will cause severe symptoms, and 1/150 c.c. will sometimes kill. Thus in testing for the degree of hypersensitiveness it is possible to inject about two hundred certainly fatal doses. By the intracranial method the certainly fatal dose is about 1/20 c.c. By the intraperitoneal route three cubic centimeters is almost certainly fatal. By the subcutaneous method it is probably impossible to reach the certainly fatal dose because of the impossibility of getting rapid absorption. As five or six cubic centimeters always develop a well-marked reaction, it is probable that from fifteen to twenty cubic centimeters, if absorbed at about the same rate, would be certainly fatal. It is impossible to use such an amount in practice. It is obvious that results obtained by one of these methods cannot be at once applied to the subject in general without most careful consideration of the values involved. In working with animals feebly hypersensitive the subcutaneous method would often show no result, while used on animals thoroughly sensitized differences in degree of reaction would be entirely masked by even 0.1 c.c. given into the circulation. For differences among animals that are all very sensitive the subcutaneous injection is capable of giving the most instructive results, unless the delicate methods are more carefully standardized quantitatively than has so far been done. To make an application of the above considerations to the problem in hand we may consider: (*a*) The incubation period; (*b*) the influence of toxin on serum sensitization.

(*a*) The incubation period is not to be considered as abruptly terminating at a given day. I have made an animal quite sick by the intracardiac injection of two cubic centimeters of serum on the sixth day after a toxin-antitoxin mixture. Those who have used the subcutaneous injection at twelve to fourteen days have not had consistent results, but by about three or four weeks the hypersensitiveness seems to reach its maximum. Holding the animals longer than this does not seem to increase the percentage of fatal cases. These rather meager facts make it appear that the antibody on which the reaction depends is produced gradually from a

time very soon after the sensitizing injection, and that the total effective quantity increases for a period of several weeks. About the sixth day, or perhaps somewhat earlier, it can be detected by the most delicate method.

(*b*) Otto published the results of a considerable number of attempts to sensitize his animals with small doses of serum alone. He found that the animals were made hypersensitive, but not to the same degree as when the serum was mixed with toxin. None died when tested by the subcutaneous method. Gay and Southard had the same experience and in order to accomplish their purpose settled on the intra-peritoneal test injection. Recently Rosenau and Anderson have published a table of results showing that toxin does not increase the sensitizing action of serum when combined with it. In these experiments they used the intraperitoneal test injection. It seems plain that the subcutaneous method of testing is the better suited to decide a point which concerns the maximum of sensitization, and I believe that the earlier opinion of Otto is the better supported by experiment.

Accepting the results of Otto, who showed that the animals which had survived the injection of diphtheria toxin alone were normal in their reaction to horse serum, I have used such animals in two groups of four each to determine what treatment, if any, with serum alone would develop the maximum grade of hypersensitiveness. As the accompanying table shows, I have confirmed Otto's observation which he did not report in detail, that repeated small doses of serum alone could do this.

The treatment with 1/1000 c.c. serum given three times on alternate days, with the injection ten days after the third treatment, was about as efficient as the treatment with the toxin-antitoxin mixture and slightly more efficient than a treatment with 1/2000 c.c. serum on ten successive days, with the test treatment ten days after the last injection. Either method is much more efficient than the single treatment with three cubic centimeters or over, or than the single treatment with 0.03 c.c. to 0.0025 c.c. serum, reported in detail by Otto.

TABLE I.—*Effect of Repeated Small Doses of Serum.*

Guinea-pig Number.	Sensitizing Treatment.	Interval Between Treatments.	Test Treatment.	Result of Test Treatment.	Remarks.
4704	1/2000 c.c. serum, 10 injections in 11 days. Total 1/200 c.c.	10 days after last injection, 21 days after first.	5 c.c. serum subcut.	Slight immediate symptoms; late induration.	Slight induration after first injection with 1/2000 c.c. serum.
4701	do	do	do	Same.	
4658	do	do	do	Same + small late ulcer.	
4689	do	do	do	Dead 2 hours.	
4699	1/1000 c.c. serum, 3 times in 6 days. Total 1/333 c.c.	10 days after last injection, 16 days after first.	do	Dead in night.	Slight induration after 1st injection with 1/1000 c.c. serum.
4722	do	do	do	Dead 4 hours.	Well marked swelling after 1st injection of 1/1000 c.c. serum.
4737	do	do	do	Slightly sick; late ulceration.	
4696	do	do	do	Very sick; late ulceration.	

II. SYNOPSIS OF EARLY EXPERIMENTS.

The records of the laboratory show that thirty-eight guinea-pigs bred from untreated mothers and themselves not treated were injected with doses of from three to five cubic centimeters of normal or antitoxic horse serum. Of these not one was noticeably affected. Examined at the end of twelve and twenty-four hours the subcutaneous tissues often show no palpable œdema or induration. The latter statement does not cover the full number of animals reported on, as such examinations were not always made. We have no contrary case, however. Our results are thus in accord with those of others in showing that practically horse serum is not a toxic substance for the normal guinea-pig. But neither is it an indifferent solution of substances which can be entirely eliminated from the subcutaneous tissues by processes which govern the removal of normal saline solution, for example. In several instances the injection of 1/1000 c.c. or 1/2000 c.c. of serum in two cubic centimeters of normal saline solution has given rise to a well-marked œdema at the end of twenty-four hours. Also, as can be demonstrated by suitable test-tube experiments, horse serum normally contained small amounts of ambocepter active between guinea-pig red blood corpuscles and the complement of guinea-pig serum. Certain of its constituents are probably always removed by complex processes similar in kind to those which prevail in the case of the hypersensitive animal, and the tissues may, under certain circumstances, be mildly injured in the course of their elimination.

Thirty-six animals treated with from two and a half to five cubic centimeters of serum as a first dose were subsequently once or twice injected with similar quantities. Of these thirty-three remained without symptom or lesion. One showed symptoms at the second treatment. One died at the second treatment, and one died at the third injection. The intervals between treatments have varied between thirteen days and four months, and have most frequently been between three and six weeks. The animals that died received their fatal treatment after an interval of sixteen days in each case. These results agree with those obtained elsewhere, and show that a single or repeated large dose of serum may render the animal susceptible to a subsequent similar dose, but that it is a

much less efficient way of inducing a hypersusceptibility. It is quite possible that the fatal results in the two cases may be due to the accidental absorption of serum directly into the circulation through a vessel injured by the injection. Or it may be that there is really a very great difference in the reaction of the individual animals to the first large dose. However that may be, our cases are evidently not comparable to those of Gay and Southard, who found that the large dose always rendered the animal hypersensitive if enough time were allowed to elapse before the test injection. As the intervals they report between the sensitizing and intoxicating injections were as a rule shorter than ours, these differences in result must also be attributed to a difference between the effects of the intraperitoneal and subcutaneous injections. In these experiments they used the intraperitoneal route for both first and second injections, and in this way avoided the great binding power which the subcutaneous tissues probably have for the toxic principle here involved.

Twenty-five guinea-pigs which had survived the treatment with a mixture of diphtheria toxin and antitoxin were injected with large doses of horse serum. In each instance in which the dose injected was one cubic centimeter or over the animal was made sick. Fourteen of them died. In our experience as well as in that of Otto and Rosenau and Anderson, it is a law without exception that treatment with a toxin-antitoxin mixture renders the animal susceptible to an acutely acting toxic substance in normal and antitoxic horse serum. The exact amount of serum injected with the mixture, if between 1/100 c.c. and 1/500 c.c., and the exact interval between the injections, if between two weeks and three months, *are indifferent matters* in the development of the reaction. The same may be said of the local lesion caused by the mixture, and of the genealogy of the animal.

The length of time that such an induced susceptibility may persist has not been fully determined so far. I have been able to test thirteen old females with intervals after sensitizing varying from eleven months to two years. Compared with those tested in less than four months after sensitization, these animals gave less reaction. Two of them, at eleven and sixteen months, respectively injected with five cubic centimeters of serum gave no reaction.

Three animals of the series died, one after an interval of twenty-two months between sensitizing and test injections. The others ranged between these extremes. The reaction is thus a slowly disappearing one which may probably in individual instances persist throughout the life of the animal.

III. HYPERSENSITIVENESS TRANSMITTED FROM MOTHER TO OFFSPRING.

Our experience in injecting large doses of serum (from three to six cubic centimeters) subcutaneously into guinea-pigs bred from mothers that had been treated with the toxin-antitoxin mixture or with horse serum alone is shown in the following table.

TABLE II.—*Transmitted Hypersensitiveness.*

Treatment of Mother.	Number of Mothers.	Number of Offspring.	Result.
Toxin + Serum, 1/100 c.c. to 1/500 c.c.	27	41	Well 17; Dead 24
Serum only. Large dose, 3 c.c. to 5 c.c.	4	6	Well 2; Symptoms 2; Dead 2

The fact that the increased susceptibility generated in a female guinea-pig by treatment with a sublethal toxin-antitoxin mixture is transmitted to her offspring as first published by Anderson, is clearly shown. It is also seen that treating the mother with a single large dose of horse serum renders the offspring hypersensitive. Gay and Southard found that the offspring of their guinea-pigs sensitized with serum alone were hypersensitive in several instances But in our experience only a percentage of the animals bred from hypersensitive mothers are abnormal in their reaction. It might be supposed that the mothers of those young which do not react are not themselves hypersensitive. We were able to test this in one case and found that the mother still gave a moderate reaction, although her offspring at the time of test gave none. Furthermore, several females have given birth to individuals of each class, the normal and the hypersensitive. In one case an entire litter (four animals) of one female was tested on the same day with the same

dose of serum (six cubic centimeters subcutaneously). Two of them died within fifteen minutes, the other two gave no reaction. It is obvious that if the statement of Rosenau and Anderson, that the hypersensitive state is always transmitted from the mother to offspring (and by inference to all of them), be essentially true, the law must be limited in its application by certain conditions. To reason from the published experiments of the last mentioned authors, from our own experience, and from analogy with the other cases of transmitted immunity reactions, it seems that hypersensitiveness is by nature transmissible. But the mother probably transmits less effectively if her own initial sensitiveness is low and if the elapsed time between her sensitizing treatment and the birth of the offspring in question is long. The young animals seem to lose their sensitiveness with some rapidity, as they increase in age and size, and it is probable that the individual variation in the rate of this loss is considerable. It must be so if it is to account for the extreme difference between animals of the same litter tested on the same day. Alternatively one could assume an individual difference due to the influence of a normal father, but this would be without known analogy, and could only be justified by prolonged experiment.

The type of reaction obtained in these guinea-pigs which have acquired their increased susceptibility from the mother is interesting and calls for explanation. The animals rendered hypersensitive by treatment with the toxin-antitoxin mixture when treated with a subsequent injection of serum usually begin to show symptoms in about half an hour. Those that die usually do so in from two to four hours. Those that recover are ordinarily most ill at about four hours after the injection. From this time they recover rather rapidly, and are to all appearances well in from six to twelve hours. Occasionally death is delayed twelve hours and complete recovery to twenty-four hours. The animals tested for a transmitted susceptibility have reacted quite differently. Those that have proved hypersensitive have usually died in from fifteen to thirty minutes after injection. Death has occurred in five minutes. In two instances out of twenty-four death took place at the end of two hours; in one instance, in the night after some hours. The animals

that do not die show almost no reaction. They frequently brush the ears and nose with the forefeet and have a staring coat for half an hour, more or less. I have not seen a case in which an animal bred hypersensitive became severely sick and recovered. Rosenau and Anderson publish protocols which do not bear out this experience, but as they have used the intraperitoneal injection altogether the results are not strictly comparable. This sharp distinction between the reaction given by different young animals, extending as it does even to individuals of the same litter, together with the more rapid reaction given by the hypersensitive offspring, cannot perhaps be clearly explained by facts definitely proven at the present writing. Several factors may be mentioned as probably influencing the results. The young animal may be more easily injured by the ultimate toxic substance when it has the capacity to form or assimilate it. Absorption from the subcutaneous tissues in the young animal in so far as it depends on physical conditions, is probably more rapid than in the older one. The subcutaneous tissues of the animal sensitized by direct injection have probably been greatly influenced by the treatment, and in such a way that there is a local hypersensitiveness induced which leads to a local specific absorption of the toxic substance, tending to protect to a degree the cells of more vital organs. This will be rather definitely developed later. Such a local reaction may be less easily transferred from mother to offspring than a more general one depending on the conditions in the blood.

The fact that there is in connection with the phenomenon of serum hypersensitiveness a definite transmission of the susceptibility to the reaction from the mother, to her offspring, is at the present time very strong evidence for the proposition that the sensitizing injection causes the formation of an anti-body with which the second or test injection reacts.

IV. PASSIVE TRANSFER OF THE HYPERSENSITIVE CONDITION.

Gay and Southard attempted to determine the mechanism of the hypersensitive reaction. Their essential experiments from this point of view may be briefly restated. They found that the serum of hypersensitive guinea-pigs mixed with horse serum, incubated

TABLE III.—*Results of the Passive Transfer of Hypersensitiveness.*

Normal Guinea-Pig Number.	Sensitizing Treatment.	Interval.	Intoxicating Treatment.	Result.
5038	27–VI–07. Serum of sensitive G. P. Inj. 1.75 c.c.; intracardiac.	1 hr.	27–VI–07. 1 hr. later. Inj. 1 c.c. normal horse serum ; intracardiac.	No symptoms or very slight.
5035	27–VI–07. Ser. of sens. G. P. Inj. 3 c.c.; intraperitoneal.	1 d.	28–VI–07. Ser. nor. horse. Inj. 1 c.c.; intracardiac. 5 c.c. intraperitoneal.	Very slight symptoms.
5044	28–VI–07. Ser. of sens. G. P. Inj. 5 c.c.; intraperitoneal.	3 d.	1–VII–07. Ser. nor. horse. Inj. 2 c.c.; intracardiac.	No symptoms.
5047	28–VI–07. Ser. of sens. G. P. Inj. 2.5 c.c.; intraperitoneal.	6 d.	4–VII–07. Ser. nor. horse. Inj. 1 c.c.; intracardiac.	Slight symptoms.
5045	28–VI–07. Ser. of sens. G. P. Inj. 1.5 c.c.; intraperitoneal.	24 d.	24–VII–07. Inj. 1.1 c.c. ser. nor. horse; intracardiac.	Dead ½ hour.
5036	23–VI–07. Ser. of sens. G. P. Inj. 2.5 c.c.; intraperitoneal.	1 d.	24–VI–07. Ser. nor. horse. Inj. 6 c.c.; intraperitoneal.	Moderate but definite symptoms.
4975	2–VIII–07. Defibrinated blood of sens. G. P. Inj. 14 c.c.; intraperit. and subcut. 4–VIII–10 a. m. Same. Inj. 5 c.c.; intraperitoneal.	2 d.	4–VIII–07, 12 m. Inj. 1.5 c.c. nor. horse serum; intracardiac.	Severe symptoms; chloroform 4 hrs.; hemorrhagic lesions.
6010	Freshly defibrinated blood of sens. G. P. 8–VIII–07: Inj. 3 c.c.; intraperit. 9–VIII–07: " 6 c.c.; " 10–VIII–07: " 6 c.c.; " Total 15 c.c.	3 d.	11–VIII–07. Inj. 1.25 c.c. nor. horse serum; intracardiac.	Dead 3 minutes.
5079	Freshly defibrinated blood of sens. G. P. 25–VII–07: Inj. 3 c.c.; intraperit. 26–VII–07: " 5 c.c.; " 27–VII–07: " 2 c.c.; " 28–VII–07: " 5 c.c.; " Total 15 c.c.	4 d.	29–VII–07. Inj. 2 c.c. nor. horse serum; intracardiac.	Dead 4 minutes.

TABLE III.—*Results of the Passive Transfer of Hypersensitiveness.*—Concluded.

Normal Guinea-Pig Number.	Sensitizing Treatment.	Interval.	Intoxicating Treatment.	Result.
6017	19–VIII–07. Freshly defibrinated blood of sens. G. P. Inj. 15 c.c.; intraperititoneal and subcutaneous.	1 d.	20–VIII–07. Serum of normal horse. Inj. 1.5 c.c.; intracardiac.	Dead 2 minutes.
6059	11–IX–07. Serum of sens. G. P. Inj. 7.5 c.c.; intraperitoneal.	1 d.	12–IX–07. Nor. horse ser. Inj. 1.5 c.c.; intracardiac.	Very severe symptoms.
6058	12–IX–07. Serum of sens. G. P. heated to 60° ½ hr. Inj. 7.5 c.c.; intraperitoneal.	1 d.	13–IX–07. Nor. horse serum. Inj. 1.5 c.c.; intracardiac.	Moderate symptoms.
		Control Experiments.		
6013	19–VIII–07. Freshly defibrinated blood of normal G. P. Inj. 15 c.c.; intraperitoneal.	1 d.	20–VIII–07. Ser. of nor. horse. Inj. 2 c.c.; intracardiac.	No symptoms.
5082	23–VII–07. *Diph. Toxin* — .18 c.c. Antitoxic serum 1/100 c.c. +. Inj. subcutaneous slight lesion.	6 d.	29–VII–07. Ser. of nor. horse. Inj. 2 c.c.; intracardiac.	Slight but rather definite symptoms.

Note. — All of the fresh guinea-pigs used in the above experiments except No. 4975 weighed from 230 to 250 grm. It is doubtful if larger animals would have reacted as well. The first five animals illustrate the ultimate sensitization by small doses of sensitive serum. The second control experiment was made for the purpose of showing that the cases in which the passive transfer of sensitive serum occupied several days do not reach the beginning of the effective incubation period for direct or active sensitization even when the latter is tested for by the intracardiac method.

and injected into the circulation of normal animals, provoked no reaction. On the basis of two such experiments they apparently drew the conclusion that the serum of the hypersensitive animal does not contain an anti-body for the toxic substance of horse serum. They further showed that 1.5 c.c. of serum of a sensitive animal injected into a " fresh " animal rendered it in turn hypersensitive after the usual incubation period of fifteen days. They also found that the blood of one sensitized and subsequently immunized animal transferred to another hypersensitive animal contains no demonstrable toxic substance. If, on the other hand, the blood of the refractory animal is transferred to a fresh animal it sensitizes it after the usual incubation period. Otto has recently published experiments showing that a fresh animal may be rendered hypersensitive within twenty-four hours by the injection of the blood serum of a hypersensitive guinea-pig. Bearing on these points I submit the following tabulated results of experiments on the passive transfer of the hypersensitive condition from animal to animal.

I have not thought it necessary to detail the history of the animals from which the sensitizing blood was drawn. They were all guinea-pigs that had been through the treatment with a toxin-antitoxin mixture some weeks previously. In order to eliminate a possible individual variation in the blood of different animals a mixture of bloods was usually employed. As it was unknown whether the intermediate substance was a labile body or not, and whether it was free in the serum or might not perhaps be bound to corpuscles, many of my attempts to transfer were made with freshly defibrinated blood including the corpuscles.

The results show definitely that there is in the defibrinated blood and in the blood serum of guinea-pigs hypersensitive to horse serum a substance which, when injected into normal guinea-pigs, renders them also hypersensitive to horse serum after a lapse of twenty-four hours.

The further study of the characteristics of this substance must be left to the future. One experiment shows that it is not destroyed by heating the serum to 60° C. for half an hour. Otto has found that it has no power to divert complement when combined with horse serum.

Gay and Southard's observation that a smaller quantity of sensitive or refractory serum can sensitize after the incubation period, I am able to confirm. I agree with them in the belief that this is a manifestation of a retained element of horse serum. But I think that this acts as an active sensitizer and is entirely distinct from the anti-body which takes part in the intoxication.

V. IMMUNITY OR ANTIANAPHYLAXIS.

It was very soon found that if a hypersensitive animal were injected with a large dose of serum but survived the reaction, a second large dose within a few days produced less reaction or none at all. Otto, who first recorded this observation, dismissed the matter in a sentence by assuming that the toxic substance is a haptin in the Ehrlich sense. It has since been found that the reaction is very different in its time relations, at least, from other immunity reactions. Twenty-four hours, or perhaps less, is all the time required to bring this immunity to its full protective force, as has been pointed out by Besredka and Steinhardt. The first of the following experiments is illustrative. Comparison of the first with the second experiment shows, too, that it is the quantity of serum injected and the point of injection that are important, rather than the fact that the animal has survived the reaction.

G. P. 4764. Sensitized Jan. 29, 1907.

$\frac{\text{Diph. Toxin .21 c.c.}}{\text{Serum 1/175 c.c.}}$ Small ulcer; paralysis.

March 28, 1907, 11 a. m. Serum of normal horse No. 93; 0.5 c.c. injected subcutaneously.

6 p. m. Quite sick.

March 29, 1907, 10 a. m. Well.

5.0 c.c. normal serum of horse No. 93; injected subcutaneously. No symptoms.

G. P. 5014. June 8, 1907. Sensitized.

$\frac{\text{Diph. Toxin .215 c.c.}}{\text{Serum 1/300 c.c.}}$ No lesion.

June 28, 1907: Normal serum of horse No. 106.

1/200 c.c. serum + 199/200 c.c. salt sol. by intracardiac injection. Severe symptoms; convulsions.

June 29, 0.1 c.c. ser. + 0.9 c.c. salt sol. by intracardiac injection. Dead after 3 minutes.

Besredka and Steinhardt have shown that it is possible to immunize in twenty-four hours against the more delicate intracranial injections and I have been able to extend this to the intracardiac injection.

The condition which makes it possible to reduce so rapidly the hypersensitiveness in the animal I believe to be a local hypersensitiveness of the subcutaneous tissues, which tends to hold the active substance of the serum at the point of injection, and so greatly to lessen its absorption rate. If the serum be so gradually introduced that this local reaction is effective, the anti-body on which the hypersensitive reaction depends may be entirely neutralized without killing the animal, or even rendering it appreciably sick, even though the test serum be introduced into the circulation. The following experiment demonstrates these points.

Three hypersensitive guinea-pigs[2] were treated as follows:

Sept. 19: 8 p. m.	0.5 c.c.	Normal horse serum, subcutaneously.	No symptoms.
Sept. 20: 8 a. m.	2.0 c.c.	Normal horse serum, subcutaneously.	No symptoms.
Sept. 20: 12 m.	5.0 c.c.	Normal horse serum, subcutaneously.	No symptoms.
Sept. 20: 8 p. m.	5.0 c.c.	Normal horse serum, intraperitoneally.	No symptoms.

September 21, 10 a. m. One of the animals received 1.5 c.c. normal horse serum by intracardiac injection; no symptoms. The other two animals were now bled, the blood was defibrinated and 15 c.c. was injected into the peritoneal cavity by a fresh normal guinea-pig weighing 240 grm. September 22, 4 p. m. After an interval of 30 hours this last animal received 1.75 c.c. normal horse serum by intracardiac injection; no symptoms.

This experiment is controlled by those on guinea-pigs Nos. 4975, 6010, 5079, 6017, 6058, 6059, of Table III. It shows conclusively that the substance on which the passive transfer of the hypersensitive reaction depends is removed from the circulation of the hypersensitive animal by the gradual introduction of large amounts of horse serum. The experiment could equally well have been considered in the section on the passive transfer of the hypersensitive state as showing that the anti-body there demonstrated was really a vital factor in the acute reaction.

As above stated, I believe that it is a local hypersensitiveness in the subcutaneous and peritoneal tissues which makes possible this

[2] These animals were the same which were used to obtain the serum to sensitize guinea-pigs Nos. 6058 and 6059. See Table III.

rapid neutralization of the anti-body without general symptoms. Under certain conditions which are not as yet fully determined this local hypersensitiveness may be greatly exaggerated. The animal is then fully protected against the acute intoxication, but its life is later sacrificed to the severe reaction in the subcutaneous tissues and abdominal organs. This type of reaction, well known in the rabbit, has not been observed heretofore in the guinea-pig, and I will, therefore, describe the cases which I have encountered in some detail.

In a number of instances I have departed for one or another reason from the usual preliminary or sensitizing treatment. In four cases I repeated, after a number of weeks, the original toxin-antitoxin mixture as nearly as might be. In twelve cases I fed serum by mouth to hypersensitive animals. In eight cases, reported above, repeated small doses of serum were given over a period of several days as a sensitizing treatment. It is not possible to discuss at present all of these experiments from the point of view from which they were undertaken. But the animals had one interesting feature in common when subsequently tested with five or six cubic centimeters of normal serum by the subcutaneous method. Several of the animals of each group died acutely with the usual symptoms. Three of the animals that received serum by mouth gave no reaction whatever. All of the remaining animals showed one or another phase of the following reaction. Acute symptoms following the injection were present or absent, but in all cases in which they were present the animal practically recovered from them in eight hours. At this time also the injected fluid was about absorbed, so that the subcutaneous tissues showed at most but a trace of thickening. From now on the animals became worse again. They became drowsy and had a staring coat with very watery eyes. Locally by the end of twenty-four hours after injection, there was a well-marked œdema, in some instances a very large one. In the milder cases the œdema was reabsorbed and the animal recovered in four or six days. In the severe cases the œdema became very large. Two animals died on the third day with a spreading œdema covering the whole abdominal and thoracic region. In the animals that lived beyond the third day, the œdema gradually became

harder, the overlying skin underwent a dry blackening necrosis, and finally the affected area sloughed out, leaving a bare ulcer varying with the severity of the case from one half to three inches in diameter. These ulcers were very slow to heal, the smaller ones taking a month, the larger ones three months from the time of injection to complete repair. These animals, as they have died, have been studied in gross and microscopically, and others have been chloroformed at one or another stage to complete the series. The results can be briefly stated.

The local lesion is at first an œdema of the subcutaneous tissues and abdominal muscles without cellular invasion. This is associated after several days with pronounced degeneration and necrotic changes in muscular tissues, connective tissues and skin. A dry superficial eschar overlying the œdematous subcutaneous tissues is formed. Through the breaks in the escharotic skin, bacteria gain an entrance. This infection developing on the fifth or sixth day calls forth a leucocytic reaction. The necrotic tissue is thrown off and leaves a bare ulcer, which, as has been said, heals very slowly. Internally one finds remains of the acute changes in the lungs and gastro-intestinal tract which have been so fully described by Gay and Southard. In harmony with the fact that the acute symptoms in these cases are slight, the lung lesions are always very small. The gastric and intestinal lesions are, on the other hand, very extensive. In two instances an irregular hemorrhagic ulceration occupying fully two thirds of the stomach wall was found. The lesion of the stomach has not been found in its stages of repair. In severe cases on the third day it is interesting to note that bacterial invasion has begun, and that a leucocytic reaction is only found at points where the bacteria have penetrated well within the necrotic gastric mucosa.

In the acute cases the lymphatic apparatus never displays definite pathological alteration, but in these cases of late reaction the spleen and mesenteric lymph nodes show interesting changes. The spleen frequently shows considerable areas of hemorrhagic necrosis. In one instance three-fourths of the organ was involved. Microscopically, the affected areas show extensive hemorrhage. The extravascular corpuscles and those in neighboring vessels are clumped,

fused, and often laked. The connective tissues and leucocytes in the affected areas are in various stages of degeneration. Where the necrotic areas border healthy tissue there is well-marked invasion of the hemorrhagic area by phagocytic endothelial cells. The healthy spleen tissue shows endothelial cell proliferation.

The mesenteric lymph nodes on the second and third day after injection show moderate œdema and congestion. The germinal areas show no alteration. The peripheral sinus and those at the hilum are dilated with serous fluid containing desquamated endothelial cells and red blood corpuscles in moderate number. In one instance a few threads of fibrin were found. The endothelial cells free in the sinuses are in stages of degeneration by lysis, and the red blood cells are agglutinated in clumps about them. The endothelial cells lining the sinuses are swollen, raised from the connective tissue cells backing them or in places are wanting altogether. In some instances the red blood corpuscles are clumped about cells that are still attached to the sinus wall. The bone marrow has been studied, but similar changes have not been found. These lesions of the lymphatic apparatus will receive more extended discussion in another paper.

Finally, I have paid particular attention to the condition of the blood in the vessels in all of the animals which I have been able to autopsy at the time of their death. The blood has frequently been drawn, suspended in salt solution and examined microscopically for evidence of agglutinative clumps. The vessels have been traced deep into the lungs in search for thrombi, and the sections have been carefully examined for the same. My conclusions are that fibrinous thrombi are never found and that such clumping of red blood cells as occurs is not enough to account for the lesions with which it is rather irregularly associated. The clumping as well as the hemorrhage are probably secondary to endothelial cell degeneration. The relation of this late reaction to the acute reaction will be further discussed with the theoretical considerations in a subsequent paragraph.

VI. GENERAL DISCUSSION OF PROBLEM.

I wish now to restate briefly the main facts in the case and to offer an explanation for them in so far as it seems possible to do so with our present knowledge.

The normal guinea-pig is not injured by the injection of normal or antitoxic horse serum into its body in any amount that the mechanical conditions at the site of inoculation will permit of. If however, a normal guinea-pig be first treated with a small amount of normal horse serum and after a time be injected with a large quantity, it will become very sick or die. If it does not die it will recover rapidly. Within certain limits of quantity and time the larger the first or sensitizing treatment, the less injurious is the second test, or intoxicating injection. If a third dose, large or small, of horse serum be given twenty-four hours or more later, it is less apt to injure than the second dose, and this applies to all subsequent subcutaneous injections of horse serum. Female guinea-pigs which have been treated with horse serum one or more times whether themselves injured or not, transmit a hypersensitiveness to their offspring. The blood or blood serum of a hypersensitive animal if transferred by injection into a normal guinea-pig in a suitable dose, renders this animal also hypersensitive. This takes place within twenty-four hours if the dose be large enough, but with a dose that is ineffective at this time the sensitization can be accomplished after the same incubation period as that required for the injection of a small dose of horse serum to become effectual. Under various conditions, which all involve repeated treatments with small doses of serum before any large dose is given, the animals may be found hypersensitive in the usual way, or they may develop a more local reaction and die or recover after a longer time. The minor facts in the case will be referred to in the course of the following discussion.

The only attempt to explain comprehensively the mechanism of this particular reaction by one who has experimented with it is that of Gay and Southard. They take the view that the sensitizing substance in the horse serum is distinct from the toxic substance, but a critical study of their experiments does not reveal an adequate basis for this assumption, which in the absence of demonstrated facts in its support is unnecessary. They suggest that chemical analysis may support their view, but at present there seems neither more nor less reason for supposing that the toxic and sensitizing elements in horse serum are distinct substances than for assuming that diph-

theria toxin is really a mixture of a toxic substance responsible for its injurious effects and another substance which stimulates the production of anti-bodies. They have admittedly demonstrated by an experiment that is easily repeated, that the sensitizing principle of the horse serum remains for a very long time in the body of the animal into which it is injected, and that it can be transferred to the body of a second animal mechanically by the transfer of blood serum. That this substance acts as a sensitizer by irritating certain cells and increasing their affinity for the toxic substance (to use their terms) can be granted. But that is less definite than the statement that by injury it stimulates these cells to the production of an excess of receptors which are in part, at least, cast off and appear in the circulating blood as an anti-body. It is more probable that the offspring of hypersensitive female animals are hypersensitive because of the presence of this anti-body than because of the presence of the irritating serum constituent, as these workers suppose. On their supposition, the young should remain sensitive throughout their lifetime and transmit sensitiveness in favorable cases to the grandchildren. If experiment has not rigidly excluded these possibilities, it has at least rendered them very improbable.

There is at present, as I understand it, but one serious biological objection to the view that the toxic substance in small quantity is the sensitizing substance. The ultimate function of the anti-body produced must be assumed to be the elimination of the toxic substance. Why, then, since it is produced in such excess, does it not do this completely, very soon, and allow the hypersensitive condition to disappear? By supposing that the toxic substance was closely united to body cells at some stage of its elimination after uniting with the anti-body, either by extreme solubility in or close chemical affinity for certain of their constituents, one could understand the retention of a residual quantity sufficient to prolong the sensitive condition. But while there is no satisfying evidence against the unity of the substance in the serum, the work of Vaughan and Wheeler on egg-white has rendered it almost certain by analogy that the substance of the serum is chemically decomposed in the body of the animal so as to leave a non-toxic residue difficult of elimination, which keeps up the sensitization.

As I would state it provisionally, there is in horse serum a substance which is actually a mild and potentially a severe toxin for the somatic cells of the guinea-pig. It is actually a mild toxin because receptors to link the toxic substance to the cells are almost wanting. By the introduction of a minimal amount of this substance, the few receptors normally present are exhausted, and subsequently regenerated in great excess. They can now be transferred passively either from mother to offspring or mechanically with the blood serum. When the intoxicating dose of serum is injected a relatively large amount of the toxin can now be suddenly brought in contact with the susceptible cells and the acute reaction is developed. It must be remembered that this is a case in which the substance involved is not toxic for cells essential to the life of the animal when gradually administered, but only becomes so when it is suddenly introduced in excess.

There has been so far no opportunity to study the characteristics of this anti-body. It seems to be necessary to introduce the sensitive guinea-pig serum some time before injecting the horse serum if the reaction is to run at a rate that will injure the animal. This would indicate that the anti-body must be united to some constituent of the body cells before the horse serum is introduced if the animal is to be effectively sensitized. I have been able to show by one experiment that this antibody is not a very labile substance, but the details must be further developed.

It will be most instructive, perhaps, to consider for a time the hypersensitive animal as though it were a normal animal very susceptible to a particular toxin. If this animal is subjected to treatment with a considerable but not fatal dose of toxin it is subsequently found to have lost its susceptibility. The first thought, reasoning from analogy, is that it has acquired an immunity in the special sense of the term. The fact that this immunity is not transferred by the mother to her offspring and that the blood of such an animal has no protective value for an animal not immune, is not an argument against the animal itself being protected by anti-bodies against the toxin or some combination or reaction product of it. The cases in which such anti-bodies can be demonstrated by passive transfer while numerous do not even cover the whole field of immunity against bacteria.

But in this instance the facts in regard to the immunity, if it is well to apply the term to the condition, are adequately explained without assuming that such anti-bodies are formed.

Another well-known although not perfectly understood reaction by which a susceptible animal can be protected against a toxic or infectious agent is by an increase in the affinity of cells not essential to the life of the organism for the toxin. This may be manifested by the leucocytes in conjunction with a special class of anti-bodies, or by the subcutaneous tissues. The mechanism of the reaction of the latter tissues is not thoroughly worked out. In this instance I believe it probable that the late reaction with necrosis before described is an example of this form of protection. By modifying the sensitizing treatment the affinity of the subcutaneous tissues is probably raised to a point where they absorb and hold so much of the toxin that very little can reach the circulation and be carried to cells more vital to the life of the organism. If they are sufficiently hypersensitive they do this to their own destruction. This case is perfectly explained in this way if it be assumed that the effective receptors are retained within the cell, and that those in the circulation represent but an unessential fraction of the total.

Nicolle experimented further with the necrosis which Arthus first produced in the subcutaneous tissues of rabbits by repeated injections of horse serum. He found that following the early treatments there was developed an anti-body which when transferred passively with the blood serum to a fresh animal caused it to react to a first subcutaneous serum injection with necrosis. He has not tried the effect of intravenous injections on either the actively or passively sensitized rabbits, but it seems probable that the reaction is essentially the same as that in the guinea-pig, with the difference that the rabbit's subcutis is easier to sensitize to a point where it will protect the animal's life at its own expense. That the "Phenomenon of Arthus" is in its essential features identical with the "Theobald Smith Phenomenon" is the recently expressed opinion of Otto also. Neither is this the only intoxication in which the rabbit's subcutis exhibits a greater binding power for the poison than does that of the guinea-pig. Morgenroth(12) showed that this was the case for diphtheria toxin as well.

In the case of this particular reaction there is a third possible explanation for the failure of the animals to react for a period. Depending for its effect in large part on a rapid reaction rate, it can be understood that if the serum is introduced very slowly all of these receptors can be satisfied without injury to the animal. The hypersensitive animal then becomes neither immune nor refractory, but is for the time being normal, or rather is a normal animal with a recent large dose of serum. This result for the animal is the reverse of that which is brought about in the course of certain procedures in the immunization against bacteria. For a short time after a given treatment of a fresh or partially immunized animal, the animal may be more than normally susceptible to infection. This is supposed to be due to an exhaustion of the protecting receptors which are not yet sufficient in quantity to protect effectively against the dose administered and still leave a surplus. It is the same in this instance, except that as the anti-body or receptor is a detrimental rather than a protective agent, its removal is salutary. That the mere exhaustion of the abnormal receptors explains the immunity, or, as they term it, the antianaphylaxis, is the view of Besredka and Steinhardt, reasoning from the fact that the animals after a time become sensitive again. For these workers, however, the whole reaction takes place in the nervous system, while my impression is that the nerve cells take little part in the reaction except as they may be subjected to actual injury by the rapid exhibition of the toxic substance in the hypersensitive animal.

It is, perhaps, needless to emphasize the point that the explanation above offered is only intended to cover the facts in this particular reaction in so far as they have been experimentally developed. Other hypersensitive reactions seem to be more complicated, and more complex explanations have been offered for them. It would be unwise to impose such theories on the phenomenon here discussed in advance of the demonstration of facts requiring them.

A few words should be said in closing about the pathological anatomy of the serum intoxication. The work of Gay and Southard was instructive in showing the rapidity with which certain definite and important pathological alterations in tissue cells may be developed. My own studies also have shown conditions which are

interesting from the point of view of the specialist in pathological anatomy. But the significance of these changes in a general consideration of the subject remains doubtful. While Gay and Southard were able to show definite cellular lesions four minutes after injection, and while hemorrhages are not uncommon at that time, yet it is true that in the cases in which death occurs most quickly, lesions are much less frequent and widespread than in those in which it is delayed for several minutes or hours. It would probably be possible to kill animals in this way without demonstrable lesion. In most of the early cases, at least, I think the cause of death is rather to be referred to the disordered function of a single organ, or broken coordination between several organs than to anatomical lesion of any one organ or cell complex. Only pharmacological methods could show whether the action is on the nervous system. the heart, or the lungs directly, or on the heart and lungs through the nervous system. Anatomical studies show that whatever may be the immediate cause of death the toxic substance in its absorption, transmission and elimination injures to a greater or less degree cells of many types.

VII. SUMMARY.

Following the divisions before used, the results presented in the preceding pages may be briefly stated.

I. The particular method of sensitization and the place where the test injection is made have an important bearing on the results obtained by various workers. Comparing the results obtained by the various methods, we may conclude that the incubation period of the hypersensitive reaction is not sharply limited, but that there is a progressive increase in sensitiveness from the sixth day, and presumably before that, extending over a period of several weeks. It seems very probable that the degree of hypersensitiveness attained where the sensitizing dose consists of a mixture of diphtheria toxin and serum is greater than when a single dose of the same small quantity of serum is given alone.

II. Our early experiments, the first in this field, are in thorough agreement with those first reported by Otto, and shortly after him by Rosenau and Anderson.

III. This hypersensitive reaction is transmissible from mother to

offspring. The transmission is probably not equally effective in all cases, and individual young guinea-pigs probably vary greatly in the rate with which they lose their ability to react. As a result, not all of the young of a hypersensitive mother react to a subcutaneous dose of five cubic centimeters of serum given when they are four or five weeks old. The reaction in the young animals differs quite markedly from that in those actively sensitized. These differences are such as to indicate that in the mother there is a considerable localization of the reaction in tissues and organs whose destruction does not cause sudden death. This local reaction is a protective factor and is not transmitted to the same degree as the factors involved in the fatal acute reaction.

IV. The hypersensitive reaction to horse serum depends on the development of a special anti-body during the incubation period, which anti-body may be passively transferred to a fresh animal. If the dose of hypersensitive serum be sufficient, and the intoxicating injection be given directly into the circulation, this passive hypersensitiveness may be enough so that the animal will die when tested. There is also in the serum of hypersensitive guinea-pigs an uneliminated horse serum element or "rest," which is distinct from this antibody, and probably without influence on the course of the acute reaction.

V. The anti-body on which the hypersensitive reaction depends may be entirely neutralized by horse serum without causing symptoms. The gradual introduction of increasing doses over a total period of twenty-four hours suffices for this. The animal is then, properly speaking, neither immune nor refractory, but is essentially in the condition of a normal animal which has recently had a large dose of horse serum. This rapid neutralization is made possible by the great binding power which the subcutaneous and other relatively unimportant tissues have for the toxic element of the serum. The so-called "Phenomenon of Arthus" is probably the same reaction for the rabbit that we have here dealt with in the guinea-pig. The fact that the manifestation is more prominently a local one depends on racial differences. I have encountered cases in the guinea-pig in which the conditions in the rabbit are closely simulated.

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