FURTHER OBSERVATIONS ON ANAPHYLAXIS TO HORSE SERUM.*

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In a previous paper (1) on the reaction of the guinea-pig to single and repeated doses of horse serum, I took the view that the violent reaction obtained at a second treatment which follows the first after a considerable incubation period, was due to the participation in the reaction of a specific reaction product or anti-body developed by the animal during the interval. In support of this position, it was shown that if a considerable quantity of the defibrinated blood of a guinea-pig which had been treated some weeks previously with a mixture of diphtheria toxin and antitoxic horse serum is injected into the peritoneal cavity of a young, normal, untreated guinea-pig the latter becomes hypersensitive to horse serum within twenty-four hours. These experiments were in agreement with those reported by Otto (2) when my paper was being prepared for the press. Moreover, there seemed to be a sharp distinction between this substance which may render the fresh animal hypersensitive within a few hours, and the substance demonstrated by Gay and Southard (3), which gives to a small quantity of the blood serum of an actively sensitized guinea-pig the property of transferring the hypersensitive condition of a "fresh" animal if an incubation period of two weeks be allowed to elapse. In further support of the view that a specific anti-body is developed was the fact first brought out by Rosenau and Anderson (4), that the offspring of a treated female animal are hypersensitive. It was reasoned that in its fundamentals the transmitted hypersensitivity resembled the more carefully studied cases of transmitted antitoxic immunity. The former seemed further to have in com-

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mon with the latter, a transient character, and a limitation of its
effect to the immediate offspring of the treated mother. Recently
I have had the opportunity to study more carefully the hypersensi-
tiveness in these young animals born of treated mothers, and it is
the results of this study which I wish chiefly to report at present.
I have also been able to make some limited observations which have
a more practical bearing on the question of serum therapy. The
brief report of these will be found in the closing paragraphs of
this paper.

The blood of the young guinea-pig hypersensitive by breeding
was first subjected to the same tests which had previously been
applied to that of animals actively sensitized. Animals of 225 to
250 grams weight were chosen. They were bled, the blood defi-
brinated and mixed. In the decisive experiments enough animals
were used at one time to give a total quantity of twenty cubic centi-
meters of defibrinated blood. This was at once injected into the
peritoneal cavity of fresh guinea-pigs of normal ancestry, weighing
from 230 to 250 grams. Two animals were used for each test:
one was injected with fifteen cubic centimeters of the blood, the
other with from one to five cubic centimeters. The animal re-
ceiving the larger quantity was treated with horse serum the next
day; that receiving the smaller amount after about twenty days.
The test injection consisted in each case of two cubic centimeters
of horse serum given directly into the circulation by the intracardiac
method. These experiments and the results obtained are described
in the following protocols:

Experiment 1.—Guinea-pigs No. 7066, 7067, 7068 were born of treated mother
No. 3931. When 3 weeks old the animals were bled. Pooled defibrinated blood,
12 c.c., were injected into peritoneal cavity of guinea-pig No. 7048 (normal, wt.
240 grm.). After 17 hours injected No. 7048 with 2 c.c. serum of Horse 106 by
intracardiac method. Dead in 2 minutes. Symptoms: typical. Autopsy: lung
and heart hemorrhages.

Experiment 2.—Guinea-pigs No. 7074, 7075, 7076, born of treated mother No.
4875. When 34 days old, bled (25-XI-07). Injection of 5 c.c. mixed defibrin-
ated blood into peritoneal cavity of normal guinea-pig No. 7071. After 30 hours
treated No. 7071 with 2 c.c. serum of Horse 106 (intracardiac). Slight but
definite symptoms characteristic of serum intoxication. 25-XI-07.—Injection
of 1 c.c. of above defibrinated blood into peritoneal cavity of normal guinea-
pig No. 7087.

14-XII-07. Treated No. 7087 with 2 c.c. serum of Horse 106 (intracardiac).
No symptoms.
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Experiment 3.—29-XI-07. Guinea-pigs No. 8035, 8036, 5 weeks old, born of treated mother No. 4877, and guinea-pig No. 7098, 6 weeks old, born of treated mother No. 4876, were bled. Blood was defibrinated and mixed. Injected 15 c.c. of mixed defibrinated blood into peritoneal cavity of normal guinea-pig No. 8003 and 2 c.c. into peritoneal cavity of normal guinea-pig No. 8031.

30-XI-07. Treated guinea-pig No. 8003 received 2 c.c. serum of Horse 109 (intracardiac). The animal was very sick from five minutes to one hour. Recovered. 2-I-08.—Chloroformed. Autopsy shows hemorrhage in lungs and erosion of stomach.

3-I-08. Treated guinea-pig No. 8031 received 2 c.c. serum of Horse 98 (intracardiac). No symptoms whatever.

Experiment 4.—Guinea-pigs No. 8093, 8104, born of treated mother No. 4868, 2½ months old. Guinea-pigs No. 8096, 8105, born of treated mother No. 4865, 2½ months old. Guinea-pig No. 8061, born of treated mother No. 4789, 3 months old.

8-III-08. Above animals were bled; blood was defibrinated and mixed. Injected at once into two young normal guinea-pigs as follows: No. 8150 received 15 c.c. intra-peritoneally. No. 8151 received 15 c.c. intra-peritoneally and 5 c.c. sub-cutaneously.

10-III-08. Treated No. 8150 received 2 c.c. serum of Horse 113 (intracardiac). Slight, but definite symptoms.

27-III-08. Treated No. 8151 received 2 c.c. serum of Horse 113 (intracardiac). No symptoms whatever.

It is shown that the blood of young animals hypersensitive by breeding has the property of rendering a normal animal hypersensitive if the quantity of blood transferred is large, and if the test injection is made within a short time. Thirty-six hours was the longest interval allowed. The degree of hypersensitiveness developed seemed somewhat less for equivalent amounts of blood transferred than that in the cases reported in my earlier paper, where the animals sensitized directly by treatment were used as the source of the blood. In Experiment 1, however, the test injection killed in a few minutes, showing that the immediate sensitiveness developed may under favorable conditions be roughly equal in the two cases. It is of interest that this most intense reaction was developed when using blood from very young animals.

The tests after an incubation period have been entirely and uniformly negative. In Experiment 4 the test of this point was made very severe. While the animal treated after thirty-six hours gave a definite reaction, the one treated after the incubation period gave no reaction whatever, although the amount of defibrinated blood used to sensitize was larger in the latter instance. These experi-
ments are in direct contrast to those performed by Gay and Southard (3), and later by myself, in which it was determined that while, as I have said, with a large amount of the blood of animals actively sensitized it was possible to transfer the hypersensitive condition within a few hours, it was much easier to accomplish this by using a small amount, one tenth to one and a half cubic centimeters, and allowing a period of two weeks to elapse before the test injection was made. Somewhat differently stated, the facts seem to be that in the blood of young guinea-pigs hypersensitive by reason of their breeding, there is a substance which renders the “fresh” animal to which it is transferred hypersensitive within a few hours. In the same blood the anaphylactin of Gay and Southard, which has the property of rendering the animal into which it is injected hypersusceptible after an incubation period, cannot be demonstrated.

Very important in an estimate of the nature of the transmitted hypersensitiveness is the question: Is it a permanent or transitory condition? In my earlier paper I gave report of results which seemed to show that it was transient. Tested by the subcutaneous method when several weeks old, only about fifty per cent. of the animals gave a reaction. It seems best to assume that all of the young bred of a hypersensitive mother acquire a measure of her abnormal condition, and that the observed differences in reaction are due to quantitative differences in the degree of transmission influenced by the rate of loss of the sensitivity. The material has not so far been available for a complete determination of the amount of variation which there may be in the initial intensity of the transmitted reaction. On the probable assumption that any such variations are dominated, in the experiments so far done, by the rate of loss of the abnormal condition, I have been able to make a few observations which more definitely fix the duration of the latter. Of two animals of the same litter, one tested with two cubic centimeters of horse serum (intracardiac), when two and a half months old, died in two minutes; the other subjected to the same test when four months old showed no symptoms. Another animal bred of another mother tested in the same way when four months old gave no reaction. This evidence, though small in amount, is consistent
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with the view that the acquired hypersensitiveness is lost within the first few months of the animal's life. It is of especial value in that the test is as severe as it can well be made.

If the conditions in the immediate offspring of treated mothers were identical, except in degree with those in the mother, it might be expected that in favorable instances the grandchildren would be found abnormal. In the earlier records of the laboratory there were found notes on several cases in which horse serum had been given subcutaneously to the grandchildren of treated mothers, the intermediate offspring having been untreated. These animals had never given any reaction, but it was felt that the tests were not perfectly satisfactory, in that the earliest litters of the mothers in question had not been used. I repeated the experiments, using the first offspring of the untreated mother at an early age, but got no reaction. On this basis, I felt justified in using the argument that transmitted hypersensitiveness did not occur beyond the first generation. Since becoming familiar with the great delicacy of the reaction when the test injection is made directly into the circulation, it has seemed worth while to reexamine this question. Eight guinea-pigs born of five different untreated mothers, descendants of five treated grandmothers, were tested. Together with these were used five control animals from a source which made it certain that none of the ancestors for several generations could have been treated in any way with horse serum. The animals were used at weights varying between 175 grams and 250 grams. The least severe test applied was the injection of two cubic centimeters of horse serum by the intracardiac method. In several instances, additional serum was administered within a few minutes into the peritoneal cavity, or subcutaneous tissue, or both. As much as ten cubic centimeters were given in several instances over and above the intracirculatory dose. The result was conclusive in showing that there is no hypersensitiveness transmitted beyond the immediate offspring.

These experiments have also been instructive in showing the limit to which horse serum can be given to normal guinea-pigs without producing symptoms of serum poisoning. In no instance where the injection was limited to two cubic centimeters into the
circulation were there symptoms produced.* When, however, this was followed by five centimeters given into the peritoneal cavity in each case there resulted a very slight reaction more or less characteristic of the earliest stage of the hypersensitive reaction. For half an hour after this maximum treatment the animals have shown short periods of uneasiness, alternating with periods of unusual quietness. They shiver somewhat, show twitching and slight involuntary convulsive movements of the limbs or diaphragm. Occasionally, they sneeze or rub the ears and nose. These symptoms pass away in a short time. In several instances where they have been most definite, the animals have been killed after a day or two. Examination, except in one case, has revealed no lesion whatever. In this case there were irregular subserous hemorrhages in the peritoneal cavity, slight swelling of one or two mesenteric lymph nodes, with some hemorrhage into their sinuses, and a few hemorrhages in the lungs. These lesions are in accord with those found in the hypersensitive animal during the phase of intoxication. Of course, not too much stress should be laid on this single case. The experience with these young guinea-pigs treated with large doses of serum is, it would seem, most consistent with the view that horse serum is not a perfectly indifferent substance for this animal, but is in reality a mild poison. The intoxication obtained in the hypersensitive animal in the light of these results is not an adventitious reaction, but is an exaggeration of a reaction which occurs when the normal animal is treated with horse serum.

It may be profitable to discuss briefly the possible manner of the hypersensitive reaction of the guinea-pig to horse serum from the point of view of the experiments with the animals of abnormal breeding. As before stated, I have held to the view that the sensitizing treatment causes a reaction on the part of the animal, in the course of which an anti-body is developed in excess. This

* In the course of these tests it was found that when so large an amount of serum as this is injected into the circulation, if chloroform has been used as a preservative it must be exhausted before use. Otherwise, there is instantly produced a profound general anesthesia, which may result in death, although it usually passes off in a few minutes. A serum which has no taste of chloroform can be used with safety.
anti-body meeting with the second injection very greatly accelerates a reaction which at one or another stage is injurious to the cells of the animal. In holding this conception of the mechanism of the reaction of anaphylaxis, I am neither alone nor original. The same idea was first expressed by v. Perquet and Shick (5), and later by Currie (6), to explain the phenomena of the serum disease in human beings. They derived the idea from theoretical considerations, obviously under the influence of current theories of immunity reactions. Nicolle (7) holds this view with regard to the reaction of the rabbit to horse serum. Otto (2) expressed the same opinion as to the process in the guinea-pig, and Richer (8) has recently indicated a similar mechanism for the reaction of the dog to mytilo-congestin. The three latter observers brought to bear experimental evidence for their opinion, in that they showed that in each instance it was possible to render a fresh animal hypersensitive within twenty-four hours, by treating it with the blood or blood serum of a hypersensitive animal.

Nor can the following partial conclusion quoted from the paper of Gay and Southard be considered as fundamentally inconsistent with this idea: “The reaction of intoxication would seem to be a cellular one, dependent upon a heightened power of assimilation on the part of cells which have been subjected to the anaphylactic substance over a definite period of incubation.” But further than this, they hold that the sensitizing and toxic actions of the horse serum are dependent on two distinct substances. On this assumption, the incubation period might be occupied with the elimination of the assimilable toxic portion, and at its completion the sensitizing, nontoxic portion, the anaphylactin in their terms, which is eliminated but slowly, would be isolated or left uncovered. The serum of the hypersensitive animal would then contain purified anaphylactin. Now, it could perhaps be argued that the purified sensitizing substance could act efficiently in developing a given degree of hypersusceptibility in a shorter or longer time, depending directly on the quantity present. In sensitizing an animal with the blood of a hypersensitive animal according to this view, when one-tenth of a cubic centimeter sensitizes in two weeks, and fifteen cubic centimeters in twenty-four hours, the inference to be drawn is not that
there are two distinct active substances in the blood used, as I have supposed, but one substance, the anaphylactin, which acts quickly in large amount, but continuously and, in the end, effectively in the smaller amount. It is not necessary at present to dwell on the indications found within the series of experiments with the blood of serum-treated animals that tended to distinguish two active substances. However plausible such an interpretation as outlined may appear to be for those animals actively sensitized, it is not at all in harmony with the experiments on the blood of the animals hypersensitive by breeding. If the sensitization of normal animals with this blood were due to the direct action of anaphylactin, the animals tested after two weeks should have been found fully as sensitive, and perhaps more so, than the animals tested at twenty-four hours, or thereabout. Guinea-pig No. 8151 of Experiment 4 should have been more sensitive than its fellow, No. 8150. The reverse was true.

On the strength of the presumably transitory character of the hypersensitive condition in the cases of acquired hypersusceptibility, the limitation of the condition to the immediate offspring of the treated mother, and especially because of the impossibility of demonstrating the sensitizing horse serum element in the blood of these animals while it is possible to transfer the condition passively to a "fresh" animal by the use of their blood, the conclusion that the acute reaction to the intoxicating injection is due to the participation in the reaction of a newly formed substance or antibody would seem justifiable. To those who have had the patience to follow the experiments and argument thus far, it will be sufficiently obvious that to have reached such a conclusion is but to have made one step toward the understanding of a most complex problem.

In closing, I wish to discuss very briefly some of the more practical questions that have arisen in connection with the study of the serum reaction in the guinea-pig, other lower animals, and man. Influenced by his work on the reaction in the guinea-pig, Besredka (9) has proposed that all antitoxic sera should be tested on the hypersensitive animal to be sure that they have no unusual toxicity in this reaction before they are marketed. In the light of a proposition of such practical importance, the following observation is of
interest. During the past winter, a sample of antitoxin was referred back to the laboratory for examination. A prophylactic injection of about three cubic centimeters of the serum had caused very severe edema of the face, pharynx, and larynx, in a healthy adult male. The effect came on within half an hour after the injection, lasted for some time, and at its height, the symptoms were very alarming. The fact that this patient's wife, who received an injection of about nine cubic centimeters of the same lot of serum, experienced no reaction, would almost be a sufficient indication that this untoward result was not due to any unusual quality in the serum. Tested on the hypersensitive guinea-pig, this serum showed a toxicity not materially different from the various normal sera which I have used in the course of this work. One two-hundredth of a cubic centimeter caused severe symptoms with recovery, when injected directly into the circulation. One one-hundredth cubic centimeter is probably a certainly fatal dose of normal serum under the conditions of this test, so that this lot of serum could not have had a toxicity of twice the normal, supposing it to have been at all above the average.

During the past winter some experiments have been undertaken on the reaction of the rabbit to horse serum. It is probable from the work of Arthus, Nicolle, and others, that this animal reacts fundamentally in the same way as does the guinea-pig. Certain distinctions become apparent at once, however. It is much more difficult to render the rabbit hypersensitive to such a degree that the intravenous injection of serum will kill the animal. The single treatment with a mixture of diphtheria toxin and antitoxin, which is so efficient in rendering the guinea-pig hypersensitive, is without any demonstrable effect on the rabbit. While the underlying principles of the reaction are probably the same in the two animals, the factors in each case are so different in their relative values that a treatment which is certainly fatal for the guinea-pig has no appreciable effect on the health of the rabbit. In attempting to determine whether a given serum treatment is or is not dangerous, evidently each species of animal must be separately considered. It is almost needless to point out that the data accumulated since 1893 on the accidents incident to the therapeutic use of horse serum, its
uncomfortable sequels, and its great benefits are of much more value, as a guide for future practice, than conclusions drawn from complex experiments on the laboratory animals. It would be a most unfortunate presentation of laboratory results on anaphylaxis which should lend itself to even a temporary or slight reaction against a therapy which has so thoroughly justified itself in the case of some diseases, and which offers such possibilities for the future in the case of others.

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