SUBCUTANEOUS REACTION OF RABBITS TO HORSE SERUM.¹

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The object of the following study is to determine the effect of subcutaneous injections of horse serum upon rabbits in order to ascertain whether this procedure can be used as a test of altered reactivity (allergy) or of acquired anaphylaxis. Heretofore most of the experimentation upon anaphylaxis has usually resulted in the death of the animal. Rabbits are known to be less susceptible than guinea pigs to foreign sera, and it was considered possible that local, but quite as definite, phenomena might be obtained in rabbits after repeated inoculations of small quantities of horse serum.

Previous Work.—But few investigators have been interested in this particular inquiry. Arthus (1) in 1903, following the ideas of Richet, first noted the effect of injections of horse serum upon rabbits. He found that the first injections produced no clinical consequences, but that after later injections intense local swellings were produced, and at times definite necrosis. In a few instances sudden death of the animal followed. This result was more frequent after intraperitoneal and intravenous injections. Later von Pirquet and Schick (2) injected rabbits subcutaneously in the ear and produced edema after the second injection, using rather large amounts of serum, one to three cubic centimeters. Whereas Arthus had considered that this production of local reaction de-

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Pended on the number of previous injections, von Pirquet and Schick showed that the interval between the first and second injections was the more important factor, and that the local swelling followed even after the second injection, provided that about ten days elapsed between the first and second inoculations. These writers found also that horse serum was specific in its action, that a previous injection of horse serum did not affect subsequent injections of other sera, and that no reaction followed the inoculation of horse serum in animals that had been previously injected with other sera. They found also that animals in which horse serum had been previously injected reacted locally after the injection of rabbit serum from an allergic rabbit.

Later Nicolle (3) produced a local edema in rabbits which had previously received a large amount of serum from an allergic rabbit, by injecting it subsequently with horse serum. Since 1907 nearly all authors have been interested chiefly in the general phenomena of anaphylaxis, and have paid but little attention to the local reaction, and, moreover, most of the work has been done with guinea pigs and not with rabbits. In regard to local reaction on guinea pigs, the observation of Paul Lewis must be mentioned. He showed that in pigs which survived the allergic shock, large areas of edema were often produced.

Description of Test.—In our first experiments twenty rabbits, ten males and ten females, of nearly equal weight, were selected (Table I). Two (Nos. 48 and 37) were reserved as controls. The remaining eighteen rabbits were inoculated in an ear vein with varying amounts of sera as follows (Table I): Nos. 55 and 53, 0.1 cubic centimeter of horse serum;2 Nos. 51 and 27, 0.1 cubic centimeter of serum and 9.9 cubic centimeters of normal salt solution; Nos. 26 and 78, 0.5 cubic centimeter of serum; Nos. 42 and 71, 0.5 cubic centimeter of serum and 9.5 cubic centimeters of salt solution; Nos. 56 and 30, 1 cubic centimeter of serum; Nos. 4 and 49, 1 cubic centimeter of serum and 9 cubic centimeters of salt solution; Nos. 14 and 35, 5 cubic centimeters of serum; Nos. 34 and 40,

1The horse serum used in the first injections was obtained by us from a superannuated but healthy horse. The serum was preserved with 0.5 per cent. carbolic acid and kept on ice. The later experiments were carried out by the serum kindly sent us from the Rockefeller Institute.
5 cubic centimeters of serum and 5 cubic centimeters of salt solution; Nos. 50 and 10 with 10 cubic centimeters of serum each.

On the sixth day after the intravenous injections, one rabbit from each group, ten in all, were inoculated subcutaneously in the ear with 0.1 cubic centimeter of a 10 per cent. solution of horse serum. This produced no local reaction in any of the rabbits. The same ten rabbits were injected with the same amounts of serum every other day for three days. After the third injection, most of the rabbits, as indicated in the table, showed a positive reaction, definite swelling, and redness of the ear at the point of inoculation. The difficulty of inoculating the serum into the scanty subcutaneous tissue of the ear without injury was recognized. It was thought that some of the redness and swelling might be due to mechanical injury, and after the twelfth day of the injection, the tests were made into the carefully shaved skin of the abdomen. As is indicated in the table, local reactions began to appear ten days after the original intravenous injection of serum, and continued with some exceptions noted in the table as long as the animals were tested; that is, the hyper-susceptibility to horse serum developed in the rabbits remained for at least two months and a half. The first test of the second rabbits of each of the ten groups was made on the fourteenth day after the original intravenous injection and consisted in the injection of 0.1 cubic centimeter of a 10 per cent. solution of horse serum into the skin of the abdomen. These second numbers of each group seemed to average about the same number of definite reactions as those which had been injected several times previously. It was, therefore, concluded that the hyper-susceptibility to the serum was the result of the first intravenous injection, and that this hyper-susceptibility developed in most of the rabbits in from ten to fourteen days.

Several interesting individual variations are to be noted. Rabbit No. 48, a control animal, which had received no serum originally, exhibited a suggestive reaction on the third and fifth day after the first subcutaneous injection, and remained definitely positive from the ninth day after this injection. Apparently this animal was unusually susceptible to horse serum and developed its anaphylaxis because of the subcutaneous injection of 0.1 cubic centimeter of a

10 per cent. solution of horse serum. Another rabbit, No. 71, showed complete insusceptibility to horse serum during two months and a half of observation. After the sixteenth day, 0.1 cubic centimeter of undiluted horse serum was used in making the intradermal tests. In general, the reactions seemed a little more constant in those animals that had originally received the larger amounts of serum, though there were exceptions to this observation. On the fifty-sixth day, 0.05 cubic centimeter of horse serum was used intradermally in making the tests. The animals seemed just as susceptible to this smaller amount of serum.

In making the tests it was always endeavored to insert the point of a small hypodermic needle into and not under the skin, and to produce by the injection a small translucent bleb.

To verify these results, a second series of animals, four in number, were each injected intravenously (ear vein) with 5 cubic centimeters of horse serum (Table II). On the sixth day they were tested by an intradermal abdominal injection of 0.1 cubic centimeter of horse serum. None of them reacted. This test was repeated on the fourteenth and sixteenth days with negative results. After a similar test on the nineteenth day, three of the animals reacted positively, but the fourth was not definitely positive until the twenty-seventh day (Table II), and was negative to three subsequent tests but remained constantly positive after the thirty-eighth day. Rabbit No. 48 of the first series, already shown to be susceptible to horse serum, was again injected with this second series as comparison. A sixth rabbit, No. 2, which had received 1 cubic centimeter of horse serum subcutaneously, reacted positively on the thirteenth day to the intradermal injections of 0.1 cubic centimeter of horse serum.

In order to determine whether even smaller intradermal injections of horse serum could not be used to determine the presence of anaphylaxis in rabbits, a third series of rabbits, twelve in number, was carried on (Table III). Of these, two were used as controls, and the others, in groups of two, were injected intravenously with horse serum as follows: first group, controls; second group, 0.1 cubic centimeter; third group, 0.5 cubic centimeter; fourth group, 1 cubic centimeter; fifth group, 2.5 cubic centimeters; sixth group, 5 cubic centimeters. On the sixth day one animal of
Subcutaneous Reaction of Rabbits to Horse Serum.

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1Control in first serum reacting well.
2Jan. 7, 1 c.c. horse serum subcutaneously.
T = .01 c.c. horse serum in ear.
T₂ = .01 c.c. horse serum in abdomen.
T₃ = .1 c.c. horse serum in abdomen.
T₄ = .5 c.c. horse serum in abdomen.
P = Precipitine test.
s = Skin reaction: one drop on shaved surface—scarified.
| Rabbit No. | Jan. 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Feb. 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|           |        |   |   |   |   |   |   |   |   |   |     |   |      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
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| 2         | 76     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 3         | 54     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 4         | 52     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 5         | 22     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 6         | 79     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 7         | 82     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 8         | 17     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 9         | 74     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 10        | 19     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 11        | 68     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |
| 12        | 75     | T | T | T | T | T | T | T | T | T | T  | T | T   | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T | T |

Rabbits 3 through 12 were injected intravenously on Jan. 19 with normal horse serum as indicated above. Rabbit 13 was injected intravenously with 1.0 c.c. normal rabbit serum on Jan. 31.

T = 1/100 c.c. normal horse serum injected subcutaneously.
T1 = 1/100 c.c. normal rabbit serum injected subcutaneously.
P = precipitine test: rabbit serum against normal horse serum.
P1 = precipitine test: rabbit serum against normal rabbit serum.

Each of these groups was injected with 0.01 cubic centimeter of horse serum intradermally. The rabbits in Groups 5 and 6, which had received the largest amounts of serum intravenously, showed definite local reaction on the seventh day. The susceptibility in these animals remained fairly constant after all subsequent injections.
On the twelfth day, all the animals were injected with 0.01 of 1 cubic centimeter of horse serum intradermally. Those receiving the test for the first time reacted nearly as readily as the other member of their group which had been tested several times before. This series, therefore, confirms the observation of Series 1 and 2 and adds the additional fact that a very small amount of horse serum is sufficient to produce a local skin reaction in a susceptible animal. A thirteenth rabbit, No. 59, was injected with 1 cubic centimeter of rabbit serum and tested on the seventh and fourteenth days with an intradermal injection of 0.01 cubic centimeter of rabbit serum with negative results.

Phenomena.—The local changes at or near the point of inoculation described as a reaction varied considerably at different times and in different animals: A typical reaction to horse serum in a sensitive rabbit may be described as follows: the bleb produced by the intradermal injection of the serum disappears in a short time, and no change is noticed at the seat of injection for ten to twelve hours. After this time the skin may become a little reddened and infiltrated, so that when it is taken up between the fingers in a fold, it seems definitely thickened as compared with a fold of skin elsewhere. The swelling and redness continue to increase and reach their height in about twenty-four hours, when there may be a definitely raised, brawny mass, perhaps one or two centimeters in diameter. The skin of the reacting area is red and increased in temperature, and the mass is definitely tender. Reactions which were less severe exhibited only a more or less diffuse infiltration of the skin at the point of inoculation with little or no redness or heat in the shaved area compared to that of the rest of the skin. In a number of rabbits the reaction was much more marked. In these the infiltration began sooner, the part affected extending five to ten centimeters, became very tense, swollen and red, and on several occasions partly necrotic. Reactions of this fulminating character often lasted for two or three weeks, whereas those which produced less severe changes diminished after twenty-four hours. On several occasions the reactions took the form of an unusually firm, sharply circumscribed round mass with less increase in local temperature and redness than was usually the case. It was thought
at one time that these firm nodules were the result of a skin test following closely the injection of a large amount (ten cubic centimeters) of horse serum intraperitoneally. We were unable, however, to reproduce later this form of reaction, and do not know to what factors it was due. As far as we could determine there was little constitutional disturbance during the time of reaction. The rabbits seemed lively and ate well. On several occasions following the injection of large amounts of serum intraperitoneally the rabbit appeared shocked. One had a short convulsive seizure followed by the passage of bloody urine. All these quickly and completely recovered. In the whole number of more than forty rabbits injected, no fatalities occurred.

Microscopic Appearances.—A typical swollen reacting area was excised for microscopic examination and may be described as follows:

The epidermis is everywhere intact and presents no abnormalities. Immediately beneath the epidermis the tissues were slightly infiltrated, but there is little if any accumulation of small round cells. Throughout the rest of the section of the skin and extending to the muscle layer, the tissues are definitely edematous, the fibrous strands being somewhat separated and compressed. In this area there is a marked increase in the number of leucocytes, many of them scattered, but the greater number gathered in small interstitial groups about the blood vessels. These cells about the vessels consisted mainly of polymorphonuclear leucocytes. The vessels are everywhere moderately injected and filled with red blood corpuscles. There is little if any evidence of marked hemorrhage, although some extravasation of red cells is noted about the smaller vessels. The muscle layer beneath the skin is unchanged. The whole appearance of this section suggested a moderately acute infiltration and inflammation.

Precipitine Test.—The serum of the rabbits was tested at intervals with horse serum for the presence of precipitines. The microscopic method was used for the most part, the results being controlled several times by microscopic test. The method used had been employed formerly by Dr. von Pirquet in agglutination of streptococci (4), and is briefly as follows:
Subcutaneous Reaction of Rabbits to Horse Serum.

On a cover slip three minute drops of horse serum, one undiluted, one diluted 1 to 10, and the third, 1 to 100, are placed in a row with a sterile platinum loop. Immediately opposite each drop of the horse serum is placed a drop of the same size of rabbit serum and the opposing sera are mixed gently together. The cover slip is now inverted over a hollow slide and placed in the incubator for three hours or for a longer time in room temperature, when under the microscope the precipitate is examined. When the precipitate forms, it is observed as a more or less extensive granular cloud. The number of times the precipitine test was made is indicated in Tables I, II, III. It will be noted that the precipitines were present in the blood of nearly all animals exhibiting local reaction, and were occasionally present where local reaction was not obtained. The dilution of the horse serum made but little difference in the intensity of the reaction.

Anti-anaphylaxis.—Having shown that the anaphylaxis, or the heightened susceptibility to a foreign serum could be demonstrated by a skin reaction, it was interesting to determine whether a lowered state of susceptibility, or the so-called anti-anaphylaxis, could be proved in the same way. Nicolle (3) first showed that experimental animals injected during incubation time not only were not killed by a second injection, but that their hyper-susceptibility was definitely lessened during this period. Besredka (5) and Rosenau and Anderson (6) working with guinea pigs found that when these animals survived a test injection they did not show general phenomena of inoculation again in twenty-four hours. In tuberculosis, also, it has been proven that after large doses of tuberculin subcutaneously, the reaction of the skin to vaccination with tuberculin (the von Pirquet test) disappears. A kind of tuberculin immunity develops. In our experiments we were unable to decrease the susceptibility of our rabbits to the intradermal injections of horse serum. Repeatedly sensitized animals which were exhibiting definite local reactions to every injection of horse serum were given large doses, five or ten cubic centimeters, intravenously or intraperitoneally, but an intradermal injection made within twenty-four hours always produced a definite local reaction. In several instances after a procedure of this kind, the localized swelling was
usually hard and circumscribed, but this form of reaction could not be regularly obtained.

Transmale of Susceptibility to Offspring.—A number of experiments were carried on to determine whether it was possible to transmit this susceptibility, which is developed in rabbits, and indicated by the local skin reaction, to their young. Anderson (7) had shown that the toxic hyper-susceptibility to horse serum, as indicated by the general phenomena of anaphylaxis and ending in death, can be transmitted in guinea pigs from a sensitized mother to her young. In several experiments the injection of horse serum into the offspring of such sensitized mothers was fatal in less than an hour, while much larger quantities of horse serum produced no effect upon young guinea pigs from unsensitized mothers. Two litters of rabbits, one six in number and three weeks old, from the offspring of a highly sensitized mother which had markedly reacted just before the birth of her young, were inoculated with 0.02 cubic centimeters of horse serum every other day intradermally into the abdomen, and on the fifth day following developed a definite but slight thickening and infiltration of the skin a few hours after the injection. At the same time a litter of five rabbits from an unsensitized mother were similarly injected and reacted positively in just the same period of time, but more vigorously than did those of the first litter. The reactions following the last injections in the second series were all accompanied by marked induration and considerable hemorrhage. Our results suggest, therefore, that the susceptibility of the sensitized rabbit, as indicated by the skin reaction, is not freely transmitted to its offspring. A comparison of the effects upon the young of these two litters would suggest, however, the possibility that the offspring of the sensitized rabbit have acquired some allergy to horse serum, that is, the property of an accelerated formation of ergines (8).

Leucocytic Count.—It was thought that it would be of interest to determine whether the leucocytes underwent any change either in their total number or differential count following the sensitizing injection of serum or following the skin reaction induced by the subsequent intradermal injection of minimal doses of serum. To determine these points four fresh rabbits of approximately the same
age and weight were selected, and before any injections were given, leucocyte counts were made as follows: at nine a. m., one p. m., and five p. m., on the first day, and at nine a. m. on each of the three successive days. After a rest of about a week, a similar series of counts were made. Another week was allowed to elapse and then sensitizing injections of serum were made intravenously. The first rabbit was given 0.1 cubic centimeter, the second, 2.5 cubic centimeters, and the third, 5 cubic centimeters, while the fourth was not injected and served as a control. The leucocytes were then counted exactly as on the two preceding occasions. After a lapse of fourteen days all four rabbits received intradermal injections of 0.01 cubic centimeters horse serum and a fourth series of counts was carried out. The total number of leucocytes and differential counts were made on each occasion, in all twenty-four total leucocytes and twenty-four differential counts on each rabbit.

The leucocytes varied through wide limits both in their total number and in the differential counts, the variations being just as great in the two series of counts made before any serum had been injected as in those series following the sensitizing injection and the subsequent test injection, and no relation could be made out between the injections and the leucocyte curves.

The detailed results of these counts are too long to include here. They will be reported in a separate paper.

CONCLUSIONS.

1. Anaphylaxis or allergy of rabbits against horse serum can be proved by subcutaneous test.

2. The test is best made in the following way. The skin of the animal, preferably of the abdomen or flank, is shaved. (This should be done a few hours before the injection.) The injection is made by means of a small hypodermic syringe and intradermally. An effort was made not to inject the serum under the skin. Those injections were considered most favorable by which the serum remained as a small bleb in the skin proper. Undiluted horse serum was used for most of the experiments. The amount injected varied from 0.01 cubic centimeter to 1 cubic centimeter. The reaction seemed as definite after 0.01 cubic centimeter as after a larger quantity.
3. The specific reaction appears in from twelve to twenty-four hours after the test is made and reaches its maximum in from twenty-four to thirty-six hours. It consists of a local swelling extending from 0.5 to 2 centimeters from the point of inoculation. The skin involved in the raised area is usually red and hotter than the surrounding skin. Macroscopically and microscopically the reacting area has the appearance of a local acute inflammation.

4. The altered reactivity (allergy) or hyper-susceptibility (anaphylaxis) sets in usually in from ten to fifteen days after the first injection of horse serum, and lasts at least three months. Individual rabbits show marked variation from the average time of the development of anaphylaxis.

5. The appearance of precipitines against horse serum in the blood of rabbits appears nearly synchronously with the allergic condition.

6. After large injections of serum the allergic rabbits still react subcutaneously. A suppression of allergy which would correspond to the so-called anti-anaphylaxis could not be proved.

7. Also in regard to the offspring of injected rabbits the subcutaneous test was not positive. The young of these rabbits did not develop a more active allergy than the young of normal rabbits.

8. Neither the injection of considerable quantities of horse serum nor the development of a marked local reaction in the skin after intradermal inoculations of horse serum in a sensitized rabbit is accompanied or followed by greater variations in the number or types of leucocytes in the circulating blood than is found in control animals.

It is a pleasure to acknowledge our indebtedness for much help to Professor von Pirquet, at whose suggestion the work was undertaken.

BIBLIOGRAPHY.
Subcutaneous Reaction of Rabbits to Horse Serum.


