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The hypersensitiveness of the guinea pig to horse serum.

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The facts in this communication are related to the "Theobald Smith Phenomenon" first described by Otto. Additional observations in regard to the reaction and its pathology have been reported by Rosenau and Anderson, Besredka and Steinhardt, and Gay and Southard.

If a normal guinea pig be treated with a dose of 1/100 c.c. of normal horse serum alone, or of diphtheria antitoxic horse serum mixed with a suitable amount of diphtheria toxin, it becomes, after an interval of ten or twenty days, very susceptible to further treatment with normal horse serum. For a guinea pig whose preliminary treatment has been a toxin-antitoxin mixture, after an interval of three weeks, 5 c.c. of serum subcutaneously administered will frequently kill and will always develop symptoms. Five c.c. intraperitoneally is an almost certainly fatal dose. One one-hundredth c.c. given directly into the circulation is probably a certainly fatal dose.

As a sensitizing treatment I have employed chiefly the toxin-antitoxin mixture. As a test treatment I have administered the serum subcutaneously as a rule, but have occasionally injected serum into the circulation by the intracardiac method. Two c.c. is a safe dose for a normal animal by the latter method.

The results of other observers show that diphtheria toxin in some way increases the sensitizing power of the single small dose of serum. With serum alone the same effect can be produced by injecting fractions of the total small dose over a period of several days. In order to show the influence of the toxin, the intraperitoneal, intracerebral, and intracirculatory methods of testing must be avoided as the more vigorous reaction obtained when they are used masks the result. By injecting 2 c.c. of serum, intracardiac, into animals sensitized by toxin-antitoxin mixtures I have obtained a definite reaction on the sixth day. Careful

scrutiny of the results obtained by the subcutaneous test seems to show that the maximum of reaction is developed about the end of the third or fourth week. The incubation period is thus not sharply limited. The reaction is probably a slowly disappearing one but it may persist for a very long time — twenty two months in one of my cases.

The hypersensitive mother guinea pig transmits her abnormal condition to her offspring. In our experience about 50 per cent. of such offspring reacted. Members of the same litter tested with the same dose on the same day may either die very quickly or show almost no reaction. The fact of transmission from mother to offspring is strong presumptive evidence for the view that the reaction depends on the development of a special antibody by the sensitizing dose which is effective at the second treatment.

Such an antibody can be demonstrated by the passive transfer of the blood or blood serum of hypersensitive guinea pigs to normal guinea pigs. I have performed this experiment repeatedly. Thus, choosing hypersensitive animals that have reached the full period of reaction, I bled and injected 15 c.c. of defibrinated blood or blood serum into the peritoneal cavity of a fresh guinea pig weighing about 230 gms. After twenty four hours 1 or 2 c.c. of normal horse serum injected intracardiac killed this animal, which exhibited the typical symptoms. This fatal reaction at twenty four hours I believe to be distinct from the reaction obtained by Gay and Southard two weeks after the transfer of from 0.1 c.c. to 1 c.c. of hypersensitive blood. This latter reaction is probably due, as they supposed, to a retained horse-serum element or "rest" which actively sensitizes the animal. The hypersensitive antibody is not destroyed by heating to 60° C. for half an hour. Thus far further study of its properties has not been made.

If the hypersensitive animal is treated with a considerable, but not fatal dose of horse serum it is for some time less or not at all hypersensitive. This refractory or immune state can be very rapidly developed. The conditions are best illustrated by the following experiment.

Three hypersensitive guinea pigs were bled. Mixed blood was tested on a normal animal and found capable of transferring

the hypersensitive condition. Nine days later the animals were all treated as follows:

Sept. 19—8 p. m., 0.5 c.c. normal horse serum subcutaneously.
Sept. 20—8 a. m., 2.0 " " " " "
Sept. 20—12 m., 5.0 " " " " "
Sept. 20—8 p. m., 5.0 " " " " intraperitoneally.

None of the animals showed any marked symptoms under this treatment.

September 21, 10 a. m., one of the three received 1.5 c.c. normal serum intracardiac. No symptoms.

The other two animals were bled and 15 c.c. of the mixed defibrinated blood were at once injected into the peritoneal cavity of a fresh normal guinea pig weighing 240 grams. After an interval of thirty hours, this pig received 1.75 c.c. normal horse serum intracardiac. No symptoms.

Thus a complete immunity to the reaction can be developed in about twenty four hours and at the same time the blood loses its power to transmit the reaction, presumably because the antibody has been exhausted. The animal is probably in the condition of a normal animal which has had a single large dose of horse serum. Preliminary experiments make it seem possible, however, that the return to the hypersensitive state may be accomplished in less than the normal incubation period. This would be in analogy with the acceleration of reaction noted in cases of serum disease in human beings.

If normal guinea pigs are treated repeatedly with very small doses of horse serum ($\frac{1}{2000}$ c.c. – $\frac{1}{1000}$ c.c.) or with repeated non-fatal toxin-antitoxin mixtures, or if hypersensitive animals are fed with horse serum, they may subsequently be found hypersensitive in the usual way. In a percentage of cases, however, they will react differently when treated with 5 c.c. of horse serum subcutaneously. Acute symptoms are slight or absent. But at twenty four hours the animal may be very sick and have a large subcutaneous edema. The edema may progress and death occur on the second or third day, or in less severe cases the animal may survive with extensive cutaneous and subcutaneous necrosis. Post mortem examination on the third and fourth day shows interesting features.

The lung lesions described by Gay and Southard in the acute cases are here very slight. The gastric necroses are often very extensive. There are frequently large hemorrhagic necroses in the spleen. The mesenteric lymph nodes show desquamation of the endothelial cells of the sinuses. There are some red blood corpuscles in the sinuses. The endothelial cells are in various stages of degeneration, show some evidence of having acted as phagocytes, and the red blood cells are agglutinated around them in rosette-like arrangement. (Demonstration.)

In the spleen bordering the necrotic areas there is very marked phagocytosis of red blood corpuscles by large mononuclear cells. As contrasted with other instances of phagocytosis of erythrocytes this case has the following features. Usually but one corpuscle is found within the phagocyte. The enclosed corpuscle at first swells and has an increased affinity for eosin. Then the color reaction fades out and the corpuscle remains as a shadow which becomes smaller. Pigment is not common within these phagocytic cells. This form of phagocytosis with intracellular lysis is not a peculiarity of the guinea pig. In other conditions in this animal one finds phagocytic cells filled with numbers of rather shrunken deeply bronzed erythrocytes or with pigment particles evidently of erythrocytic origin. Comparison of the two classes of cases indicates, either that intracellular blood destruction in cells of endothelial type may be accomplished in ways that are fundamentally distinct, or that the factors of dissolution of the erythrocyte and segregation of the pigment constituents are independent and may run at different rates.

Recurring to the picture in the mesenteric lymph nodes, it seems possible that the endothelial cells may be the source of origin of certain serum hemagglutinins. It is an obvious suggestion also that agglutination functionally considered may be a constant factor in phagocytosis, serving to bring the subject cell, in this instance the erythrocyte, in contact with the phagocyte, in this case the endothelial cell.

This sharp reaction of the subcutaneous tissues resulting frequently in necrosis is closely analogous to the reaction which Arthus obtained to repeated subcutaneous doses of horse serum in the rabbit. It seems probable that it is a protecting factor so far as the animal is concerned.