ALLERGIC IRRITABILITY.

II. Anaphylaxis in the Guinea Pig as Affected by the Inheritance.

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(Received for publication, November 3, 1924.)

In a recent paper (1) we defined "allergic irritability" as "a general characteristic of the animal on the basis of which it reacts to stimuli of the antigenic class, whether they be helpful, injurious, or indifferent to bodily health." Here as there we have been concerned with the determination of this character in a stock of guinea pigs of which it has been shown by Wright and Lewis (2) that variations in the natural immunity, or resistance, to tuberculosis are partially determined by inheritance. We have now made the observation that these animals vary by families in their anaphylactic reactions. The purpose of this paper is to present our experiments bearing on this point. Reference may be had to our previous communication (1) for a detailed discussion of the background of the work. In view of the complexities of the subject as there outlined, it is apparent that a final interpretation must await the fuller development of several lines of work in themselves having little in common. We shall, therefore confine ourselves here to a presentation of actual observations and to such discussion as present knowledge of anaphylaxis may permit. The experiments to be detailed have a possible bearing on the problem of asthma and this will likewise be briefly considered.

The observations of Wright and Lewis (2) were made on five inbred lines of guinea pig cited by number in order of decreasing resistance to tuberculosis as follows: 35, 2, 32, 13, and 39. Of these, No. 39 has not recently been available and the present experiments have concerned only the first four strains. As controls we have

introduced at times a number of crossbred animals of the stock of this Department in no way nearly related to the inbred lines.

During the past year we have been interested in observations on the production of anti-sheep hemolysin in these inbred animals. In one experiment we gave to previously untreated animals 5 cc. of a 20 per cent suspension of washed red blood corpuscles of the sheep intraperitoneally and 5 cc. of the same suspension subcutaneously. On the 17th day succeeding, the same quantities of the same strength suspension were again given, also in both injection sites. The result of the second injection from our present point of view was as follows:

Family 35.—9 animals injected. 2 showed slight symptoms suggesting anaphylactic shock.

Family 2.—10 animals injected. 3 showed similar slight symptoms.

Family 32.—4 animals injected. 2 showed similar slight symptoms.

Family 13.—8 animals injected. 7 died within 2 hours, the 8th was very sick. Postmortem examination showed the fully expanded lungs, with slight edema and punctate hemorrhages, characteristic of immediate anaphylactic shock in the guinea pig. The blood cells in the peritoneal cavity were completely hemolyzed. The amount of absorption was not estimated. The blood in the subcutaneous tissues was partly hemolyzed.

The records of the test for anti-sheep amboceptor which had been made the previous day show that the following average serum dilutions were able to hemolyze completely 0.1 cc. of 1 per cent sheep red corpuscles in the presence of an excess of guinea pig complement. Individual determinations were carried out to the nearest 1/100 dilution.

Family 35: 1/374.
Family 2: 1/455.
Family 32: 1/600.
Family 13: 1/693.

We had expected that many of the animals might die suddenly following the injection, as this has been common experience. But we had anticipated that the deaths would be the effect of the *hemolysin-red corpuscle* reaction and that in consequence the lungs would be found collapsed and congested rather than expanded and pale. It is, however, now recognized that the condition of the lungs alone is not a safe criterion for judging of the existence of the anaphylactic reaction, even in the guinea pig, in which it develops its most characteristic features. Doerr (3) refers to various authors who have shown that the injection of toxic precipitates, anaphylatoxin, toxic normal or immune sera, when so administered as to cause sudden

death, may leave the guinea pig lung in the fully expanded, pale condition. The further fact that the severity of the reaction in our case paralleled the average hemolytic titer of the serum points to a possible interpretation of the reaction as anaphylactoid in nature rather than true anaphylaxis. We have, therefore, carried out classical anaphylaxis experiments with horse serum as antigen in order to clear the way to a more definite interpretation.

Ten animals of the Institute crossbred stock, and ten of each of the inbred families were selected. They were all treated with normal horse serum subcutaneously as follows: July 14, 1924, 0.01 cc.; July 16, 0.001 cc.; and July 18, 0.01 cc. On the basis of previous experience with an entirely different stock of guinea pigs, it was expected that 2 weeks following the last injection or any time shortly thereafter the fatal dose of horse serum administered intravenously would be about 0.01 cc. and that 0.005 cc. would cause severe symptoms but not kill. Accordingly, between the 5th and 8th of August, inclusive, all the animals were given intracardiac injections of horse serum. Some were given 0.01 cc., some 0.005 cc., and a few intermediate quantities were administered. In general the animals reacted less regularly and less severely than we expected. A number died within the week following.

On August 29, all of the remaining animals were given 0.5 cc. of normal horse serum, either subcutaneously or intraperitoneally, for the purpose of reenforcing and possibly equalizing the sensitization. Two animals died of immediate anaphylaxis. One other was quite sick. In the rest symptoms were slight or absent. 20 days later the survivors were again treated, being given 3.0 cc. of horse serum intraperitoneally as an intoxicating injection. On this occasion the reactions were about what we expected of fully sensitized guinea pigs.

The results of the whole experiment come out most clearly perhaps when the last injection is first considered. Table I gives the response of those remaining of the different groups at this time. The deaths as shown in the table are numbered also under the severe reactions.

This result is supported by a consideration of the reactions in the earlier periods of the experiment.

Thus, of the Institute stock, the four animals recorded in Table I as having a slight reaction or none were at no time more severely affected than this. Four of ten animals thus failed to give an anaphylactic response to horse serum under the conditions prevailing. (40 per cent negative.)

Of Family 35, one animal died before any intoxicating test was given. Of the remaining nine, three of those recorded as giving no symptoms or slight never gave definite reactions. (33⅓ per cent negative.)

Of Family 2, one animal which died 8 days after the 0.5 cc. dose gave only slight symptoms at the two tests to which it was exposed. All the others gave marked reactions. (10 per cent negative.)

Of Family 32 and Family 13, all gave marked immediate reaction at one time or another. (None negative.)

It thus appears that the regularity with which the animals react in the anaphylaxis experiment to horse serum varies from family to family. The order, in this rather small series at least, happens to follow the order of resistance to tuberculosis. If the experiment with horse serum is considered with relation to the result with blood corpuscles there is partial agreement. In the latter case the dividing line was between three resistant groups and one much more susceptible. In the experiment with horse serum, if one consider

TABLE I.

Reaction to the Final Injection of Horse Serum.

	No. remaining.	Reaction.				Deaths.
		None or very slight.	Slight.	Medium.	Severe.	
Institute stock.	6	3	1	—	2	1
Family 35	6	3	1	—	2	1
" 2	6	—	—	2	4	2
" 32	4	—	1	—	3	3
" 13	5	—	—	1	4	2

only the inbred animals, the line of demarcation is between one resistant family and three showing a higher degree of susceptibility. Family 35, judged by either experiment, appears to be definitely more resistant to this general form of intoxication. In the horse serum experiment, Family 35 manifested the same reaction as the usual crossbred stock of guinea pig, assuming the Institute stock to be such.

There would appear to be several possible ways in which animals might differ with the result that they would react variably toward the intoxicating dose of protein as in this series. They may conceivably differ in the rate at which they become sensitive after a first treatment, or they may differ in the maximum degree of sensitiveness attained. If the sensitiveness be considered in terms of

the amount of antibody formed in response to the first injection, a lesser degree to all appearances might result from the development of either less or more antibody than that optimal for the most severe reactions. Finally, the animals might differ in their susceptibility to intoxication with a hypothetical poison which may be formed as the result of the antibody-protein combination within the cell.

An experiment devised to determine the rate at which the families become sensitive is described in the following paragraphs, chiefly because of its confirmatory value.

TABLE II.

Response to Second Injection (1/100 Cc.).

Family.	Slight or negative.	Moderate to severe.
35	12 = 54.5 per cent.	10 = 45.5 per cent.
2	7 = 41.1 " "	10 = 58.9 " "
13	7 = 13.7 " "	44 = 86.3 " "

TABLE III.

Response to Third Injection (1 Cc.).

Family.	Used.	Survived.
35	20	3 = 15 per cent.
2	18	6 = 33⅓ " "
13	50	3 = 6 " "

The animals were first given 1/1,000 cc. of normal horse serum subcutaneously each day for 4 successive days. On the 8th day after the last injection, two of Family 35, two of Family 2, and four of Family 13 were given 1/100 cc. of normal horse serum by intracardiac injection. Similarly constituted groups were given the same treatment on succeeding days until all had been injected. 16 days after the second injection each animal was again given an intracardiac injection of 1 cc. of normal horse serum.

In response to the second injection (1/100 cc.) reactions of varying severity occurred and there were a few deaths. The reactions, grouping the slight with the negative, and the moderate with the severe (deaths included), are shown in Table II.

The deaths were two in Family 13, and one in Family 2, the latter possibly due to traumatic hemorrhage.

The result of the third injection (1 cc.) is shown in Table III. The fatalities were all immediate, the animals dying in about 4 minutes. There was no significant difference between the families in this respect.

This experiment agrees with the first experiment with horse serum in showing a definite contrast in the reactivity of Families 35 and 13. Family 2 is more like Family 35 in the second experiment, more like Family 13 in the first.

As we point out in the introduction, a discussion of the general significance of these observations must await an accumulation of evidence of other kinds. In special, an evaluation of the observed parallelism between the resistance to horse serum anaphylaxis and that to tuberculosis must be reserved. It may be recalled, however, that the tuberculin reaction, which seems to be so very important as a feature of infection with the tubercle bacillus, is a reaction of hypersensitiveness, and it is not difficult to imagine that further work may justify placing more emphasis on the relationships here developed.

Any attempt to elaborate the results in their bearing on the general problems of allergic irritability would have to be carried out in the light of some particular conception of the phenomenon of anaphylaxis. The most generally accepted theory in explanation of this may be stated somewhat as follows: The first protein injection gives rise to antibody formation, part of the antibody being found in the circulating blood, part remaining or becoming sessile in certain cells. A second injection, after an interval, encounters this antibody with various results, depending on the quantitative relationships and distribution of the components of the reaction. If the circulating antibody is excessive, the animal might not, theoretically, be intoxicated at all, as none of the protein would reach susceptible cells in uncombined form. If the antibody is deficient, the animal could not react. There is then, supposedly, a condition of high cellular content in antibody, with low humoral content, which is optimal in that it makes for extreme sensitiveness. If the second administration of the protein is so conducted that the animal survives, the antibodies are more or less completely exhausted, and until they are re-formed the animal is in what has been termed a condition of anti-anaphylaxis.

Applying this concept to our experiments, one may assume that Family 35 shows itself to be relatively less affected by the procedure either because the anaphylactic antibody is produced in excess or

because it was in less quantity than was the case with Family 13. Those individuals wholly failing to react, in special after the intensive treatment of the final portion of the last experiment, may have so failed because they are less easily sensitized or because they are more easily rendered antianaphylactic. It is probable that attempts at a quantitative determination of the actual state of affairs would fail because of technical difficulties; and in any event the material is lacking for such an experiment at present.

Accepting our inability to carry this study as far as might be wished in the direction of an understanding of the nature of the observed phenomena, these still have a very real interest. Particularly it seems clear that the differences in reaction between the strains or families do not rest on the complete absence of the reacting factors in any group. Individuals have been encountered in which sensitiveness has not been demonstrated. These are encountered in each group and would seem to represent the extremes in a continuously varying series rather than discontinuous instances. The result is in harmony with our other studies on allergic irritability, that is to say the capacity to be immunized or sensitized, and with our results on resistance to tuberculosis. In so far as determinable differences exist between our families of guinea pig, they are differences in the mean about which there exists a wide range of individual variations. We feel that a difference in allergic irritability with reference to anaphylaxis, resting on the basis of family and hence of inheritance, is at the least very strongly suggested by the results of the experiments here presented.

Superficially considered, these experiments seem to have a direct bearing on the questions arising from the conception that asthma depends on an anaphylactic phenomenon and that the tendency to asthma is inherited.

Adkinson (4) and Spain and Cooke (5) have recently brought together evidence from the records of asthmatic cases bearing on this point of view. Adkinson finds that asthma as a familial manifestation exists independently of any relation to the special proteins to which individuals are found hypersensitive, or indeed to the presence or absence of protein hypersensitiveness. Whether associated with demonstrable hypersensitiveness or not, "the asthmatic condition is found not to be congenital or transmitted by the mother to the fœtus or through the milk,

but it behaves as a true inherited trait, transmitted in the germ plasm of both parents alike, and following closely in the family histories the theoretical expectation of a Mendelian character recessive to the normal condition." Adkinson further considers that the nature of the inherited factor is unknown and that: "It is the tendency or power to develop asthma whether caused by sensitization to proteins or not, which is transmitted and not the condition itself."

The data of Spain and Cooke, extending still earlier figures of Cooke and Vander Veer (6), are in essential agreement with those of Adkinson. They found more normal offspring of matings where both parents were sensitive and as a consequence are unable to accept the recessive nature of the character. They suggest that it is a dominant, or that perhaps the condition is determined by multiple characters.

In our case, in so far as inheritance is a factor it is certain that this is in some way bound up with the ability to be sensitized. Each of our inbred strains of guinea pig shows this capacity, the degree of sensitization resulting from like treatment being the variable. We are not, evidently, dealing with the presence or absence of a single unmodified character. Approaching the matter from the point of view of the animal experiment, it seems possible that those who have dealt with the human material have not, in seeking a precise genetic interpretation, allowed sufficiently for the varying play of the actual sensitization process which must supervene before the underlying constitutional qualities become manifest.

The possibility remains that when the asthma problem is more closely approached through animal experiment the outcome may be different. For in the human we are dealing with variable sensitizing doses which, taken by and large, are minimal. Such individuals as fail to develop clinical asthma may be in part those whose absorption system is so constituted that they are never sensitized, and in other part those who respond to an ordinary sensitizing dose insufficiently for clinical symptoms at later exposure. The presence or absence of the underlying factors which make asthma possible in human beings may conceivably be simulated in animals. Indeed it was so simulated in our experiment with blood corpuscles. It is possible that a modification of our experimental procedure might throw more light on this angle of the question.

As to the significance of our results from the genetic point of view, this too awaits the accumulation of material. Crosses between our

families have been tested but in insufficient numbers and with unsuitable familial distribution to permit decision as to whether or not the differences between the families rest on a Mendelian basis.

SUMMARY.

Inbred lines of guinea pig which have previously been observed to differ in their susceptibility to tuberculosis differ in their anaphylactic responses as well. The families that are relatively resistant to tuberculosis appear also to be somewhat more resistant to some one or more of the phases of the anaphylactic reaction complex.

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