ALLERGIC IRRITABILITY.

IV. The Capacity of Guinea Pigs to Produce Antibodies as Affected by the Inheritance and as Related to Familial Resistance to Tuberculosis.

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Wright and Lewis (1) found that certain inbred families of guinea pigs differed in their resistance to experimental tuberculosis. The work as published has been carefully repeated and considerably extended by them with particular reference to amplifying the results of cross-breeding the various families. It thus became apparent that the observed variations in familial resistance must depend on the varied inheritance of a number of factors or factor groups. The operation of at least three or four separately inherited qualities was suggested by the genetic results.* An analysis of the known physiological and structural pecularities of the families was presented by Wright and Lewis (1) and later in great detail by Wright (2, 3), it being shown that such qualities as color, ability to grow at a certain rate, weight at a certain age, fertility as measured either by the number of young born or the percentage of young raised to the weaning age, or, finally, general vigor as estimated by these several qualities combined could be of but minor significance with relation to resistance. Thus of the total observed variation in resistance between the families about 40 per cent appeared to be dependent on characteristics which can be accounted for at present. All of the above characters combined account for but 7 per cent out of the 40 per cent mentioned leaving somewhat over 30 per cent as due to inheritance. It might

* It is expected that the amplifying data here referred to will be published in detail in the near future.

therefore, within a range of the observed variation of about 30 per cent, be hoped to discover by appropriate experiment one or more inheritable qualities which could be considered influential as determinants of resistance. The present authors set themselves this problem.

Consideration made it clear that our problem was intimately involved with the uncertainties of knowledge in regard to the natural resistance to infectious diseases in general and tuberculosis in particular. It has been our plan to fix on certain qualities, which might conceivably be influential, and to determine for each so far as possible the variability of the inbreds by family. As the result of a succession of such studies it is hoped to piece together a picture which may suggest either the inherited qualities concerned or more definitive experiments to reveal them.

Among the general biological reactions considered in this connection is that of the capacity of animals to acquire an artificial immunity or sensitization in response to treatment with antigenically active materials, that is to say their *allergic irritability* (4–6). For it must be true generally with infectious diseases that the initial stages of infection elicit immunity responses and that these influence the course of the disease, its character, and termination.

In the light of the great accumulation of knowledge with regard to specificity of immunization reactions it is possible on one view to presuppose that the quantitative relations within any such group of animals as that under consideration might vary for each antigen and that the only significant reactions for our purpose would be those connected with the tubercle bacillus or its products. But at the time this problem was taken up it was quite doubtful whether the guinea pig had ever been successfully immunized against tuberculosis, and it seemed that the effort to acquire the desired information directly would be attended with many uncertainties, if it were indeed practicable in any degree. Provisional experiments in this direction were undertaken however and may be reported upon later.

On the other hand it seemed not impossible that while immunity reactions speaking generally are so highly specific, the underlying qualities in the animal upon which they depend might be much less or not at all so; that in other words within any species an animal easy to immunize with one substance might be relatively easy to immunize

with any other, or with some others. As to this the literature afforded little information and hence experiments were undertaken with various immunity reactions which were easy to execute. We have thus studied the formation of hemolytic amboceptor for sheep and beef corpuscles, agglutinins for *Bacillus typhosus* and *Bacillus abortus* (Bang), and the reaction of anaphylaxis to sheep red blood corpuscles and to horse serum. From the beginning of the work the results have been suggestive with relation to our problem. Incidental results of considerable interest with reference to allergic irritability in general were also obtained and have been the subject of publications by Lewis and Loomis (4–6).

It was found (4, 6) that preexisting infectious disease, especially tuberculosis had a very marked stimulating influence on the quantity of antibodies produced by antigens unrelated to those involved in the disease process. This observation is pertinent to the present discussion in that it implies that a more or less common mechanism underlies the highly specific reactions of immunity with which we are more familar and on which we are technically dependent for information in this field.

We found (5) that when the complete experiment used for the classical demonstration of the phenomena of anaphylaxis was carried through on our guinea pigs the outcome varied by families and that there was a parallelism between the reactions of the families in this experiment and their resistance to tuberculosis as determined by Wright and Lewis. Those most resistant to the disease were the least responsive in the anaphylaxis experiment.

The series of papers (particularly Lewis and Loomis (4)) also give in detail the methods which we have employed in the estimation of antibody production, some discussion of the principles underlying our problem, and a review of the pertinent literature.

Our present purpose is to present and discuss briefly such observations as we have accumulated bearing on the varying capacities of the inbred families of guinea pigs to produce antibodies, keeping in mind especially the question as to whether there is any parallel to be observed between this capacity and resistance to tuberculosis.

EXPERIMENTAL.

In the first instance groups of animals from each family were injected simultaneously with sheep red blood corpuscles and a heat-killed culture of *Bacillus typhosus*. The antibodies, anti-sheep amboceptor and anti-*typhosus* agglutinin,

TABLE I.

Order of Families Relating Antibody Production to Resistance against Tuberculosis.

T. B. resistance	Anti-sheep hemolysin		Anti-beef hemolysin		*B. typhosus* agglutinin		*B. abortus* agglutinin		*B. abortus* fixation
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
35	35 (616)	35 (3000)	35 (524)	2 (487)	13 (400)	13 (60)	35 (32)	35 (94)	35 (940)
2	13 (280)	13 (2700)	2 (343)	35 (460)	35 (93)	35 (13)	2 (17)	2 (80)	2 (658)
32	2 (182)	2 (1200)	13 (331)	13 (280)	2 (90)	2 (13)	13 (15)	13 (61)	13 (237)
13	32 (92)		32 (226)	32 (220)	32 (20)	32 (6)	32 (14)	32 (14)	32 (110)

TABLE II.

Family No.	Individual No.	Natural amboceptor undiluted serum	Acquired amboceptor—dilutions					
			1:3	1:5	1:10	1:20	1:30	1:40
35	1	++++*	0	0	0	0	0	0
			++++	++++	++++	+++	+	0
(1/100)	2	++	++++	++++	++++	+++	+	0
			++++	++++	++++	+++	0	0
	3	±	++++	++++	++++	+++	+	
			++++	++++	++++	+++	++++	++
	4	++++	0	0	0	0	0	0
			++++	++	0	0	0	0
(1/1000)	5	±	0	0	0	0	0	0
			d.	−	−	−	−	−
	6	0	0	0	0	0	0	0
			++++	++	0	0	0	0
13	1	0	++++	++++	++++	++++	++	0
			++++	++++	++++	++++	++	0
(1/100)	2	++++	++++	++++	++++	++++	+++	++
			++++	++++	++++	+++	++	0
	3	++++	0	0	0	0	0	0
			++	0	0	0	0	0
	4	0	0	0	0	0	0	0
			0	0	0	0	0	0
(1/1000)	5	0	0	0	0	0	0	0
			+	0	0	0	0	0
	6	0	0	0	0	0	0	0
			+	0	0	0	0	0
32	1	±	0	0	0	0	0	0
			d.	−	−	−	−	−
(1/100)	2	+++	++++	++++	+++	+	0	0
			d.	−	−	−	−	−
	3	±	0	0	0	0	0	0
			0	0	0	0	0	0
	4	±	+	0	0	0	0	0
			d.	−	−	−	−	−
			+	0	0	0	0	0
(1/1000)	5	++++	0	0	0	0	0	0
			+	0	0	0	0	0
	6	0	0	0	0	0	0	0

*++++ = complete hemolysis; 0 = no hemolysis; d. = dead.

Of the two readings shown for each individual the first is for the 9th, the second for the 22nd day after injection.

TABLE II—*Concluded.*

Family No.	Individual No.	Natural amboceptor undiluted serum	Acquired amboceptor—dilutions					
			1:3	1:5	1:10	1:20	1:30	1:40
2	1	0	0	0	0	0	0	0
			++++	++	0	0	0	0
(1/100)	2	0	0	0	0	0	0	0
			+++	+	0	0	0	0
	3	0	0	0	0	0	0	0
			+	0	0	0	0	0
	4	++++	0	0	0	0	0	0
			0	0	0	0	0	0
(1/1000)	5	0	+	0	0	0	0	0
			0	0	0	0	0	0
	6	+	0	0	0	0	0	0
			+	0	0	0	0	0

were determined on the 9th day after the first treatment. The same animals were then treated simultaneously with beef red blood corpuscles and a heat-killed culture of *Bacillus abortus* (Bang). Again the respective amboceptor and agglutinin titres were determined on the 9th day after injection. There were three animals in each family group. Table I in the 1st column under each subhead shows the results obtained in this experment. The experiment was repeated using groups of ten animals for each family. The results are shown in the 2nd column under each subhead. The arrangement is by family numbers in order of decreasing titre from top to bottom. The actual average titres are shown in parenthesis and where these are considered to differ by insignificant amounts they are bracketed together. In connection with the second experiment the titres of the sera for *Bacillus abortus* in the complement fixation reaction are also shown as they emphasize the significance of the differences among sera of low agglutinin titre (last column Table I).

This type of experiment was carried through once more using beef corpuscles and the heat-killed culture of *Bacillus bovisepticus* (Strain Pn. I). Agglutinins did not develop and Family 32 was not represented. The result with reference to anti-beef hemolytic amboceptor in the terms used in the table was first, 35 (3000); second, 13 (2700); third, 2 (1200).

Several experiments were done in which much larger and repeated doses of sheep cells were given. It was thought that the higher titres of amboceptor so induced might give better definition to the place of the families. This proved not to be the case. In general either Family 13 or Family 35 were the high producers but the irregularities rather than the resemblances were emphasized in this type of ex-

periment. Family 32 was represented in but two of this group of experiments and it took a high place being highest once and second to Family 35 once.

Since the exaggeration of the antibody production seemed, as has been said, to emphasize irregularities in the relations and probably to introduce additional factors it was thought possible that the repetition of the study using the minimal amount of antigen necessary to a response might be instructive. Two experiments were conducted on this basis as follows:

In the first of these, six animals were taken from each family. They were tested individually for natural hemolysin and then injected with washed red corpuscles of the sheep in amount equivalent to 1/100 cc. and to 1/1000 cc. of whole defibrinated blood, three animals of each family being used for each dose. The animals were bled on the 9th day and on the 22nd day after injection. The titres of the serum for anti-sheep amboceptor were determined in the usual way in the presence of an excess of guinea pig complement. The results are shown in Table II.

Findings.

It was found that with reference to our problem no significance is to be attached to the presence of natural amboceptor. This is very small in amount at most and frequently undemonstrable. Each of the families shows instances of its presence in maximum and its complete absence. When present it apparently does not influence the formation of specific amboceptor as instances appear of animals with the maximum of natural amboceptor giving no increase on treatment and of those with no natural amboceptor giving a good response to the injection.

Considering the acquired antibodies by families two clearly defined groups appear. The animals of Families 35 and 13 produced much more antibody than did those belonging to Families 2 and 32. Within the groups Family 35 did somewhat better than did Family 13. No distinction of value appears between Families 32 and 2.

This experiment was repeated in its essentials with the same general result. The grouping by families appeared as before. The only difference was that within the groups on repetition Family 13 produced definitely more amboceptor than Family 35.

The animals used in the second experiment were now given a second injection of 1/100 cc. of sheep corpuscles. The results were in accord with those previously obtained when repeated doses or larger amounts of corpuscles were administered. In this response to a second injec-

tion Family 13 continued to lead but the level of Family 35 was essentially equaled and actually overtopped by a small margin by Families 2 and 32.

In connection with some of the experiments reported in Table I and those when larger and repeated injections of antigen were given attention was paid to the curve of antibody production. As already reported (4) it was found that in the guinea pig the curve of anti-sheep amboceptor production in response to any injection usually presents two peaks, one at about the 9th the other at about the 22nd day. Attention has been directed to possible variants in this curve with relation to family. In the main features and especially with reference to first injections no suggestion of any difference has appeared. The maxima are found at the 9th and 22nd days for each family. There is something to suggest that with reference to the second or subsequent injections the rise to and fall from the maxima is somewhat more abrupt in the case of Family 32 as compared to the others.

Interpretation.

Classified by their capacity to produce hemolytic antibodies for sheep or beef corpuscles, or agglutinins for *Bacillus typhosus* and *Bacillus abortus* (Bang) the inbred guinea pigs we have been able to study fall into two clearly defined groups; Families 13 and 35 are high producers, low are Families 2 and 32. It is found that this grouping appears most clearly when minimal doses of antigen are used in the form of a single treatment (Table II). When the amount of antigen is somewhat increased the grouping is still presented, possibly somewhat less clearly (Table I). When the treatment is in the form of much larger single doses, or repeated injections even of those minimal in amount the groups are no longer well defined. It seems justifiable to consider that the responses to the first minimal impact with antigen most truly represent the latent capacity of the animal to react with antibody production.

In the case of injections repeated at intervals it must be true that the result is a complex made up of the initial response on the basis of latent capacity and a secondary response on the basis of a stimulated

or acquired capacity. There is no *a priori* reason why this secondary reaction should not develop on the basis of inherited characteristics as the primary one seems to do. With equal probability in anticipation it might be foreseen that the reactions of the two periods should either be closely correlated (that is dependent on the same set of characters) or quite independent.

In general if the correlation were close the results in the two periods should be of the same order and it might even be expected that those of the second period with high absolute titres of antibody should be the more distinctive. That this is not the case lends something to the view that the reaction characters underlying the two phases are at least partially distinct.

The regularity with which Family 32 takes a low place when given a single treatment of antigen in small and moderate amounts suggests that some special significance should be attached to those instances when in response to repeated treatments this family takes a high place. It may well be that the reactions peculiar to the second phase of antibody productions are also conditioned by inherited characters but somewhat different from those influential in the initial response.

It seems very likely however that the results in the second period are rendered obscure and irregular by some other influence. A scrutiny of the individual records suggests that individual differences in reaction may be relatively more pronounced in this phase. The number of animals for which individual records are available is too small to enable us to decide this point. Many of the data included in the study were based on results with serum pooled by families rather than on the average of individual tests.

The order of familial resistance to infection with the tubercle bacillus, as determined by Wright and Lewis (1), from high to low is 35, 2, 32, 13. Within this group 2 is more like 35 and 32 more closely allied to 13. Comparing the order of tuberculosis resistance with the allergic irritability, arranging the families from high to low in each case and indicating the grouping by parenthesis, gives the following:

Tuberculosis resistance (35, 2) (32, 13).
Allergic irritability (35, 13) (2, 32).

It is therefore apparent that allergic irritability as defined or recognized by the capacity to produce antibodies against the antigens here used is not perfectly correlated with and hence cannot be wholly responsible for the differences in resistance. With so much understood there are still observable relations which seem to be of interest. Thus if we were to set aside Family 13 in both cases the order of the remaining families would approximate 35, 2, 32 in each relation. This seems to be sufficient to suggest that the latent capacity to produce antibodies freely may be of some significance to the natural resistance against tuberculosis. Family 13 would in this interpretation be regarded as peculiar. In later papers we hope to be able to show that this family is also peculiar in other respects which may be significant.

In our previous paper in which anaphylaxis was used as a criterion for the allergic irritability the agreement between the results and the order of resistance was also suggestively close. If it be considered that the reaction of anaphylaxis is solely determined by the abundance of antibodies produced in response to the sensitizing injection there is developed a definite conflict between results of the two types of experiment. For whereas Family 13 which is now shown to be generally a high producer of antibodies was also found the most susceptible in the anaphylactic reaction, Family 35 which is also a high producer of antibodies was definitely the most resistant to anaphylactic shock. Two equally reasonable explanations suggest themselves. Either the two kinds of antibodies are independently produced, or there is in the anaphylactic response some other and hitherto unrecognized factor which serves to protect Family 35 in spite of its presumably high content of antibodies. This matter could be decided were it possible to make satisfactory quantitative determination of the passive transfer of anaphylaxis. This we have not so far been able to do and the nature of the differences revealed by the experiments in question remains problematical.

It may not be redundant to point out once more that in this work whenever the inbred families are found to differ significantly with respect to any quality or capacity this is to be regarded as evidence *per se* that the character is influenced by the inheritance either directly, or indirectly through other inheritable interacting characters. As

all inheritance is now held by most specialists to be Mendelian such qualities and capacities are likewise to be thought of as subject to Mendelian principles. The direct determination of the Mendelian attributes however must depend on the results obtained with intercrosses between the families. In the instance here considered, the ability to produce antibodies, we have so far been unable to trace significant differences through the reactions of the crossbreds. So that while we are able to conclude that the allergic irritability as expressed in the capacity to produce antibodies is high or low according to the inheritance we can only infer that the inheritance itself is in accord with the Mendelian conception.

SUMMARY.

The allergic irritability of closely inbred guinea pigs as represented by their capacity to produce hemolytic antibodies for beef and sheep corpuscles, and agglutinins for *Bacillus typhosus* and *Bacillus abortus* (Bang) differs by families and therefore is at least partly dependent on inherited characteristics.

These differences show an imperfect but suggestive correlation with the differences in resistance of the same families to inoculation tuberculosis as previously determined by Wright and Lewis.

The differences in antibody production also show an imperfect correlation with the differences in response in the anaphylactic reaction complex as previously determined by Lewis and Loomis.

These studies suggest very strongly that the allergic irritability is one of the several inheritable characters which form a partial basis for the natural resistance to tuberculosis.

The antibody-producing capacity is only satisfactorily defined when minimal or moderate amounts of antigen are used and this in single treatments. The irregularities in experimental result when repeated treatments or very large single treatments are used suggest that antibody production in the second or "acquired capacity" phase may rest on a somewhat different fundamental basis than the latent or potential natural capacity. There is some very slight evidence that production in the second phase may also be influenced by inherited qualities.

BIBLIOGRAPHY.

1. Wright, S., and Lewis, P. A., *Am. Naturalist*, 1921, lv, 20.
2. Wright, S., *U. S. Dept. Agric., Bureau Animal Ind., Bull. 1090*, 1922.
3. Wright, S., *U. S. Dept. Agric., Bureau Animal Ind., Bull. 1121*, 1922.
4. Lewis, P. A., and Loomis, D., *J. Exp. Med.*, 1924, xl, 503.
5. Lewis, P. A., and Loomis, D., *J. Exp. Med.*, 1925, xli, 327.
6. Lewis, P. A., and Loomis, D., *J. Exp. Med.*, 1926, xliii, 263.