Genetic Constitution in the Rabbit and Antibody Production*

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IT is not a new observation that wide variation in immunologic response may occur among several animals inoculated with the same antigen. Several explanations for the variations have been proposed, and it seems certain that age, the physiological condition of the animal as influenced by diet and living conditions, and inherited characteristics, are factors influencing immunization. For example, it is common laboratory experience that two or three rabbits should be immunized to be sure of obtaining a precipitin serum sufficiently potent for forensic tests. For the production of diphtheria antitoxin certain horses which have slight normal antitoxin are more suitable than others.

In the case of immunization of rabbits with human blood cells it was noticed that certain rabbits failed to respond to the injection of A cells, and the reason was shown by Witebsky[1] to be due to the presence of an A antigen in the tissues of these rabbits. This situation is similar to that for the human O, A, B, and AB blood groups, where the presence of antigen in the body tissues determines the absence of the corresponding antibody from the serum. Furthermore, rabbits lacking the A antigen possessed normal anti-A agglutinins in their sera.

In the course of some work at Brown University with the anti-human rabbit sera it was noticed that one stock of rabbits consistently yielded good titering immune specific anti-A agglutinins while other rabbits failed to do so. At the time, a good sized rabbit colony was being maintained for genetical work, and it was decided to test these animals for normal agglutinins for human blood cells of the various groups. Some 40 or more rabbits were immunized with A cells, and specific antibody formation was found to correlate with the presence of normal specific A agglutinins.[2] Among 9 inbred, or closely bred families in which the animals were distributed[3] one family was found in which the animals were all of one type—all possessed A agglutinins—and consequently could yield potent A antibodies after immunization.

The data suggested that the ability to immunize was inherited, and possibly in a simple Mendelian fashion. Subsequently, a few cases have been found in which atypical, and for the most part temporary, A agglutinins were present. These were unlike the usual normal agglutinins and occurred even when an A antigen was present in the rabbit tissues. Breeding tests were carried

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out, in conjunction with other genetical work, to determine the mechanism of inheritance of the ability to yield strong anti-A agglutinins (as indicated by the presence of normal A agglutinins, or by the absence of A antigen in the rabbit serum shown by a negative complement-fixation test with the appropriate antiserum). Good evidence for ininheritance was found.[4] Results of the breeding tests to date are summarized in Table 1.

TABLE 1

Inheritance of the A Character

Genotype	*Normal Agglutinin*	*Immunizing Capacity*
A_nA_n	absent	—
A_na_n	absent	—
a_na_n	present	+

Type of Mating	*Type of Progeny*	*Number of Progeny*	*Expected Ratio*	*Chi Square*
$\frac{A_n}{A_n} \times \frac{A_n}{A_n}$ or $\frac{A_n}{a_n}$	A_n	30	30	
	a_n	0	0	
$\frac{A_n}{a_n} \times \frac{A_n}{a_n}$	A_n	205	209.2	0.22
	a_n	74	69.8	
$\frac{A_n}{a_n} \times \frac{a_n}{a_n}$	A_n	208	219	1.10
	a_n	231	219	
$\frac{a_n}{a_n} \times \frac{a_n}{a_n}$ *	A_n	0	0	
	a_n	225	225	

* This mating includes 150 animals in Family I.

The mechanism of inheritance is illustrated at the top of the table; A_n representing the dominant and a_n the recessive factor. Only the homozygous recessive animal possesses normal specific A agglutinins and can yield strong immune antibodies. The first type of mating involves parents, one or both of which may have been a homozygous dominant since no negative (lacking A agglutinins) offspring occurred. The double heterozygous mating gives a ratio of 205 to 74 which is close to the expected 3 to 1. The 439 animals in the backcross matings yielded 208 negative to 231 rabbits with A agglutinins—well within the chance variation from the 219 expected. From the homozygous recessive mating 225 progeny were obtained. This includes 150 rabbits which belong to the Family I strain, Family I being a pure recessive stock. The deviations of the experimental from the theoretical ratios, although within chance error, have always been toward an excess of alpha animals. The significance of this is uncertain. A rare exception from the expected type of offspring could be theoretically possible due to mutation.

In the case of the human group B blood cell antigen, the situation is different. The B factor is a multiple antigen,[5] a part of which occurs in the erythrocytes of all rabbits. Consequently upon injection, antibodies could be produced in rabbits only against the B components not in the rabbit cell. Immunizations have shown,[6] however, that although all rabbits possessing normal B antibodies produce immune agglutinins, some but

TABLE 2

Inheritance of the M character

Genotype	*Normal Agglutinin*	*Immunizing Capacity*
M M	absent	+
M m	absent	+
m m	absent	—

Type of Mating	*Type of Progeny*	*Number of Progeny*	*Expected Ratio*	*Chi Square*
M/M x M/M or M/m	M	13	13	
	m	0	0	
M/m x M/m	M	36	39.8	1.21
	m	17	13.2	
M/m x m/m	M	13	11.5	0.37
	m	10	11.5	

not all individuals without the agglutinins also produce immune B antibodies. Furthermore, B antibodies in general are less easily produced than A agglutinins. If immunizing capacity for the B antigen is inherited it apparently is not by a simple mechanism but probably by multiple factors.

Another serological characteristic was studied—the ability to produce anti-M agglutinating sera. Instances cited in the literature showed that about 30 per cent of the rabbits that were immunized yielded suitable M typing fluids. Injections of animals from our stock showed considerable variation in response to the M antigen. A further series showed that the immunizing capacity was inherited [7] and, so far as the data went, inheritance could again be explained by a single pair of factors.

Table 2 summarizes the breeding data to date for the M character. In no case are normal specific M agglutinins present in the rabbit sera. With the M antigen, in contrast to the A, the ability to produce antibodies is the dominant (M) factor and only the homozygous recessive animal (mm) is incapable of forming M agglutinins. Progeny have been tested from three types of matings and, although the numbers are not great because of the long procedure needed to test each animal, the figures closely approximate the expected ratios for a simple Mendelian interpretation of inheritance. No progeny from the double recessive mating have been available for testing.* While the ability to produce M agglutinins definitely seems to be an inherited characteristic in rabbits, no final conclusions can be drawn until the double recessive mating has been made and until larger numbers of progeny have been tested.

The reason for the failure to produce M antibodies is not apparent. M antigen has not been conclusively demonstrated in the tissues of rabbits. It is not in the erythrocytes,[8] and in the few cases which we have examined we have not found it in the tissues. A serious difficulty in working with M and N agglutinins is nonspecific absorption. This seems to be overcome by the new technic of Kosjakov and Tribulev,[9] and possibly by this method M antigen will be demonstrated in the tissues of rabbits which fail to yield M antibodies.

* Mr. Sleeper has several litters of young rabbits which should be ready for testing within a few months.

Besides these studies with the human blood cell antigens a few additional facts have been noted in connection with the stock of rabbits, particularly with respect to Family I which is a strain of New Zealand whites, large (8–10 lb.) white rabbits which was started in 1932 with 3 sibs and now has been closely bred for 7 generations. Fifteen of these animals were tested by Dr. Landsteiner at Rockefeller Institute and consistently found to give considerably higher titers for Forssman antibodies than the stock commonly used. Another interesting fact was observed in connection with immunizations done at Brown University with coliform organisms. Because of shortage of Family I rabbits discarded animals from genetic experiments were used for most immunizations. With one culture attempts to produce agglutinins in 6 animals failed; the maximum agglutinin titer was 1:160. One Family I rabbit was used and after a short course of injections gave an agglutinin titer of 20,000.

For several reasons no attempt has been made to extend the studies to other antigens, bacterial or otherwise, but it seems likely that, possibly with a strain of the present stock, or by selection of animals and breeding to bring out the characteristic, strains of rabbits could be had that were especially suitable for specific immunological purposes. The Family I animals, for example, are excellent for the production of anti-A agglutinins or heterogenetic antibodies against the human A- or sheep cell antigens. Possibly this, or another strain would also prove particularly good for anti-pneumococcus sera, or antisera against the enteric organisms.

That the individuality of the rabbit is a factor in both the qualitative and quantitative aspects of antibody production should be borne in mind. While many factors such as length of immunization, route of injection, age and physical condition of the animal, etc., influence the reactive qualities of the serum produced, especially when complex cellular antigens are used, the " constitutionality " of the rabbit is important, and sometimes critical. We have shown that some of the " constitutional attributes " of the animal are inherited and probably in simple Mendelian fashion. Other serological characteristics also seem to be inherited although the manner of inheritance, at present, is not so apparent.

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