THE INHERITANCE OF ANTIBODY RESPONSE TO TOBACCO MOSAIC VIRUS IN RABBITS

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Summary

In a crossbred population of rabbits it was found that the level of secondary antibody response to a routine injection of T.M.V. was highly heritable ($h^2 = 0.876$, S.E. 0.09).

I. Introduction

It is common experience among immunologists that animals of the same species vary widely in their ability to produce antibodies. Comparisons between the responses of different inbred lines indicate that at least some of the variability is genetic, as shown in guinea pigs by Lewis and Loomis (1928), in rabbits by Lurie (1941), and in mice by Gorer and Schutze (1938), Davidson and Stern (1949), and Fink and Quinn (1953). Little work has been done to study the inheritance of response. Kleczkowski (1939) presented suggestive evidence for a single gene control over the level of response to small injections of human serum into rabbits, but the data are limited. Scheibel (1943) when selecting for and against the ability to produce antitoxin to a standard dose of diphtheria toxin in guinea pigs, obtained good evidence of a single gene control over the ability to 'produce' or 'not produce' antitoxin. There was little or no evidence, however, of a genetic control of the level of response of the 'producers.' Carlinfanti (1948) measured the levels of human isoantibodies anti-A_1 and anti-B quantitatively in 51 families with 159 children, and found them to be almost entirely genetically determined. This interesting study does not, however, contribute information to the inheritance of response to introduced antigens; the presence of isoantibodies not having been satisfactorily explained as yet.

The results described in this paper are an approach to the quantitative study of the inheritance of antibody response.

II. Experimental

(*a*) *Antigen*

Tobacco mosaic virus (T.M.V.) was selected as a suitable antigen. This antigen has an extremely high molecular weight: 33,000,000 (Schramm and Friedrich-Freska 1941), 50,000,000 (Kausche and Ruska 1939); and is strongly

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antigenic. The T.M.V. used in the present work was kindly supplied by Dr. A. Kleczkowski of Rothamsted Experimental Station. Injections were given by the intravenous route to avoid the possibility of introducing variables independent of antibody response, e.g. skin thickness or local blood supply. Each rabbit received a total dose of 0.5 mg T.M.V. given in two injections, each consisting of 2 ml 0.0125 per cent. T.M.V., with an interval of a week between injections. Rabbits were injected in the left ear and bled from the right ear, the marginal ear vein being used in both operations. Bleedings were made on the tenth and thirteenth days after the last injection.

(*b*) *Antibody Response*

The equivalence zone method was used throughout. Some preliminary tests were made to find the range of response at the injection dose selected. The method, as it was finally adopted and used throughout the experiment, is given below. Eight 5-ml tubes were set up for each serum to be tested and 2.5 ml of the appropriate antigen dilution was added to each (Table 1).

TABLE 1

EXPERIMENTAL SCHEME ADOPTED

Tube No.	2·5 ml T.M.V. (% dilution)
1	0·04
2	0·02
3	0·01
4	0·005
5	0·0025
6	0·00125
7	0·000625
8	0·0003125

To each tube was then added ½ ml of test serum diluted by half. The tubes were incubated in a 40°C water-bath for half an hour and left overnight in the refrigerator. They were then centrifuged and the supernates removed and divided in two, half into each of two tubes and the tube number retained for each pair of tubes. To one half was added 0.5 ml of strong anti-serum diluted by half, and to the other 0.5 ml 0.01 per cent. T.M.V. These were then placed in the water-bath and the equivalence zone estimation read when precipitation was complete. The zone is indicated by a pair of tubes in which no precipitation occurs, i.e. there is neither excess antigen nor antibody. Where the zone was wide, or overlapping, the estimate of response was obtained by interpolation. The scale of values measuring antibody response is given in Table 2.

The antigen dilutions are in a geometric progression and the tube number for any equivalence estimation represents the inverse log of the antibody re-

sponse. The extremes, 10 and 1, are not strictly to scale, but they cover the present range of response adequately. This method of measuring the response is convenient and also useful in view of the evidence of Carlinfanti (1948) and Holt (1951) that the scale of antibody response is logarithmic.

TABLE 2
SCALE OF VALUES

Scale	10	9	8	7	6	5	4	3	2	1
Tube No.	>1	1	2	3	4	5	6	7	8	<8

The secondary time response curves, as illustrated in Table 3, all reach a peak by 7 days, after which they fall off very gradually.

TABLE 3
SECONDARY TIME RESPONSE CURVES

Serial No.	Response at the Following Days After Last Injection						
	2	4	7	10	13	17	20
399	5	5	5·5	5·5	5		
400	4	4·5	6	5	5		
401	3	4	4·5	4	4·5		
402	1·5	4·5	5·5	5·5	5·5		
403	1·5	5	5	5	5		
404	2	5	6	5	5		
405	1	1	1	1	1		
406	1	1	1	1	1		
407	1	1	1	1	1		
408	1	1·5	2	2	2		
409	1	1	2·5	2	2		
410	4·5	6	7	7	6	5	5
411	5	7	8	7	6	5·5	5·5
412	3·5	4·5	4	5	4·5	5	4·5

The estimated experimental error of measurement is ±0.6 when calculated on the assumption that the variance of the difference between 10- and 13-day responses is twice the experimental error for any individual observation. Within the limits of this error the order of ranking can be regarded as being the same whether judged by response at 4, 7, 10, or 13 days. The variation is greatest at 7 days but only slightly reduced at 10 and 13 days, and these were selected for the present investigation. By taking two measurements it was possible to estimate the experimental error of measurement and to limit the probability of missing any unexpected variability of time response. All calculations relating to heritability are based on the response at 10 days.

(c) *Rabbits*

The parent-offspring comparisons include only measurements made on the F_1 and F_2 of a Flemish Giant (F.G.), Ermine Rex (E.R.) cross. Some measurements were made on E.R. rabbits and these are included for purposes of comparison and frequency distribution only.

The animals were housed in ventilated, unheated buildings throughout the year. Up to 9 months of age animals were fed pellets and thereafter a mixture of oats and pellets. All animals were injected at between 5 and 6 months of age, at which age they reach sexual maturity, and antibodies passively acquired from the mother will have disappeared. The pre-injection sera of 25 rabbits, whose dams had received the routine injection of antigen before being mated, showed no demonstrable passive antibodies to the antigen.

III. Results

Measurements made in the parent generation included an unselected F_1 population of F.G. × E.R. cross, and an unselected population of E.R. rabbits. The frequency distributions of response of these two populations are given in Figure 1*A*,*B*.

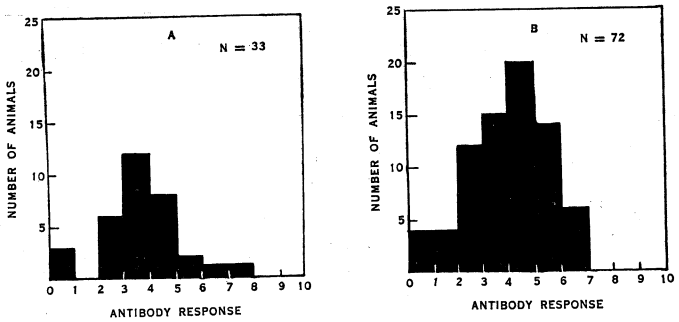

Fig. 1.—Frequency distribution of secondary response to routine injection of T.M.V. in two populations of rabbits. *A*, an E.R. population ($\bar{x} = 4.12$), and *B*, an E.R. × F.G. cross population ($\bar{x} = 4.57$). The means are not significantly different ($t_{103} = 1.49$).

In view of possible genetic differences between the populations, these distributions were compared statistically. They show a variance ratio of 1.185, which is not significantly different at the 5 per cent. level and justifies a comparison of their means by a t test. This gave $t_{103} = 1.49$, which is not significant at the 10 per cent. level, and the two populations can be regarded as being the same in their response to T.M.V. To increase the data on the frequency distribution of response the data from the two populations were added; this result is shown in Figure 2.

This distribution is obviously normal, even to the extent of being almost suspect, and justifies the use of a logarithmic scale in the measurement of response.

The heritability of response to T.M.V. was measured by a mid-parent offspring regression; 213 animals, comprising 32 litters, were measured. This regression, shown in Figure 3, gave a heritability $h^2 = 0.876$, S.E. 0.09.

In spite of the relatively low number of points making up the regression, these data clearly demonstrate the secondary response to T.M.V. to be highly heritable.

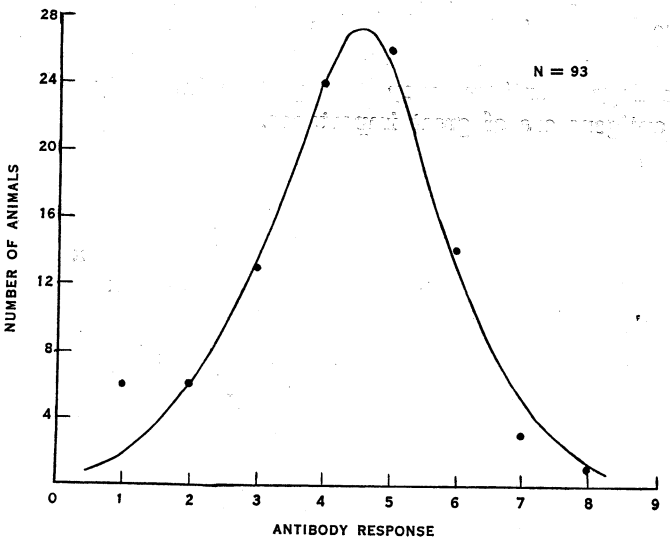

Fig. 2.—Frequency distribution from the combined data in Figure 1. $\bar{x} = 4.43$, $s\bar{x} = 0.14$, $s = 1.44$. Coefficient of variation = 32.5 per cent.

IV. Discussion

These results indicate that ability to produce antibodies to T.M.V. is highly heritable and it would be a relatively simple matter to select lines of animals with a high or low secondary response to this antigen. It was shown by Sobey (1953) and Sang and Sobey (1953), using a double diffusion technique in agar, that under the conditions prevailing in the present work, T.M.V. was apparently acting as a single antigenic fraction and producing a single specific antibody response. It was further shown that with a more prolonged antigenic stimulus T.M.V. elicited more than one antibody response; these could not be individually distinguished by the routine equivalence zone method used but only by the more delicate technique of agar diffusion. Thus had a greater antigenic stimulus been employed as a routine, the method of estimating antibody response would still have given a single measurement of what was in fact more than one antibody response. There are no grounds for assuming that these responses would be correlated, and if they were not, the resulting confusion in measurement would be likely to give a low or non-significant measure of heritability. Nevertheless, the individual antibody responses could be

as completely under genetical control as that found to the single antigenic T.M.V. fraction in this study.

These arguments raise fundamental issues regarding any study of the significance of antibodies in disease resistance. Pathogens generally are antigenically complex and may be expected to stimulate more than one specific antibody response. Of these antibodies it is often only one which plays a major part in immunity; in pneumococcal infection antibodies to the specific polysacchonides afford a high degree of specific immunity, whereas those to the nucleoprotein antigens have little protective value (Topley 1929; Topley and Wilson 1948; Bailley 1950). The evidence of Felix (1924) indicates that in motile bacilli the antibodies to flagella antigens play little part in immunity, whereas those to the somatic antigens are of great importance.

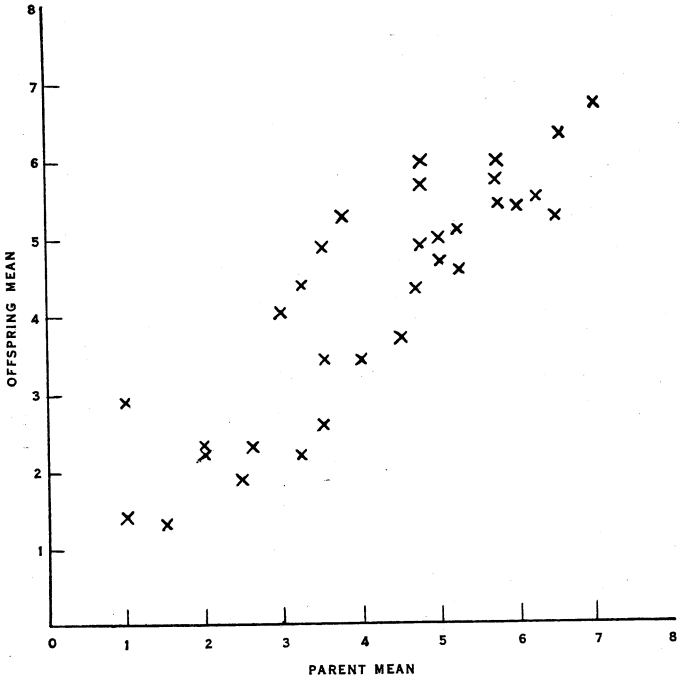

Fig. 3.—The regression of mean secondary response of offspring on mean secondary response of parents to routine injection of T.M.V. 213 Animals, comprising 32 litters, were measured. The estimated heritability was $h^2 = 0.876$, S.E. 0.09.

The relationship between the quantitative antibody response to the specific antigens concerned in resistance and the degree of resistance is not yet fully understood. There is evidence of a positive correlation (Smith 1932; Carlinfanti and Cavalli 1945; Bailley 1950), but this does not always justify the acceptance of a causal relation (Gorer and Schutze 1938; Weir, Cooper, and Clark 1953). Another factor concerning the individual antibody responses which has received little attention is the variability of their time responses, and whether or not speed of reaction is concerned in resistance.

To ensure a clear understanding of the inheritance of antibody response and of the relation of antibodies in resistance, it would appear necessary to study in detail the individual antibody responses involved. Measurements failing to differentiate the individual antibody responses are likely to be misleading in any quantitative study involving complex antigens.

If specific antibody responses are generally as highly heritable as that demonstrated with T.M.V., it might be possible to select lines of animals with a high or low antibody response to each of the individual antigenic fractions of a pathogen, provided these could be measured. Such lines of animals would afford useful material for the study of the problems raised.

V. Acknowledgments

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