INHERITANCE OF ANTIBODY RESPONSE

IV. HERITABILITY OF RESPONSE TO THE ANTIGENS OF RHIZOBIUM MELILOTI AND TWO STRAINS OF INFLUENZA VIRUS

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Summary

A heritability of $-0 \cdot 012$ is obtained in mice to injections of *Rhizobium meliloti* as measured by the Vi antigen and $0 \cdot 78$ by the O antigen.

The heritability of antibody response to injections of influenza virus strain MEL is $0 \cdot 60$ and $0 \cdot 16$ and to strain LEE $0 \cdot 30$ and $0 \cdot 10$ when measured by two methods.

These differences are discussed and suggestions offered to account for low heritabilities.

I. INTRODUCTION

The inheritance of the amount of antibody produced in response to injections of antigens has been studied by Sang and Sobey (1954), Sobey (1954), Sobey and Adams (1955), and Claringbold, Sobey, and Adams (1957).

Sobey and Adams (1955) suggested that low heritabilities of antibody production in response to injections of complex antigens could be expected if the titre recorded was that of the component antibody in highest concentration. Measurement of the titre of individual antibodies or measurements of the total of all antibodies present was necessary for accurate determination of heritability of antibody production.

Two antigens were recommended for further investigation, *Rhizobium meliloti*, containing two antigens, and influenza virus. Influenza virus was suggested as it was believed that the technique of measuring neutralizing antibody to influenza virus would measure one specific antibody only (Fazekas de St. Groth, personal communication).

The use of these antigens together with an improved design of experiment for measurement of heritability (Claringbold, Sobey, and Adams 1957) was expected to give additional useful information about this problem.

II. MATERIALS AND METHODS

(*a*) *Antigens*

Rhizobium meliloti, strain "Sydney University 277/1" and the mouse-adapted strains of the two influenza virus strains MEL and LEE were used in this investigation.

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(b) Serological Techniques

(i) Rhizobium meliloti.—This organism contains a Vi-like and an O antigen. It has been demonstrated (Vincent 1953) that the Vi antigen of *Rhizobium*, unlike that of *Salmonella typhosa*, does not completely inhibit the demonstrable O antigen–antibody reaction when unheated antigen is used in testing, though some inhibition may be experienced. For this reason, the antigen was used unheated for measuring response to the Vi antigen and heated at 100°C for 30 min to destroy the Vi antigen when measuring response to the O antigen.

A stock suspension of the organism was kept at a concentration of 5×10^8 organisms/ml.

Methods of bleeding and separating of serum were as described by Sobey and Adams (1955). Serum was not inactivated before testing. Using a pipette drawn to deliver 30 drops/ml, two drops of serum were delivered serially into precipitin tubes containing two drops of calcium–magnesium saline, thus diluting the serum twofold at each step. One drop of antigen was added to each tube, the tubes then incubated in a water-bath at 37°C for 4 hr, left at room temperature overnight, and the reaction recorded the following morning. Readings were made in a specially constructed and illuminated black box with the aid of a hand lens.

The end-point was found to be clear cut. The relative agglutination titre of a serum was recorded on a log scale simply by taking tube number as the titre values. Titres of antibodies to the Vi and O antigens were separately measured on each antiserum. In order to choose the best dose of antigen for injection and the best bleeding time, the following factorial experiment was undertaken, measuring the titre of antibodies to the O antigen only. The antigen was given at three levels, 1 ml of 5×10^8, 5×10^7, and 5×10^6 organisms/ml, in two intravenous injections of 0·5 ml, each spaced by 4 days. The mice were bled $3\frac{1}{2}$, 7, $10\frac{1}{2}$, 14, and $17\frac{1}{2}$ days after the last injection. All the mice used came from a randomly bred albino stock maintained in this Laboratory. Mice were housed four to a box and supplied mouse cubes and water *ad lib*. Four mice were allocated at random to each treatment group. This gave a 3×5 factorial with four mice per treatment group, making a total of 60 mice. The design, results, and the analysis of variance are given in Table 1.

Antibody titre was seen to increase log-linearly with dose. Higher titres were recorded at the bleeding time of 7 days and with the dose of 5×10^8 organisms/ml.

(ii) *Influenza Virus Strain MEL and LEE.*—Methods of bleeding and separating of serum were as described in Sobey and Adams (1955).

Antisera produced in reponse to injections of both strains of virus were measured for antihaemagglutinin and neutralizing antibody.

Measurement of antihaemagglutinin was done in plastic trays. After inactivation and destruction of non-specific inhibitions by R.D.E–citrate–65°C treatment (Fazekas de St. Groth 1949), twofold dilutions were made up with Takatsy loops in 0·25 ml saline. A standard drop (0·025 ml) of 5% fowl cells was then added, and finally a drop containing exactly 4 agglutinating doses of the test virus. The trays were shaken, and the pattern of cells read after half an hour at room temperature.

Relative titres were expressed as the log of the antihaemagglutinin titre by taking well numbers from the plastic trays as titre values.

Neutralization tests were carried out in plastic trays using the method described by Fazekas de St. Groth, Withell, and Lafferty (1958). Titres were expressed as the "mean neutralizing potency" or pN value, a score derived by Fazekas de St. Groth (1961).

TABLE 1

DESIGN, RESULTS, AND ANALYSIS OF VARIANCE OF THE RHIZOBIUM DOSE–TIME RESPONSE EXPERIMENT

Bleeding Times (days after last injection)	Dose of *Rh. meliloti* ($2 \times 0{\cdot}5$ ml injections, 4 days between injections)			
	5×10^8/ml	5×10^7/ml	5×10^6/ml	Totals
$3\frac{1}{2}$	7	4	0	
	8	7	0	
	8	2	0	
	1	2	0	
Totals	24	15	0	39
7	10	8	5	
	9	9	4	
	10	7	0	
	9	9	0	
Totals	38	33	9	80
$10\frac{1}{2}$	5	0	5	
	6	0	5	
	8	0	0	
	0	8	3	
Totals	19	8	13	40
14	7	7	0	
	8	2	5	
	2	2	3	
	2	0	0	
Totals	19	11	8	38
$17\frac{1}{2}$	4	4	0	
	10	7	0	
	9	4	0	
	6	0	2	
Totals	29	15	2	46
Grand totals	129	82	32	243

TABLE 1 (*Continued*)

Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square	*F*
Doses	(2)		
Linear	1	235·2	35***
Deviations	1	0·1	1
Bleedings	(4)		
Linear	1	6·5	1
Quadratic	1	4·7	1
Cubic	1	69·0	10**
Quartic	1	25·7	3·8
Interactions	8	10·1	1·4
Error	45	6·7	

** $P<0·1$. *** $P<0·01$.

(*c*) *Design of Heritability Experiments*

(i) Rhizobium meliloti.—128 daughter/dam pairs of adult albino mice weighing about 25 g, obtained from a randomly bred stock maintained in this Laboratory, were used. The dams were housed in 32 boxes, and they and their daughters were prepared as for an earlier experiment (Claringbold, Sobey, and Adams 1957). For convenience, the experiment was divided into four blocks (*BL*). Each block comprised 32 boxes of dams (*D*) and 32 corresponding boxes of daughters (*d*). The mice were given standard mouse cubes and water *ad lib.*

1 ml 5×10^8 organisms/ml was given in two 0·5 ml intravenous injections spaced by 4 days. Mice were bled 7 days after the last injection. The treatment of successive blocks was commenced at weekly intervals but was otherwise identical.

(ii) *Influenza Virus.*—The same animals and design were used for this determination. Mice were injected with each of the two virus preparations simultaneously, 0·25 ml of both MEL and LEE were administered by the intraperitoneal route. Mice were bled 14 days after injection for MEL antibody titration and 18 days after injection for LEE titration. The first two blocks of animals were injected together and the second two blocks were injected 21 days later.

III. RESULTS

Heritabilities were obtained by doubling the correlation of daughter with dam scores. The results of the *Rhizobium* titrations are presented in Table 2. A heritability of —0·012 for Vi and 0·78 for O antigen was obtained.

The results of the influenza virus titrations by both antihaemagglutinin and neutralizing potency tests are presented in Table 3. Heritabilities of 0·60 (0·43–0·74) by antihaemagglutinin and 0·16 (0–1·0) by the neutralizing potency test were obtained for MEL virus, and heritabilities of 0·30 (0·06–0·51) by antihaemagglutinin and 0·10 (0–1·0) by the neutralizing potency test were obtained for LEE virus.

IV. Discussion

Methods of measuring antibody response are probably open to limitations where more than one antibody is concerned. By serial dilution of antiserum, antibodies with low titres will become too low in concentration to contribute to the reaction, and the end-point giving the measure of response will be determined by the

Table 2

VARIANCE AND COVARIANCE FOR THE RHIZOBIUM TITRATIONS

Antigen	Variance Dam	Variance Daughter	Covariance	r	h^2	95% Limits
Vi	222·43	363·56	−17·76	−0·006	−0·012	—
O	407·74	329·47	138·88	0·39	0·78	0·13–0·90

titre of the antibody which is in greatest concentration. It is unlikely that the different antibody titres are additive in respect of the end-point, the consequence being that in no instance would total antibody response be measured; the measure would be of antibody in highest concentration. This has been shown to be true of the A, B, and O human antigens (Sobey and Adams 1955). Since the same antibody would not necessarily be present in greatest concentration in all individuals, varia-

Table 3

VARIANCE AND COVARIANCE FOR THE INFLUENZA VIRUS TITRATIONS

Virus Strain	Test*	Variance Dam	Variance Daughter	Co-variance	r	h^2	95% Limits
MEL	N	1531	297	56	0·08	0·16	0–1
MEL	A	7603	7935	2335	0·30	0·60	0·43–0·74
LEE	N	1280	1555	75	0·05	0·10	0–1
LEE	A	7130	4399	823	0·15	0·30	0·06–0·51

* N = neutralizing potency test; A = antihaemagglutinin test.

tion observed would not be variation of a single specific antibody and this would result in an apparent lack of genetic variability. For example, when testing for response to Vi of *Rhizobium*, both the Vi and O antigens are present, and heritability is low, as demonstrated from Table 2; this is not surprising and is in accord with previous arguments. When the Vi antigen is destroyed by heating, the only measurable response is that to the O antigen and here the heritability of response is high, $h^2 = 0 \cdot 78$, and is close to that found for tobacco mosaic virus by Sang and Sobey (1954), where apparently a single antibody response is measured.

From the influenza virus data in Table 3, low heritabilities are recorded for both strains of virus as measured by the neutralizing potency test, whilst both strains give a higher heritability as determined by antihaemagglutinin titration. If the neutralizing potency test did measure only a single response, as then believed, then, on the assumption of a high degree of genetic control of individual antibody responses, no valid explanation could be put forward to support the low heritability obtained. Further work by Lafferty on this problem showed that the neutralization test measured not a single antibody but a complex of antibodies (Lafferty, unpublished data).

It has been shown by Claringbold (unpublished data, 1961), using the above assumptions in Monte Carlo runs in the electronic digital computer SILLIAC, that with increasing numbers of antigenic components, each with a heritability of 100% and no correlation, the measured heritability decreases such that with 10 antigens the heritability is 50%. Further, where one antigen is included in the model with a low heritability the measured heritability approaches this value regardless of the heritability values attributed to the other antigenic components in the model. These results from the computer do not include any environmental variation, which in actual observations would further lower heritability.

It is suggested that these findings support previous arguments concerning the complexities of antigens and responses to them, and that still more studies on the inheritance of antibody response are required using purified antigens which will elicit a single antibody response or using some means to identify and measure an individual response from within a complex response pattern.

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