INHERITANCE OF ANTIBODY RESPONSE

III. HERITABILITY OF RESPONSE TO SHEEP RED CELLS

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Summary

The heritability of antibody response to a priming dose of sheep red cells is 41·8 ± 19·4 per cent. and to a secondary dose −7·4 ± 20·7 per cent.

With this antigen it would appear that the factors governing response to a priming dose are considerably different from those governing response to a secondary dose.

It is stressed that adoption of a block replicated experimental design with this experimental material results in considerable gains in precision of experimentation.

I. INTRODUCTION

In the first paper of this series (Sobey and Adams 1955) an estimate of heritability (h^2) of response to sheep red cells (S.R.C.) was obtained using the well-known technique of mid-parent offspring regression. In obtaining this estimate 228 parents and 456 offspring were tested. At that time it was not known that considerable secular variation in response occurred, and as a result the estimate suffered a large sampling error. One purpose of this paper is to study a different approach to the estimation of h^2 using an experimental design which enables the elimination of the secular component, and also the study of other causes of variation.

II. MATERIALS AND METHODS

(*a*) *Serological Technique*

Methods of preparing red cells for injection, collection of immune serum and complement, and the routine of testing immune serum have been described in a previous paper (Sobey and Adams 1955).

(*b*) *Preparation of Animals*

In all, 256 female albino mice were used in this investigation; of these, 64 were dams obtained from a line of mice selected for increased sensitivity to oestrogens for six generations (Biggers and Claringbold 1955), and another 64 were dams obtained from a line selected for decreased sensitivity. The remaining 128 mice were daughters of these dams (one daughter per dam) by sires from the two selection lines. The basic unit in the experimental design is a daughter-dam pair of animals.

The mice were housed in 64 boxes, four mice per box, and were supplied standard mouse cubes and water *ad lib*. The dams of each line occupied 16 boxes with

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four dams selected at random in each box. The boxes were serially numbered. Individual dams were identified both by an ear clip and a colour code. Thus each dam is identified by her box number and her ear clip or colour. The daughter corresponding to a particular dam was put into a box similarly numbered, and received the same ear clip and colour coding as her dam. To distinguish boxes of dams from those of daughters, the box card colour was varied and dams and daughters were clipped in opposite ears. Subsequent manipulations were carried out with daughter-dam box pairs as units, boxes of daughters being treated in parallel with boxes of dams.

(c) *Experimental Design*

Since it was impossible to determine the antibody responses of the 256 animals simultaneously the experiment was carried out in four blocks each of 32 dams and their corresponding daughters, i.e. of 32 experimental units. The treatment of the first block will be described in detail. The remaining blocks are simply replicates of this, the treatment of successive blocks being started at weekly intervals.

TABLE 1

SCHEMATIC LAYOUT OF THE EXPERIMENTAL TREATMENT OF ONE BLOCK

	High-selection Line				Low-selection Line			
	Intravenous Route		Intraperitoneal Route		Intravenous Route		Intraperitoneal Route	
Dose (ml)	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Dose (%)	0·1	0·01	0·1	0·01	0·1	0·01	0·1	0·01
No. of units	4	4	4	4	4	4	4	4

Each block of 32 experimental units was made up of 16 units from each oestrogen-selection line. Of each 16 units, eight were injected with S.R.C. by the intravenous and eight by the intraperitoneal route. Two levels of dose were given to each subgroup of eight units, four units receiving 0·5 ml of a 0·1 per cent. suspension of S.R.C. and four a 0·01 per cent. suspension. The layout of the experimental treatment of one block is shown in Table 1.

The antibody responses of the animals were measured 6 days after the injection of the antigen using the standard technique.

The experiment for the study of primary response is thus a 4×2^3 factorial experiment with four experimental units per treatment combination. Two dependent variables were measured in each experimental unit, namely the responses of the dams and of the daughters. The body weight of all animals was measured to the nearest half gram at the time of testing as an additional concomitant variable. Thus a correlation between response of daughter and dam could be calculated independent of the effect on response of line, dose, route of injection, weight, and time of testing.

Four weeks after receiving the primary dose of antigen, a second injection of S.R.C. was given. The four experimental units within each treatment combination described above and shown schematically in Figure 1 were allocated to an additional four treatment combinations. Two of the units were injected by the intraperitoneal route and two by the intravenous route, a high dose and a low dose being given to one unit by each route. Six days later the secondary response of the animals was determined.

The experimental design for secondary responses is thus a 4×2^5 factorial experiment with one experimental unit per treatment combination. The factors are blocks (*Bl*), lines (*L*), primary injection route (*Rp*), primary dose (*Dp*), secondary injection route (*Rs*), and secondary dose (*Ds*). Since all animals were mature at

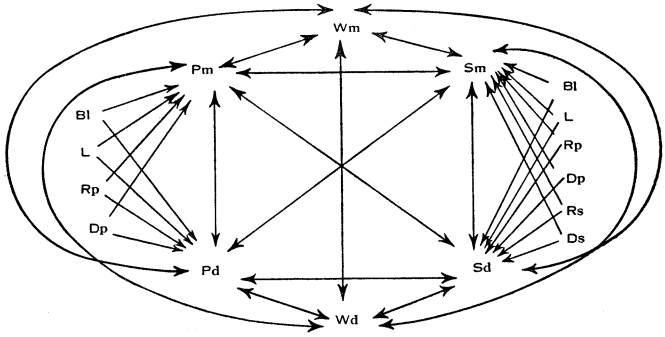

Fig. 1.—Diagrammatic representation of the complete experiment in which double-headed arrows indicate possible correlations in the experimental material. The computed correlations are given in Table 1.

the time of testing, body weight (*Wm* for dams, *Wd* for daughters) was only determined once during the course of the experiment. The dependent variables are four in number: primary response of dams (*Pm*), primary response of daughters (*Pd*), secondary response of dams (*Sm*), and secondary response of daughters (*Sd*).

III. Results

The statistical analysis of the data falls into two parts: (1) the effect of the independent variables (treatments and body weight) on both primary and secondary response, and (2) the estimation of genetic components of variation (h^2) for primary and secondary response, and the genetic covariances between primary and secondary responses. The complete experiment is represented diagrammatically in Figure 1 in which the double-headed arrows indicate possible correlations in the experimental material. Possible effects of the independent variables are represented by single-headed arrows. A standard analysis of variance and covariance (Fisher 1954) is made of the body weights and primary responses of the daughters and dams. The error term of this analysis enables estimates to be made of the correlations between these variables (see Table 2). Since both dam and daughter have been placed in the same experimental unit, the analysis of variance cannot be directly extended to

study any differences in the mean response of daughters compared with dams or any differential response of them to the treatments. The sum of the responses of the dams and daughters in each experimental unit is a measure of the joint response of both individuals while the difference between them should be zero (on the average) if the animals are responding similarly to treatment. The variance of a sum or a difference may be directly computed for the variances and covariances of this variable (cf. Rao 1952, p. 241), thus enabling the analyses to be completed.

TABLE 2

CORRELATIONS WITHIN THE EXPERIMENTAL MATERIAL

For definition of symbols, see text

Correlation Coefficient	*Sm*	*Sd*	*Pm*	*Pd*	*Wm*	*Wd*	
r_{93}	1	−0·037 1	0·087 0·179	−0·018 0·176	0·040 0·128	0·094 −0·031	*Sm* *Sd*
r_{108}			1	0·209* 1	0·065 0·101 1	−0·313** −0·256** 0·018 1	*Pm* *Pd* *Wm* *Wd*

* $0{\cdot}05>P>0{\cdot}01$. ** $0{\cdot}01>P>0{\cdot}001$.

The analysis of variance of the sum of the daughter and dam primary responses indicates large block differences, thus confirming previous work on which the experiment was designed. Both daughter and dam responses are very strongly dependent on dose, and significance of the daughter-dam difference mean square shows the dose-response slope to be a function of age, since the daughters and dams differ alone in this respect. While the average effect of route of injection is not significant, the highly significant interaction of this variable with lines demonstrates that the intraperitoneal route led to higher response in the high-sensitivity line and a lower response in the low-sensitivity line, whereas the lines do not differ with intravenous route of administration. The remaining significant item in the analysis of variance table (Table 3) is the interaction of blocks with lines for the daughter-dam difference. This finding is difficult to explain and may be due either to non-additivity of the responses over blocks (although if this were serious other interactions should be significant) or chance. Having removed variance and covariance due to all known sources of variation the remainder in the "error" term may be used to calculate heritability.

From Table 3 it is apparent ($\chi^2_{(L)}=0{\cdot}004$, $0{\cdot}05<P<0{\cdot}1$) that the variances of the primary response of daughters and dams are not significantly different. In this case the correlation coefficient gives an estimate of heritability $h^2(\%)=200r$. Therefore the heritability of primary response is $41{\cdot}8\pm19{\cdot}4$ per cent. where the standard error follows the $\pm$ sign.

The analysis of variance of the secondary responses follows the same pattern as that of the primary responses. Further correlation coefficients may be determined,

however, since both secondary and primary response have been determined in the same individuals. These correlation coefficients complete Table 2. Since none of

TABLE 3

ANALYSIS OF VARIANCE AND COVARIANCE OF PRIMARY RESPONSES CORRECTED FOR BODY WEIGHT

For definition of symbols, see text

Source of Variation	D.F.	*PmPm*	*PmPd*	*PdPd*	$(Pm+Pd)^2$	$(Pm-Pd)^2$
Bl	3	23,802	15,237	10,145	64,421***	3,473
L	1	29	−222	1,672	1,257	2,145
Dp	1	44,258	31,294	22,127	128,973***	3,797*
Rp	1	60	−139	316	98	654
Bl × *L*	3	6,142	−919	140	4,444	8,120*
Bl × *Dp*	3	4,438	−796	747	3,593	6,777
Bl × *Rp*	3	2,238	−148	640	2,582	3,174
L × *Dp*	1	3	33	439	508	376
L × *Rp*	1	1,648	3,181	6,139	14,149***	1,425
Dp × *Rp*	1	606	804	1,068	3,282	66
Error	107	51,046	7,571	50,362	116,500	86,266

* $0{\cdot}05>P>0{\cdot}01$. ** $0{\cdot}01>P>0{\cdot}001$. *** $P<0{\cdot}001$.

the additional coefficients appear significant, little will be gained by correction of secondary responses for this concomitant variation in the analysis of variance (see

TABLE 4

ANALYSIS OF VARIANCE AND COVARIANCE OF THE SECONDARY RESPONSES

The grouped interactions were tested separately and none found significant. For definition of symbols, see text

Source of Variation	D.F.	*SmSm*	*SmSd*	*SdSd*	$(Sm+Sd)^2$	$(Sm-Sd)^2$
Bl	3	4,340	6,630	13,677	31,277***	4,757***
L	1	1,041	1,540	2,278	6,399**	239
Dp	1	1,333	2,220	3,698	9,471***	591
Rp	1	3,190	4,443	6,188	18,264***	492
Ds	1	5,113	3,274	2,096	13,757***	661
Rs	1	410	455	504	1,824	4
Bl × *L*	3	1,576	1,490	1,739	6,295*	335
Bl × *Dp*	3	1,638	1,345	1,155	5,483*	103
Bl × *Rp*	3	1,073	−986	6,057	5,158*	9,102**
Bl × *Ds*	3	3,515	−1,764	1,096	1,083	8,139**
Bl × *Rs*	3	759	648	1,146	3,201	609
Other first-order interactions	10	2,678	−42	2,994	5,588	5,756
Error	94	22,668	−978	31,166	51,878	55,790

* $0{\cdot}05>P>0{\cdot}01$. ** $0{\cdot}01>P>0{\cdot}001$. *** $P<0{\cdot}001$.

Table 4), which is presented in the same form as that for primary responses. Significant time trends occurred during the course of the experiment as evinced by the

block effect. That these trends were not constant over both dams and daughters is shown by significance of the block effect in the daughter-dam difference analysis. Further time trends of a more complex nature are seen in the interaction terms of the analysis of variance. This indicates that the various treatment effects were not of the same magnitude over the whole experiment.

It is clear that the variances of the secondary responses of the dams and daughters are not significantly different ($\chi^2_{(L)}=2{\cdot}63$, $0{\cdot}1<P<0{\cdot}2$). Comparison of the mean variances of the primary responses with that of the secondary responses indicates that the latter are considerably less variable than the former ($\chi^2_{(L)}=12{\cdot}77$, $P<0{\cdot}001$). As above, we may estimate the heritability of secondary response from the correlation coefficient given in Table 2, and $h^2=-7{\cdot}4\pm20{\cdot}7$ per cent. based on figures in the "error" term.

The correlation between the primary response of dams and the secondary response of daughter or vice versa is a measure of the *genetic determination* of secondary response by primary response. The correlation between primary and secondary response in the same individual obviously includes environmental factors as well as genetic. It is obvious, however, that none of these correlations are of any significance in our material (Table 2).

IV. Discussion

This paper illustrates a convenient way of obtaining an estimate of heritability while at the same time examining other causes of variation. The accuracy of the method in a given situation is determined by the number of experimental units which are employed. A number of estimates obtained in a series of experiments of this kind may be combined to give estimates of greater precision. Estimates of heritability from controlled experiments must be interpreted cautiously since the value obtained is to some extent under the control of the experimenter. Heritability is defined as the percentage of the total variance of a character taken up by the additive genetic variance. By means of planned experimentation certain components of variance such as the effect of time, age, dose, route of administration, and additive genetic variance, etc. can be separately determined. Depending on the definition of total variance, different estimates of heritability are obtained, indicating the arbitrary nature of this concept. In comparing different estimates care must therefore be taken that estimates are obtained in similar circumstances. The estimates of heritability given above are maximal or approaching this since all known components have been removed from the total variance leaving only experimental error and additive genetic variance. It would be expected, therefore, that in the field, under less controlled conditions, the heritability of response to this complex antigen will be very small. (From Table 3 additive genetic variance is $[4\times(7571)^2]/51046=4492$ which is comparable with other mean squares for the primary responses of the mothers or daughters.) Under the less-controlled conditions of the first paper of this series the daughter-dam correlation of primary response ($h^2=11{\cdot}0\pm11{\cdot}7$ per cent.) was not found significant for this reason, although roughly three times the number of animals were used in the determination.

The various block differences found with both primary and secondary response are indicative of secular changes in both the level of response and in the magnitude of differences induced by some treatments. Findings of this nature are common in other biological material, for example the secular changes in median effective dose and dose-response line slope reported by Biggers (1953) with reference to the Allen-Doisy oestrogen-assay technique. The findings emphasize the need for all comparisons to be made at the same time, and, if an experiment is so large that this is impracticable, an appropriate block replicated experimental design used.

With primary response (Table 3) there is no difference between the lines in overall response, but examination of the route-line interaction shows that while both lines react equally to intravenous injection, with intraperitoneal injection the high oestrogen-selection line gives a higher response than the low oestrogen-selection line. Whether this effect is due to genetic correlation of primary response with oestrogen-sensitivity or due to genetic drift cannot be ascertained from the present data. Irrespective of route of administration there is a significant difference between the lines in their secondary response, with the low oestrogen-sensitivity line having a higher response than the other line. Taken together with the absence of significant correlation between primary response and secondary response this difference between the responses strengthens the view that the factors governing the response of an animal to a priming dose of a complex antigen are considerably different from those governing response to a subsequent encounter with that antigen. Further evidence in support of this view is that the variability of primary responses is about twice that of secondary responses.

It was found that the route of injection of the priming dose affected the response to a secondary dose of antigen in that those primed by the intravenous route responded more to a secondary dose than those primed by the intraperitoneal route, suggesting that this antigen when administered intravenously impresses itself more permanently, or strongly, upon the antibody-forming mechanism than when administered intraperitoneally.

V. Acknowledgments

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VI. References

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