STUDIES IN ANAPHYLAXIS

III. RE-EXAMINATION OF SCORES FOR ANAPHYLAXIS USING FOUR INBRED LINES OF MICE

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[*Manuscript received January* 13, 1958]

Summary

Two significant scores were calculated from anaphylactic tests in four inbred lines of mice. The dominant score proved to be highly correlated ($r = 0{\cdot}966$) with the score derived in Part I of this series (Claringbold and Sobey 1957). The implications of the second significant score are discussed.

Differences between inbred lines with respect to anaphylactic score were found and the significance of this is discussed.

Female mice were found to be more susceptible to anaphylaxis than male mice.

I. Introduction

In Part I of this series (Claringbold and Sobey 1957), scores were calculated for different symptoms of anaphylaxis. These scores maximized the dependence of response on log-linear increment of both sensitizing and shocking doses and enabled a quantitative study of anaphylactic shock to be made. The scores were calculated on a limited amount of data, 64 random-bred mice, and may well have limited application. For any given population a score may depend on such factors as sex, genetic structure, and the type of antigen used in testing it. The present work was undertaken to examine the effect of these factors in a more detailed study.

II. Experimental Methods and Results

(*a*) *Mice*

Equal numbers of male and female mice from the four inbred lines A_{Fa}, $C57_{Fa}$, $C3H_{Fa}$, and CBA_{Fa} were used in the experiment. The mice were aged from 12 to 20 weeks, housed four per box, and given standard mouse cubes and water *ad lib.*

(*b*) *Antigen*

Bovine gamma-globulin (Armour fraction II) was used as an antigen throughout. The shocking dose, bovine gamma-globulin in 0·5 ml saline, was administered intravenously 14 days after a single, intramuscular, sensitizing injection of alum-precipitated antigen.

(*c*) *Experimental*

The experiment was of the $4^3 \times 2$ factorial type (Cochran and Cox 1957) with two mice per treatment group (i.e. 256 mice were used in the experiment). Both sensitizing and shocking dose were given at four different levels in each of the four inbred lines and two sexes. The symptoms scored are those described in Parts I and II (Sobey and Adams 1957) and are tabulated below together with the estimated scores for the observed symptoms.

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Designation	*Scores*			*Description*
	*1**	*1*	*2*	
0	0	0	0	No effect.
x	29	37	−30	Animal exhibits vigorous scratching of ears and face and nibbling of anal region.
y	38	44	−46	Animal displays absence of any desire to move, huddles in a corner, with laboured breathing and infrequent convulsive movements. The animal will move when stimulated.
z	59	56	− 7	Paralysis, partial or complete. The animal does not move when stimulated, or moves slowly, dragging its hindquarters.
a	65	60	− 1	Death.

Here *1** is the score estimated in Part I, *1* is the dominant score, and *2* is the second score. The results of the experiment are shown in terms of the symptoms in Table 1, and in terms of the scores in Table 2.

III. Statistical Analysis

The experimental data obtained are more extensive than those obtained in Part I, and manual calculation of the preliminary analysis of variance in terms of the unknowns x, y, z, and a is very time-consuming. For the purposes of calculation, covariance analysis may be used where the observations are scored in terms of four formal variates (x_1–x_4), thus:

	x_1	x_2	x_3	x_4
0	0	0	0	0
x	0·5	0	0	0
y	0	0·5	0	0
z	0	0	0·5	0
a	0	0	0	0·5

giving the matrices discussed in Part I. In this way it was possible to use a SILLIAC programme to carry out the calculations (Claringbold, unpublished data). The sums of squares and products of the observations were partitioned into between- and within-group components, and the between-group component was further broken down into main effects and first-order interactions, leaving higher-order interactions as a residual term.

Matrix

Main effects = **M**

	X_1	X_2	X_3	X_4	D.F.
X_1	0·657				10
X_2	0·168	1·374			
X_3	−0·618	−1·011	2·206		
X_4	−0·226	−0·243	0·672	0·399	

Within group = **W**

$$\begin{array}{c} \\ X_1 \\ X_2 \\ X_3 \\ X_4 \end{array} \begin{array}{c} \begin{array}{cccc} X_1 & X_2 & X_3 & X_4 \end{array} \\ \begin{bmatrix} 3{\cdot}500 & & & \\ -0{\cdot}875 & 4{\cdot}000 & & \\ -1{\cdot}500 & -2{\cdot}375 & 6{\cdot}125 & \\ -0{\cdot}375 & -0{\cdot}250 & -1{\cdot}500 & 2{\cdot}500 \end{bmatrix} \end{array} \quad \begin{array}{c} \text{D.F.} \\ 128 \end{array}$$

TABLE 1

FREQUENCY OF THE SYMPTOMS OBSERVED IN THE EXPERIMENTAL GROUPS

Line	Sensitizing Dose (mg)	Female				Male			
		Shocking Dose (mg):				Shocking Dose (mg):			
		20	5	1·25	0·31	20	5	1·25	0·31
A_{Fa}	4	$x+a$	$x+z$	$y+z$	$x+y$	$2y$	$x+z$	$x+y$	y
	1	$y+z$	$2x$	$2y$	x	$x+z$	$x+a$	$2x$	$2x$
	0·25	$x+z$	$2a$	$y+z$	$2y$	$2y$	$2z$	$x+y$	$2x$
	0·06	$y+z$	$2y$	$2y$	$2y$	$x+y$	y	$2y$	0
$C3H_{Fa}$	4	$y+z$	$z+a$	$x+z$	$y+z$	$y+z$	$2x$	a	a
	1	$2y$	$2x$	$x+y$	$2y$	$y+z$	$y+z$	a	$z+a$
	0·25	$2y$	$2z$	$x+z$	$y+z$	$2y$	z	z	0
	0·06	$x+a$	z	$x+z$	0	$2y$	$2y$	x	0
$C57_{Fa}$	4	$z+a$	$2z$	$y+a$	$2y$	$2y$	$x+z$	$2y$	x
	1	$2z$	$x+z$	$x+y$	$2y$	$2y$	$x+z$	$y+z$	0
	0·25	$2z$	$2z$	$y+z$	z	$y+z$	z	$2x$	x
	0·06	$x+y$	y	$x+z$	0	$2y$	x	$x+z$	0
CBA_{Fa}	4	$z+a$	$z+a$	$z+a$	$2z$	$y+z$	$2y$	$z+a$	$y+z$
	1	$z+a$	$x+a$	$z+a$	$2z$	$y+z$	y	$y+z$	0
	0·25	$y+z$	$z+a$	$2z$	$2z$	$y+z$	$z+a$	$2y$	$2y$
	0·06	$y+z$	x	z	0	$2y$	$2z$	$2x$	$2y$

The calculation of scores ($\mathbf{b}^T$) requires solution of the matrix equation

$$\mathbf{b}^T [\mathbf{M}-\theta\mathbf{T}] = 0,$$

where $\mathbf{T} = \mathbf{M}+\mathbf{W}$, $\mathbf{b}^T$ is a vector of estimates, T indicating transposition, and θ is a root of the determinantal equation (Rao 1952)

$$|\mathbf{M}-\theta\mathbf{T}| = 0,$$

which is obtained using a SILLIAC programme.

The roots and their test of significance are given in Table 3. The estimated scores are shown above, the first column giving those obtained in Part I. The score corresponding with the dominant root of the present investigation (θ_1) is given in the second column and is clearly similar to the first estimate.

Since the variance of any score is obtained using the within-group matrix, thus:

$$\mathbf{b}^T \quad \mathbf{W} \quad \mathbf{b}/w,$$

and the covariance between a pair of scores (**b** and **c**) may be obtained in the analogous fashion,

$$\mathbf{b}^T \quad \mathbf{W} \quad \mathbf{c}/w,$$

where w is the within-group degrees of freedom, a correlation coefficient between

TABLE 2

EXPERIMENTAL DATA IN TERMS OF THE DOMINANT SCORE

Components of each score as in Table 1

Line	Sensitizing Dose (mg)	Female				Male				Totals
		Shocking Dose (mg)				Shocking Dose (mg)				
		20	5	1·25	0·31	20	5	1·25	0·31	
A_{Fa}	4	97	93	100	81	88	93	81	44	
	1	100	112	88	37	93	116	74	74	
	0·25	93	120	100	88	88	112	81	74	
	0·06	100	88	88	88	81	44	88	0	2704
$C3H_{Fa}$	4	100	116	93	100	100	112	60	60	
	1	88	112	81	88	104	100	60	116	
	0·25	88	112	93	100	88	56	56	0	
	0·06	97	56	93	0	88	88	37	0	2542
$C57_{Fa}$	4	116	112	104	88	88	93	88	37	
	1	112	93	81	88	88	93	100	0	
	0·25	112	112	100	56	100	56	74	37	
	0·06	81	44	93	0	81	37	93	0	2457
CBA_{Fa}	4	116	116	116	112	100	88	116	100	
	1	116	97	116	112	100	44	100	0	
	0·25	100	116	112	112	100	116	88	88	
	0·06	100	37	56	0	88	112	74	88	2936
Totals		1616	1537	1514	1147	1475	1360	1270	718	10,637
		5814				4823				

scores may then be calculated in the usual way. The correlation coefficient between the scores *1** and *1* is 0·966, which clearly demonstrates the equivalence of these scores.

A second latent root (θ_2) is also found to be highly significant (Table 3) and the score corresponding to this root is given in the column designated *2* above. This score has zero correlation with the dominant score, a property of the method of

TABLE 3

LATENT ROOTS AND THEIR TEST OF SIGNIFICANCE

Latent Root	Degrees of Freedom	χ^2	P
$\theta_1 = 0{\cdot}5085$	13	92·5	<0·001
$\theta_2 = 0{\cdot}3011$	11	46·7	<0·001
$\theta_3 = 0{\cdot}1291$, $\theta_4 = 0{\cdot}0405$	16	23·4	0·2–0·1

estimation. Information supplied by this score may be considered independently from that supplied by the dominant score. It is seen that symptoms x and y are the only ones scoring highly in the second variate. This indicates that irritation

TABLE 4

ANALYSIS OF VARIANCE OF THE DATA OF TABLE 1 USING THE SCORE ESTIMATED IN PART I (*1**), THE DOMINANT SCORE (*1*), AND THE SECOND SCORE (*2*)

Source of Variation	Degrees of Freedom	Mean Square		
		*1**	*1*	*2*
Sex	1	565***	380***	293*
Lines	3	105**	68*	419*
Shocking dose	(3)			
Linear	1	1171***	1129***	440*
Quadratic	1	295**	199**	447*
Cubic	1	7	62	1557***
Sensitizing dose	(3)			
Linear	1	840***	656***	58
Quadratic	1	172*	130*	50
Cubic	1	139*	97*	29
Sex × lines	3	58	11	572***
Sex × shocking dose	3	12	27	464**
Sex × sensitizing dose	3	54	43	147
Lines × shocking dose	9	42	37	191
Lines × sensitizing dose	9	31	28	134
Shocking × sensitizing dose	9	37	39	183
Residual error	47	29·3	25·7	113·3
Within-group error	128	27·5	21·6	74·7

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

of the mucous membranes and the discomfort symptoms are controlled by some factors which are independent of the train of symptoms leading to death.

In Table 4 an analysis of variance is made of the data of Table 1 using the three scores. The first two columns of this table are very similar, a reflection of the high degree of correlation between the scores used. Both sexes and lines respond

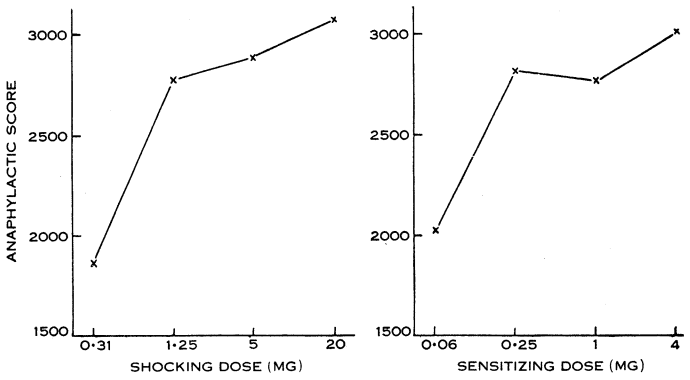

Fig. 1.—Dose response lines for shocking and sensitizing doses plotted in terms of anaphylactic score, using the dominant score.

differently to the anaphylactic reaction, females being more sensitive than males. Significant departures from linearity of response of both shocking and sensitizing dose were found. The shocking dose–response line is apparently the right-hand side

TABLE 5

ANALYSIS OF VARIANCE OF THE DATA OF PART I USING THE SECOND SCORE (*2*)

Source of Variation	Degrees of Freedom	Mean Square
Shocking dose	(3)	
Linear	1	517*
Quadratic	1	81
Cubic	1	14
Sensitizing dose	3	98
Interaction	9	264
Error	48	113

*$P<0{\cdot}05$.

of a sigmoid curve since the quadratic component is significant. A similar relationship of response to log-sensitizing dose was found (see Fig. 1). Since no first-order interactions were significant, these findings are consistent over lines and sexes.

Column 3 of Table 4 gives the analysis of variance of the data of Table 1 in terms of the second score. Sex and line differences are also observed with this variate, and the sex difference is not constant over lines, as evinced by the interaction

between the factors. This score has no dependence on sensitizing dose but marked dependence on shocking dose. The form of this dependence varies between the sexes.

Since only one score was found significant in Part I, the data were re-analysed using the second score (Table 5). Bearing in mind that a different antigen is being studied, a dependence (albeit linear) of response on the shocking dose is again found. Presumably the amount of information in the first experiment (64 observations) was insufficient to detect significant variation in other than the dominant score.

IV. Discussion

The score determined in Part I and the dominant score from the present work are very highly correlated. For practical purposes, as demonstrated by comparing the first two columns in Table 4, they are interchangeable, and can be used regardless of sex or genotype. They are also valid for the two different antigens used, namely bovine plasma albumin and bovine gamma-globulin.

A second significant score, independent of the dominant score, and accentuating the values of x and y was found. The secondary nature of certain symptoms in anaphylaxis in dogs is reported by Dragstedt (1941). The symptoms observed were dyspnoea, vomiting, salivation, general weakness, diarrhoea, and a marked fall in blood pressure which accompanied and paralleled in degree the severity of the other symptoms. Anaesthesia prevented the vomiting, diarrhoea, etc., but did not prevent the fall in blood pressure, from which it was concluded that these reactions were probably a sequel to and less important than the vascular reaction. The second score may be of some value in physiological studies of anaphylaxis.

The shocking dose for bovine gamma-globulin is approaching a maximum at 20 mg, whereas with bovine plasma albumin a comparable value is 0·05 mg, indicating that different antigens may be expected to have different optimal shocking doses. Further work is being undertaken to examine this aspect of anaphylaxis.

Sobey and Adams (1957) have defined the score conferred on an animal after anaphylactic shock as the anaphylactic score. In so far as this score is dependent upon the state of the animal prior to the shocking dose, it will depend upon the extent to which the animal has been primed (by the injection of antibody or the sensitizing dose of antigen) and its innate susceptibility. This state was defined as the animal's anaphylactic potential. Different animals having the same level of available antibody may have different anaphylactic potentials because of their individual innate susceptibility to the whole anaphylactic process. This innate susceptibility was defined as the animal's anaphylactic sensitivity. If these terms are accepted, then differences in anaphylactic score between the inbred lines found here, and those reported by Fink and Rothlauf (1954) to egg albumin, indicate genetic variability in anaphylactic potential. Anaphylactic potential is probably the outcome of an interaction between the ability to produce antibodies and anaphylactic sensitivity. Since each of these may be under separate genetic control, line difference *per se* do not allow us to distinguish between them.

Females were found to be more susceptible to anaphylaxis than males. It is tempting to associate this with the greater antibody producing ability of females (Gorer and Schutze 1938; Sobey and Adams 1955) but the explanation is unlikely to be as simple as this since Pittman (1951) has shown females to be more sensitive than males to histamine shock, and the part played by histamine in anaphylaxis is still controversial.

V. Acknowledgments

We are indebted to Dr. B. F. Short and Mr. K. M. Adams for their assistance in carrying out the experimental work.

VI. References

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