THE PATTERN, SENSITIVITY AND PRECISION OF THE RESPONSE TO INSULIN IN RANDOM BRED, INBRED AND HYBRID STRAINS OF MICE

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The study of the pattern of the response to insulin, its sensitivity and precision in hybrid, random bred and inbred mice has confirmed that while the qualitative and quantitative differences in response for each strain are specific and must be determined empirically, there is considerable general correlation in this species of the average weight of the strain of mouse and the sensitivity and precision of its response.

THE British Pharmacopoeia of 1932 introduced as an official test for the assay of insulin, the convulsive responses in mice previously deprived of food. Such responses were therefore considered a suitable tool for the investigation of specific differences between random bred, inbred and hybrid strains of mice.

MATERIAL AND METHODS

The test animals were bred at the Laboratory Animals Centre and issued soon after weaning so that mice aged six weeks ± seven days could be used for the first test. The weight distribution limit of 5 g. imposed in the official routine assay could not be applied to these less readily available strains without severely curtailing the numbers in each group.

TABLE I

MEAN AND STANDARD DEVIATION OF THE AVERAGE OF THE MEAN WEIGHTS OF THE MICE USED IN EACH OF THE FIRST TESTS ANALYSED IN TABLE II

Strain	M	F
LAC grey	23·8 ± 3·2	20·6 ± 2·2
A2G	18·4 ± 2·8	15·7 ± 2·1
C57Br/cd	18·3 ± 1·9	14·5 ± 1·2
CBA	17·5 ± 2·9	14·7 ± 1·8
DBA/1	13·0 ± 1·1	12·1 ± 1·6
A2DB/1F_1	17·8 ± 3·6	14·1 ± 1·5
A2CF_1	18·8 ± 1·6	15·6 ± 1·0
CA2F_1	17·9 ± 0·5	15·8 ± 0·4
A2BrF_1	19·9 ± 2·5	16·6 ± 0·8
BrA2F_1	16·4 ± 1·3	14·4 ± 0·7

Mice from each strain were therefore segregated first by sex and then by weight into groups. The several groups of mice for each strain were randomised into boxes—for any one strain three for males and three for females. Doses of insulin were adjusted so that each mouse received the exact equivalent per kg. mouse of the dose allotted to it, to correct within strain variation in weight. The average of the mean weights of mice used for the first tests and the standard deviation of the average for each strain is given in Table I. Whenever sufficient mice were available three groups

TABLE II

THE COMPARISONS OF DEFINITE AND SEVERE RESPONSES TO DOSES OF INSULIN IN MICE: 1. DIFFERENCES IN PATTERN OF RESPONSE BETWEEN THE SEXES OF EACH STRAIN; 2. DIFFERENCES IN PATTERN OF RESPONSE BETWEEN ALL OTHER STRAINS AND THE CONTROL LAC GREY AT DOSES GIVING A SIMILAR PERCENTAGE OF TOTAL REACTIONS. P = PROBABILITY, D = DIFFERENCE, −P. >05, +P.05, ++P.01, +++P.001

Expt.	Strain	Severe Reaction		Definite Reaction		No Reaction		Percentage reaction	Difference between sex response pattern			Difference between strains response pattern and LAC grey response pattern		
		M	F	M	F	M	F		χ^2	P	D	χ^2	P	D
1	LAC Grey ..	8	19	90	130	125	75	55·2	24·2	<0·001	+++			
	A2G	37	74	59	75	128	82	57·3	24·4	<0·001	+++	72·7	<0·001	+++
2	LAC Grey ..	3	14	31	53	54	26	55·8						
	A2G	22	39	27	27	40	31	52·0	6·1	0·05	+	37·4	<0·001	+++
	DBA/1	24	23	17	21	40	20	58·6	2·0	0·5	−	35·8	<0·001	+++
	A2DB/1F_1 ..	10	15	19	25	41	30	45·4	3·5	0·2	−	13·9	ca. 0·02	++
3	LAC Grey ..	27	41	126	143	90	53	70·2	13·4	ca. 0·001	+++			
	A2CF_1	27	47	40	38	48	25	67·6	12·6	0·01–0·001	+++	40·1	<0·001	+++
	CA2F_1	23	59	42	33	53	26	66·5	26·1	<0·001	+++	56·5	<0·001	+++
	BrA2F_1	51	46	26	34	43	40	65·4	1·4	0·7	−	88·9	<0·001	+++
4	LAC Grey ..	4	5	39	52	50	30	55·6						
	A2BrF_1	16	25	20	22	51	42	53·2	2·9	0·2	−	34·9	<0·001	+++
5	LAC Grey ..	2	1	18	29	30	20	50·0						
	C57Br/cd ..	22	16	8	4	20	27	51·5	3·2	0·05–0·1	−	55·7	<0·001	+++
6	LAC Grey ..	2	7	27	28	21	15	64·0						
	CBA	6	21	16	20	23	7	67·7	17·2	<0·001	+++	15·9	0·01	++

A2DB/1F_1 = A2G (F) × DBA/1 (M) A2CF_1 = A2G (F) × CBA (M) CA2F_1 = CBA (F) × A2G (M) BrA2F_1 = C57Br/cd (F) × A2G (M)
A2BrF_1 = A2G (F) × C57Br/cd (M)

of 10 males and three groups of 10 females, that is 60 mice were used for each strain per test, and in no test was each group less than 8 or the total number of mice less than 48.

One batch of crystalline insulin, kindly supplied by Dr. G. A. Stewart of the Wellcome Chemical Works, Dartford, was used for all tests and about six strains of mice were tested on one day, always including the LAC grey mice as control. All doses of insulin used were in the range 500–2,812 milliunits/kg. with a dose ratio 1 : 1·334. The middle dose was chosen to give a response of about 50 per cent.

The mice were allowed to convulse in a room thermostatically controlled at 20° and illuminated by one central strip light. Glass jars measuring 17 cm. in diameter by 12 cm. in depth were used to house the mice under test and not more than five or less than four mice were put into any one jar. Frank convulsions were recorded as severe and the convulsed mice were injected with 0·5 ml. of 15 per cent glucose and returned to their original container. Convulsions causing hind leg paralysis and general immobility were recorded as definite and the mice remained with the non-reactors in the test jar. This simplified the final recording. Reactions were noted for 2 hr. because the lower temperature used compared with that used for the official test made the interval between injection of insulin and reaction longer.

Results

Differences in Pattern of Response to Injections of Insulin in Random Bred LAC Grey Mice, Inbred and Hybrid Strains

For doses of insulin to which between 50 and 70 per cent of the mice injected gave positive responses in strains LAC grey, A2G, CBA, A2CF, and CA2F, there was a significant difference between the sexes in their pattern of response, the females being the more reactive (see Table II). The hybrid strains $A2CF_1$ and $CA2F_1$ followed the same pattern as their parent strains A2G and CBA. The hybrid strains $A2DB/1F_1$, $A2BrF_1$ and $BrA2F_1$ for which there was no sex difference in the response pattern each had one parent with little or no sex difference, namely DBA/1 or C57Br/cd.

All strains were found to differ significantly from LAC grey mice in the pattern of the quality of their response because this strain had always a higher proportion of definite than severe reactions (see Table II). In other words the LAC grey strain showed qualitatively less response than all others.

Among hybrid strains the pattern of response for the $BrA2F_1$ mice, which were tested at the same time as the hybrid mice $A2CF_1$ and $CA2F_1$ (Table II, experiment 3), was significantly different in quality from these ($P = 0{\cdot}02$ and $P = 0{\cdot}001$). There were more severe reactions than definite reactions, that is, qualitatively more marked response.

Finally for the strains of experiment 2 in Table II, parents with their hybrid, the pattern of response of the hybrid mice $A2DB/1F_1$ was not in these tests found to be significantly different from that of either parent ($P = 0{\cdot}05$–$0{\cdot}1$ for each parent), although differences approach significance.

TABLE III

THE COMPARISON OF THE PARALLELISM OF THE REGRESSION BETWEEN THE CONVULSIVE RESPONSE AND THE DOSE OF INSULIN BY MEANS OF THE χ^2 SUMMATION FOR THE FIRST TESTS ONLY: 1. WITHIN STRAIN COMPARISON FOR ALL FIRST TESTS MADE: 2. COMPARISON BETWEEN LAC GREY MICE, INBRED STRAINS AND HYBRID STRAINS; 3. COMPARISON BETWEEN A2G MICE, DBA/1 MICE AND THEIR HYBRID A2DB/1F:; 4. COMPARISON BETWEEN DBA/1 MICE AND HYBRID A2DB/1F_1. P = PROBABILITY, D = DIFFERENCE

Strain	No. of tests	Approx. ED50 milliunit/kg. mouse	Mean slopes	Within strain comparison		Between LAC grey mice and other strains		Between A2G, DBA/1 and their hybrid mice		Between DBA/1 and A2DB/1F_1	
				P	D	P	D	P	D	P	D
LAC grey	6	1,880	1·018	0·5	–						
A2G	6	953	1·929	0·5	–	0·01–0·001	+++				
LAC grey	3	2,335	0·742	0·3	–						
CBA	3	1,125	0·905	0·1–0·2	–	0·2–0·3	–				
LAC grey	3	1,875	0·904	0·99	–						
A2CF_1	3	1,190	1·815	0·2–0·3	–	0·1–0·2	–				
CA_2F_1	3	1,100	0·985	0·8–0·9	–	ca. 0·99	–				
BrA2F_1	3	885	1·825	0·99	–	ca. 0·05	+				
LAC grey	2	1,965	0·929	0·99	–						
A2BrF_1	2	1,110	1·625	0·2–0·3	–	0·7–0·8	–				
LAC grey	2	1,845	1·280	0·8–0·9	–						
A2G	2	885	1·962	0·1–0·2	–	0·1–0·2	–				
DBA/1	2	875	2·842	ca. 0·5	–	0·02–0·05	+	0·1–0·2	–		
A2DB/1F_1	2	890	1·283	ca. 0·8	–	0·99	–	0·1–0·2	–	0·01–0·02	++

Parallelism of the Regression Between the Convulsive Response and the Dose of Insulin for First Tests only by means of χ^2 Summation and using Random Bred, Inbred and Hybrid Mice

It is important that the within strain analyses (Table III) indicate that for first tests on each strain the regression lines between convulsive response and insulin dose are parallel. There are, however, significant

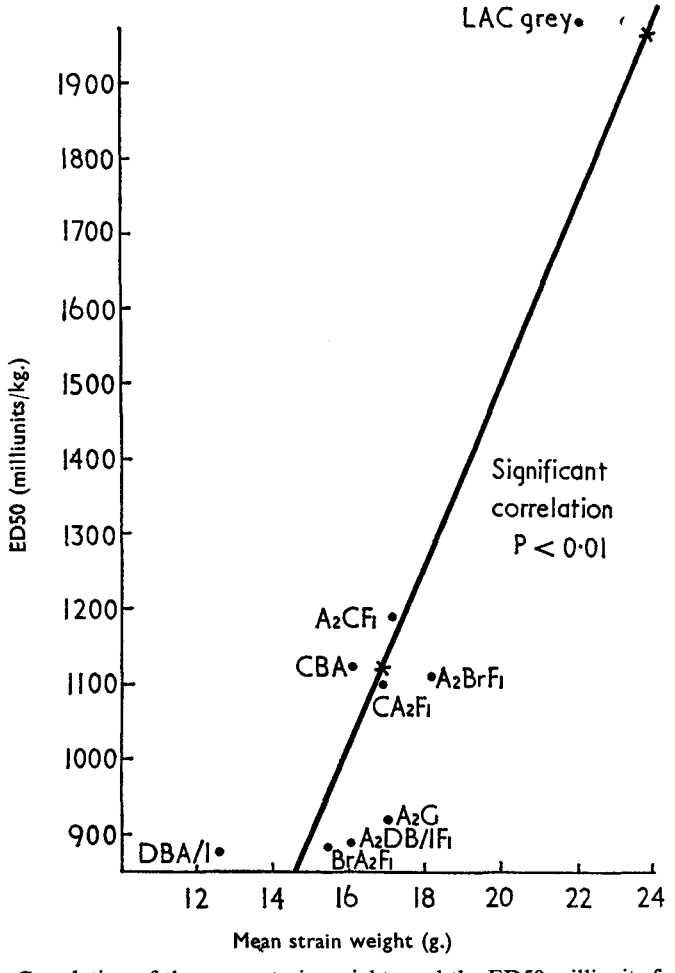

FIG. 1. Correlation of the mean strain weights and the ED50 milliunits for insulin.

differences in the precision of response between strains. The regressions between the convulsive response and the insulin dose for DBA/1 and $BrA2F_1$ mice and for the first group of A2G mice are significantly steeper than that for LAC grey mice (see Table III). The amount of within strain variation in slope may affect the probability of parallelism between

two strains. Thus of the hybrid strains $A2CF_1$ and $BrA2F_1$ the former, with a mean slope of 1·815, having greater within strain variation of slope (or less probability of parallelism) does not show a significant difference in slope from the LAC grey mice, while the latter strain $BrA2F_1$ with a mean slope of 1·825 shows a significant difference. The results for the hybrid mice $A2CF_1$, as also for the second group of A2G mice tested, show that, in spite of considerable variation, that is, less probability of

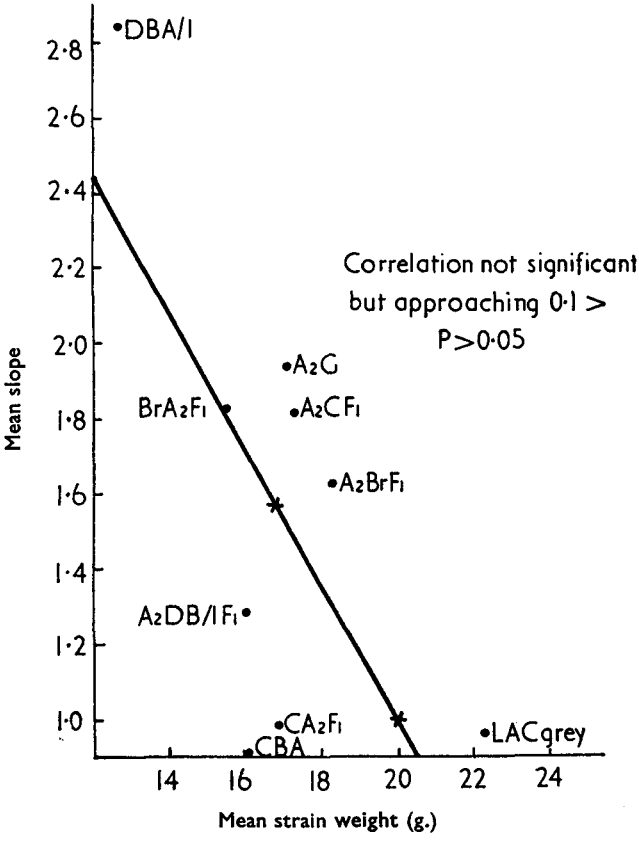

FIG. 2. Correlation of the mean strain weights and the mean slope of the regression of the response to insulin.

within strain parallelism, the difference in slope between the regression for both these groups of mice and the LAC grey mice is towards significance ($P = 0·1–0·2$).

The group of strains comprising hybrid mice with their parents is also interesting. The slope of the regression for the hybrid is less than that of either parent and significantly less than that of the DBA/1 strain. The within strain variation for this hybrid, however, is much less than that for either parent strain.

Correlation of Mean Strain Weights, and ED50 milliunits/kg. of Insulin and of Mean Slope of the Regression of the Response to Insulin. Also the correlation of ED50 milliunits/kg. and Mean Slope in the same Strains of Mouse

The combined test results are plotted against mean strain weight in Figs. 1 and 2 and show that strains derived by mutation or selection in this species (*Mus musculus*) react with insulin in such a way that there is a

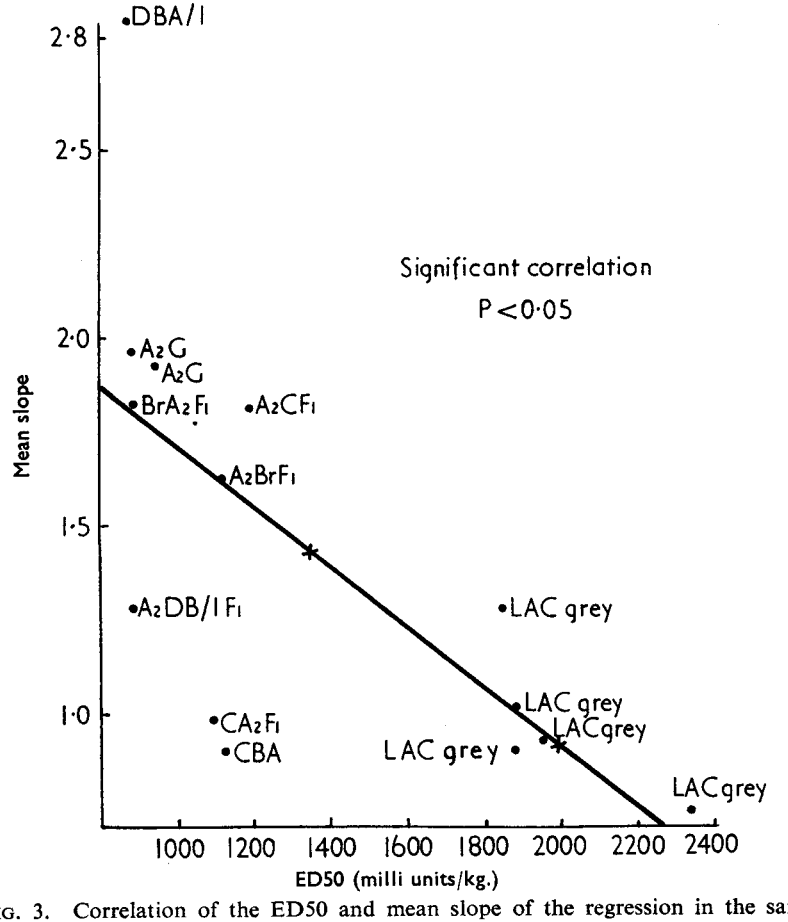

FIG. 3. Correlation of the ED50 and mean slope of the regression in the same strains of mouse.

significant general correlation of ED50 and the mean weight of the strain used, and an almost significant correlation of mean slope of the regression of this reaction and the mean weight of the strain. The results are in contrast to those for within strain variation published by Young and Stewart (1952) in that the lighter strains are more sensitive and more precise, whereas in the analysis of a larger number of mice from a random bred strain the heavier mice were the more sensitive.

Young and Stewart made no statement about the precise age of these mice. The third figure showing the correlation of mean slope and ED50 for these strains is interesting, emphasising the unique position of the DBA/1 strain and also the difference between the F_1 strains $A2CF_1$ (maternal strain A2G) and $CA2F_1$ (maternal strain CBA), each more closely resembling its maternal parent (cf. Walton and Hammond, 1938, and McClaren and Michie, 1956).

Discussion

As the response to doses of insulin in mice has been an official assay test since 1932 it has been often investigated. The present work which has been done in a constant environment such as was deemed necessary in the work of Sellar and Smart (1959), shows that the response is in pattern, sensitivity and precision specific to the strain of mouse employed.

The strain specificity in pattern of reaction is evident both in differences between male and female convulsion rates and in the predominance of either severe or definite reactions rates in the reactions of any one strain.

The sensitivity of the mice to insulin is strain specific and with the strains used the ED50 varied from approximately 900 milliunits/kg. mouse to approximately 2,000 milliunits. From the correlation of the average weights of the mice used its value would seem to be inversely related to the average weight of the strain of mouse employed.

Although there is a general correlation of the sensitivity of the mice and the slope of the regression between their response and the dose of insulin received, the specific reactions of individual strains are still manifest. This is well illustrated by the plots of the results from the hybrid $A2DB/1F_1$ and its parents on the correlation graph. The hybrid plot is well outside that of its parents (cf. Chai, 1960). All have approximately the same sensitivity but the parent strains show greater precision of response, that of DBA/1 being significantly greater than the hybrid. There is no doubt that the general correlations are the outcome of the genetic relationship between the strains.

It would appear that the sensitivity and pattern of response may bear some relation to each other. The very insensitive LAC grey mice have a pattern of response significantly different from all other strains at doses giving similar percentages of reaction. At all doses studied the mice of this strain give more definite than severe reactions, a qualitative difference of less response than all other strains.

The within strain variation in precision, that is, in the parallelism of the regression between its convulsive response to doses of insulin, is not dependent on whether the strain is inbred or hybrid. The hybrid strains $A2CF_1$ and $CA2F_1$ with the hybrid strains $A2BrF_1$ and $BrA2F_1$ illustrate this. For both pairs of hybrids the strain out of A2G females is the more variable.

Specifications most suitable for biological assay, as illustrated by response to doses of insulin, cannot be said to appertain either to all hybrid mice, to all inbred mice or to all random bred mice. Each strain of mouse must be assessed empirically in the environment where it will be

used, and for routine insulin assay this was not the environment I was able to provide. The random bred strain used in this work is entirely unsuitable for insulin assay and would probably be unsuitable in any environment, but this does not condemn all other random bred strains. Of the hybrid and inbred strains tested, the $BrA2F_1$, DBA/1 and A2G strains were those most suitable in the conditions which I imposed.

Similar conclusions are illustrated in the work of Chai (1960), on hormone response in mice, and in other work on this subject which he reviews. It has been further confirmed in the work on the response in hybrid, inbred and random bred mice to pentobarbitone sodium (Brown, 1961) and to histamine acid phosphate (Brown, 1959).

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