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Variations in Sensitivity to Anaphylaxis and to Histamine in Inbred Strains of Mice.* (20873)

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Following a study demonstrating that there was genetic variation in the ability of mice to form circulating antibody(1), experiments were designed to determine whether or not similar variation could be demonstrated for the ability to react with anaphylactic shock; and to determine the extent that anaphylaxis was dependent on circulating antibody. This further led to an inquiry concerning the basic mechanism of anaphylaxis in the mouse, especially to a re-examination of the possibility of a relationship between anaphylaxis and histamine sensitivity.

Materials and methods. Anaphylaxis. Every attempt was made to operate under conditions identical to those prevailing in the study on circulating antibody(1). The same 5 inbred strains of mice—DBA/1 Jax, BALB/C Jax, C3H/Jax, C57BL/6 Jax, and A/He Jax, all sufficiently inbred to insure genetic uniformity in succeeding generations, were used. They were of mixed sexes, and over 4 months old. The amount of alum precipitated egg white, the intra-abdominal route of injection, and the time interval did not vary between the 2 experiments.

Mice were given a challenging intravenous injection of 15 mg of crystalline egg albumin in 0.25 ml of 0.85% NaCl, an amount estimated to furnish an antigen excess. The mice were carefully observed for the characteristic signs of anaphylaxis, and the findings were recorded in accordance with the following: convulsions with or without death, *positive,* severe; muscular spasms or paralysis without death, *positive,* mild; no effect or very slight agitation, in absence of above, *negative.* The time of death was recorded for each animal

TABLE I. Effect of Circulating Antibody on Sensitivity to Anaphylaxis.

	DBA	BALB/C	C3H	C57BL/6	A/He
No. with circulating antibody* / No. injected	28/28	23/27	22/22	26/27	23/24
Rank*†	Excellent-good	Excellent	Good	Excellent	Fair
Anaphylaxis:					
Severe with death	4	12	4	5	24
" without death	0	3	0	0	8
Mild shock	0	0	1	1	0
Negative	28	10	26	36	1
Total	32	25	31	42	33

* Results from (1).
† Based on a statistical analysis considering both number positive and titer.

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TABLE II. Comparison of Sensitivity to Histamine and to Anaphylaxis.

	DBA	BALB/C	C3H	C57BL/6	A/He
No. with anaphylactic shock*† / No. injected	15/32 (47%)	23/27 (85%)	7/31 (23%)	15/42 (36%)	32/33 (97%)
Median reaction time—min.	25 (6-360)	85 (11-960)	87 (46-150)	26 (13-120)	23 (15-47)
No. reacting to histamine† / No. injected	15/16 (93%)	14/16 (88%)	1/16 (6%)	12/16 (75%)	15/16 (94%)

* Survivors of first shocking inj. were tested 7 days later. These figures are total No. reacting in the two test periods.

† No statistical difference due to sex was ever apparent.

dying of the shock.

For every strain, 5 to 10 normal control animals were injected intravenously with the antigen. In no case did an animal show any effect from this injection. Results of this anaphylaxis experiment are recorded in Table I, where they are compared with the amount of circulating antibody.

One week after the first shocking injection, survivors were injected intravenously with the same amount of antigen. The total number of animals reacting in both of the two trials in each strain is recorded in Table II.

Histamine. An Eimer and Amend preparation of crystalline histamine diphosphate was used. Ten per cent solutions were made in 0.85% NaCl daily, immediately prior to use. In preliminary tests, it was found that by the intravenous route a minimum of 0.5 mg per gram of body weight was necessary to elicit a reaction. This standard dose was then employed in determining the sensitivity of the different strains.

Two-months-old mice of the same strains were employed. This age was selected to make the results comparable to other work on histamine sensitivity in the mouse(2). Eight males and 8 females of each strain were tested, except in the A/He strain, in which 11 males and 5 females were used. A positive reaction was recorded when the animal suffered a frank convulsion, with or without death. No mild reactions were observed.

Results. Table I shows, for each of the 5 mice strains, the percentage of animals having circulating antibody, and the incidence of anaphylactic shock after the same time interval, and under the same conditions of immunization. The two sets of data were rank-ordered in terms of the amount of circulating antibody present and the severity of anaphylactic shock, respectively, and were compared by using Spearman's rank-difference correlation method. A Rho of –0.8 was obtained. A rough test of the significance of correlation was obtained by comparing it with its standard error of 0.16. This comparison showed the correlation to be significant below the 1% level of confidence. This statistical analysis indicates an inverse relationship between ability to shock anaphylactically and the circulating antibody titer. On examination of the data for individual strains, however, it is seen that this relationship does not hold for every strain.

Table II contains data showing the extent of sensitivity to histamine and to anaphylaxis for each of the 5 mouse strains. The extent of inter-strain variability was determined by the method of Chi square. Each strain was compared with every other strain in both cases.

The results expressed in Table II indicate the following: a) a genetic influence on the ability to shock; and b) a strain variation in the sensitivity to histamine. With respect to the former, strains A/He and BALB/C were not significantly different from each other, but were different from the other 3 strains; strains DBA and C57BL/6 were not different from each other, but were different from A/He and BALB/C; strain C3H was significantly different from DBA, but not from C57BL/6.

A strain difference in sensitivity to the intravenous injection of histamine is also apparent, with the C3H strain significantly different from all the others, being the most resistant.

When the results of the two determinations are compared, it is seen that the C3H strain, the most resistant to histamine, was among the three strains most resistant to anaphylaxis; and the A He strain, one of the two strains most susceptible to anaphylaxis, was one of the strains most susceptible to histamine. Beyond this, the prediction of a relationship between histamine and anaphylactic sensitivity will not hold.

Discussion. A genetic variation in the ability of mice sensitized to egg albumin, to respond to a subsequent intravenous injection of this antigen by anaphylactic shock, is apparent from the results presented in Table II.

No direct relationship exists between the circulating antibody titer and the sensitivity to anaphylaxis. On statistical analysis the possibility of an inverse correlation is indicated, but it remains for future work to determine the significance of this finding. Further suggestions that these two phenomena are not related in the mouse are found in the work of Weiser *et al.*(3) who found that anaphylactic sensitivity persisted long after precipitins were no longer demonstrable; and by Malkiel *et al.*(4) who were unable to demonstrate precipitable or non-precipitable antibody, or passively transferable antibody, in the circulation of actively sensitized mice. Active anaphylaxis has also been used to demonstrate the antigenicity of homologous malignant tissue in the mouse(5), again in the absence of demonstrable circulating antibody. These findings, together with the observation herein reported that circulating antibody and anaphylaxis are not positively correlated, support the thesis that the antibody responsible for mouse anaphylaxis does not reside in the circulation.

The mechanism of anaphylaxis in the mouse, although it has received some investigation, remains obscure. The repeated findings(2,6,7) that enormous doses of histamine are necessary to produce shock in the mouse, plus the suggestion that the entire body of the mouse is incapable of yielding sufficient histamine to produce the reaction(6) is evidence that the shock syndrome is not mediated through the action of histamine. It may not be coincidental that the results reported in Table II show that the C3H strain of mice, the most resistant to anaphylactic shock, is also the most resistant to histamine. On the other hand, the A/He strain, the most susceptible to anaphylaxis, is no more sensitive to histamine than the other strains studied. Further work on pertinent differences between these two strains is in progress.

In contrast to the intra-abdominal route of injection of histamine, as employed by many workers, the intravenous injection as used here requires a smaller quantity of the drug to produce a reaction. The finding that there is no statistical difference in the reaction of the two sexes to histamine is in disagreement with previously reported observations(2). This may or may not be due to the different route of injection.

Summary. 1. Based on the reactions of mice of 5 inbred strains sensitized to egg albumin, evidence is presented which indicates a genetic variation in the ability of mice to respond with anaphylactic shock. 2. The response by anaphylaxis was not found to be positively correlated with the amount of circulating antibody, and it is suggested that anaphylaxis in the mouse is not dependent on this antibody. 3. One inbred strain of mouse, C3H, which was found to be significantly more resistant to anaphylaxis, was also more resistant to histamine. A second strain, however, very susceptible to anaphylaxis, was no more sensitive to histamine than any other. 4. No sex difference in sensitivity to histamine or to anaphylaxis was demonstrable.

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