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Use of Mice in Testing of Antigenic Power of Tetanus Toxoid.

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A simple and accurate method is herein described for testing the antigenicity of tetanus toxoid, using mice instead of guinea pigs. Preliminary experiments with Swiss mice have indicated that the following factors have to be considered in order that uniform results in repeated antigenicity tests may be obtained. (1) Source of mice. Antibody response varied according to source. (2) Route of immunization. Intraperitoneal route gave optimum response. (3) Sex of mice. Female mice showed better response than male. (4) Immunization period. An interval of 21 days between a single injection of toxoid and the test injection of 10 mouse MLD seemed to be the shortest time to insure reproducible results.

After due consideration of the above factors a tetanus toxoid, previously tested on guinea pigs was adopted as provisional standard for use in repeated tests for immunization of Swiss mice. The results of 4 tests show a high degree of uniformity. From 93 to 96% of mice immunized with a provisional standard toxoid resisted the test with 10 M.L.D. of toxin in 4 separate experiments involving a total of 251 mice. The effect of varying amounts of toxoid (made up to 1 ml with peptone diluent) on the antibody formation of Swiss mice of the same source can be seen in Table I. A detailed description of two alternative methods of practical application is given here.

Procedure I. Swiss mice from a constant

source are standardized for their antigenic response by repeated tests using the provisional standard toxoid as immunizing agent. This standard toxoid may be either a liquid or dried toxoid of proven constant antigenicity. At least 20 female and 20 male mice of 18 to 20 g weight are injected intraperitoneally with 1 ml of the standard toxoid, rested for 21 days and exposed to a subcutaneous injection of 1 ml containing 10 Mouse MLD (= 2 Guinea Pig MLD) of an aged tetanus toxin of constant titer. Five non-immunized control mice are exposed to 1 Mouse MLD of toxin and should die within 96 hr. The exposed mice are observed for 7 days and the average number of protected males and females recorded. This figure represents the "standard value of protection" for the chosen strain of Swiss mice. This figure should be checked every few months. This value serves as comparator for testing toxoids of unknown antigenic titer which should show as good or better protection than the standard.

Procedure II. This method does not involve a standardization of mice from a known source. Each test of tetanus toxoid of unknown titer includes a control of mice which received an intraperitoneal injection of 1 ml of the standard toxoid. After 3 weeks the 2 groups of mice are challenged with 10 MLD, and the non-immunized mice with 1 Mouse MLD of toxin respectively. The protection resulting from the use of the

TABLE I.
Effect of Varying Amounts of Toxoid on Resistance to Toxin in Swiss Mice.

Amt of toxoid A inj., ml	Route of immunization	Immunization period, weeks	No. of mice exposed to 10 MLD toxin	Avg % protected
1.0	Intraperit.	3	41	95
0.5	"	3	42	70
0.25	"	3	41	30
1.0	Subcutan.	3	57	95
0.5	"	3	58	64
0.25	"	3	64	28

TABLE II.
Comparative Antigenicity Tests Using Mice and Guinea Pigs.

Toxoid	Mice			Guinea pigs		
	Immunization period, weeks	No. of animals tested	% protected	Immunization period, weeks	No. of animals tested	% protected
Stand. A	3	41	95	6	13	92
No. 1	3	38	96	6	8	87
	3	40	93			
No. 2	3	40	95	6	16	75
				6	10	90
No. 3	3	39	92	6	13	92
No. 4	2	69	100	6	12	92
No. 5	2	67	96	6	13	85
No. 6	3	40	77	6	10	40

unknown toxoid is then compared with that of the standard toxoid tested simultaneously. The technic of the test is otherwise the same as described under Procedure I.

Using Procedure I we have compared the degree of resistance attained in mice and guinea pigs and have obtained the data listed in Table II.

Summary. Swiss mice can be used as test animals in place of guinea pigs for the deter-

mination of the antigenic power of tetanus toxoid. The advantage in the use of mice lies in two facts: (1) the immunization period necessary is only 3 weeks instead of the usual 6 weeks; (2) lower cost of mice permits the use of a larger number of animals and animals of greater uniformity than guinea pigs, which contributes to a greater statistical significance and accuracy of the test.

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Activity of Desoxycorticosterone Acetate upon Oral Administration.

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Comparative studies of the activity of desoxycorticosterone acetate upon parenteral and oral administration have been carried out repeatedly. Grollman found 1 mg of this hormone effective both in maintaining life and promoting growth of adrenalectomized rats, regardless of the mode of administration.¹ On the other hand, more re-

cent studies indicated that the effects produced by injection of the hormone were considerably superior to those following oral administration.² A comparison of the technics of oral administration in the earlier and in the later studies indicated a difference which appeared of possible significance. Grollman mixed the hormone with the diet and thus ensured prolonged or almost continuous intake of the hormone. On the other hand, Kuizenga gave the hormone, dissolved

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¹ Grollman, A., *J. Pharm. and Exp. Therap.*, 1939, **67**, 257.

² Kuizenga, M. H., Nelson, J. W., and Cartland, G. G., *Am. J. Physiol.*, 1940, **130**, 298.