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Influence of Dietary Factors and the Adrenal on Hyaluronidase Spreading Reaction in the Rat.* (20427)

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The influence of adrenalectomy and adrenal cortical hormones on the spreading reaction of hyaluronidase has been studied in mice and rabbits(1). Conflicting results have been reported in the rat(2,3). The present study is concerned with observations on the intradermal spread of hyaluronidase in intact, cortisone treated intact, in adrenalectomized or sham operated rats, and the influence of nutritional factors on the reaction.

Materials and methods. A total of 185 adult male and female rats, bred in the laboratory, were used in these experiments. Both intact and adrenalectomized rats received a 1% sodium chloride solution as drinking water. No hormonal therapy was given the adrenalectomized rats. One group of intact rats was given 5 mg of cortisone intramuscularly daily for 4 days prior to the skin test. One hour before the skin test was performed another dose of cortisone was given. The rats received one of the following *diets*: 1) a dog chow diet; 2) an experimental diet adequate in all nutritional substances for the rat; 3) the experimental diet supplemented with excessive amounts of calcium pantothenate (43.6 mg per 100 g of diet); 4) the experimental diet supplemented with large amounts of vit. B_{12} (50 μg of B_{12} concentrate/100 g diet); and 5) the experimental diet deficient in pantothenic acid. The composition of the experimental diet (per 100 g diet) was as follows: vitamin free casein 22 g, sucrose 62 g, primex 9 g, salt mixture (ferric citrate 2.3%, calcium phosphate dibasic 44%, potassium citrate 42%, magnesium citrate 11.6%, copper sulfate 0.8%, and potassium iodide 0.01%) 5 g, cod liver oil 2 g, thiamine 0.3 mg, pyridoxine 0.3 mg, riboflavin 0.5 mg, folic acid 0.1 mg, biotin 0.05 mg, calcium pantothenate 1.0 mg, niacine 1.0 mg, choline 30.0 mg, inositol 5 mg, mixed tocopherols 7.5 mg, and synthetic vit. K 5 mg. The animals on the dog chow diets were divided into 4 groups. *One group* was skin tested as a control group, *one* received cortisone intramuscularly for 4 days before the skin test, and the *third* group was bilaterally

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adrenalectomized and skin tested at varying intervals of time postoperatively. A *fourth* group fed dog chow was sham operated and skin tested at similar postoperative intervals.

The animals on the synthetic diets were divided into 5 groups. One group received the pantothenate deficient diet for 30 days; one-half of this group was bilaterally adrenalectomized on the 24th day of the diet. The remaining 4 groups received the control experimental diet for 10 days. At this time one-half of each group was bilaterally adrenalectomized, and all the animals thereafter received one of synthetic diets throughout the remainder of the study. The rats consumed approximately 10-15 g daily of their ration. The *hyaluronidase spreading reaction* was measured 6 to 21 days after adrenalectomy. Food and fluids were withheld 18 hours prior to the injection of hyaluronidase. The test was carried out as follows: a solution of India ink was added to an equal volume of hyaluronidase† in physiological saline so that the concentration of the enzyme was 15 mg/ml. Saline was substituted for the enzyme in the control solution. The animals were lightly anesthetized with ether, the flanks were shaved, and 0.08 ml of the hyaluronidase-India ink solution was injected intradermally into the left flank. An equal volume of the control solution was intradermally injected into the right flank. The injection time was constant at 8 seconds. Six hours later, the animals were sacrificed with illuminating gas and were skinned. The skin was stretched on a flat surface, and 24 hours after the injection the areas of spread were outlined on cellophane and traced onto paper. The area in square centimeters was determined with a planimeter. The results are expressed in square centimeters as the spread of the hyaluronidase-India ink solution minus the spread of the saline-India ink.

Results. The effect of adrenalectomy or the intramuscular injection of 5 mg of cortisone on the spreading reaction in rats fed a dog chow diet is compared with that of control rats in Table I. Control intact rats fed the dog chow diet had a mean area of spread of 8.05 sq cm; adrenalectomized rats 14 days postoperatively had a mean area of spread of 0.47 sq cm; intact rats treated with cortisone had a mean area of spread of 2.29 sq cm. In the rat, both the withdrawal of adrenal hormones and the addition of cortisone appeared to decrease the spreading reaction. Throughout the study no sex difference was found.

TABLE I. Effect of Adrenalectomy or Cortisone Injections on Hyaluronidase Spreading Reaction in the Rat on a Dog Chow Diet.

	No. of rats	Area of spread in cm^2 (mean ± S.E.)
Intact	23	8.05 ± .81
Adrenalectomized	7	.47 ± .13
Cortisone treated, intact	13	2.29 ± .39

In order to evaluate the effect of the operative procedure, *per se,* 34 rats on the dog chow diet were sham operated and the spreading reaction measured at intervals of 2, 5, 16, and 22 days later. The spreading of the sham operated rats was compared with that of rats on the dog chow diet, adrenalectomized for the same periods of time. At 2 days after operation, the mean area of spread in square centimeters for 5 sham operated rats was 1.90 ± 0.47 compared with 2.05 ± 0.43 for 12 adrenalectomized rats. At 5 days the corresponding values were 2.25 ± 0.44 sq cm for 6 sham operated rats and 1.41 ± 0.22 sq cm for 5 adrenalectomized animals; at 14 days, 1.59 ± 0.14 sq cm for 11 sham operated and 0.47 ± 0.13 for 14 adrenalectomized rats; and at 22 days 3.76 ± 0.65 sq cm for 12 sham operated and 2.16 ± 0.47 sq cm for 5 adrenalectomized animals. All these values are significantly less than the mean area of spread 8.05 ± 0.81 sq cm of intact rats on the dog chow diet. The results indicate that sham operation and adrenalectomy had similar effects on the hyaluronidase spreading reaction in rats.

The effect of various diets upon the spreading reaction in both intact and adrenalectomized rats is shown in Table II. In all groups except those fed a high vitamin B_{12} diet, the adrenalectomized animals had a significant decrease in the areas of spread ($P < 0.05$). Neither a high pantothenic acid diet nor a

† Wyeth Co. lot No. W-108-A.

TABLE II. Effect of Diet upon Hyaluronidase Spreading Reaction in Intact and Adrenalectomized Rats.

Diet	Intact			Adrenalectomized			
	No. of rats	Days on diet	Area of spread in cm^2 (mean ± S.E.)	No. of rats	Days on diet	No. days post-op.	Area of spread in cm^2 (mean ± S.E.)
Control experimental	8	30	5.19 ± 1.32	10	30	21	.96† ± .19
High pantothenate	7	27	4.21 ± 1.21	11	17	17	1.33† ± .39
Pantothenate deficient	11	29	7.35 ± .93	10	30	6	.13† ± .16
High vit. B_{12}	8	20	1.72* ± .27	8	20	20	1.05 ± .18

* Significant difference ($P < .05$) from intact control value.
† " " " between intact and adrenalectomized rats on same diet.

pantothenate deficient diet in intact rats significantly altered the area of spread from the control value of 5.19 sq cm. Intact rats fed a high vit. B_{12} diet had a significant decrease ($P < 0.05$) in the area of spread when compared to the control intact rats. The area of spread was reduced from 5.19 sq cm in the control rats to 1.72 sq cm in the animals fed the high vit. B_{12} diet. Adrenalectomized rats fed the high vit. B_{12} diet had a mean area of spread of 1.05 sq cm, which was not significantly different from the intact rats fed the same diet.

Discussion. Adrenalectomy had the opposite effect on the spreading reaction of hyaluronidase in rats than has been reported for mice and rabbits(1). Furthermore, in the rat, sham operation caused as great and as prolonged a decrease in the area of spread as did adrenalectomy. This suggests that the operative procedure is itself an important factor in the spreading reaction in this species, and that the effect of adrenalectomy may be non-specific. The injection of cortisone decreased the spread of hyaluronidase and in this respect the rat behaved as do other species(1). The fact that adrenalectomy and injection of the adrenocortical hormone both reduced the area of spread also supports the suggestion that the effect of adrenalectomy on the spreading reaction was not specific.

The spreading reaction was also decreased in intact rats by the administration of excessive amounts of vit. B_{12} in the diet. The explanation of this reaction is not clear. We did observe that the skin of the rats on vit. B_{12} appeared to be thicker than that of the rats on other diets. However, no measurements of skin thickness were made. Hayes *et al.*(4) have noted that prolonged local therapy of the skin with adrenal cortical extract causes thinning of the dermis and epidermis of the rat, and under these conditions they noted an increased spread of hyaluronidase.

In the rats on the pantothenate acid deficient diet, there was a moderate increase in the area of spread following the injection of hyaluronidase. Pantothenic acid deficiency causes atrophy of the hair follicles and bulbs in the rat(5) and obvious thinning of the skin. One might, therefore, anticipate that under these circumstances the intradermal injection of the hyaluronidase solution would result in a greater spread than in animals on a normal diet. The rats in this experiment were maintained on the deficient diet for only 29 days, and possibly prolonging the period of the deficiency would have resulted in a more pronounced effect on the spreading reactions. Pantothenate deficiency also affects the integrity of adrenal cortical cells(6,7), but from the present data there is no way of evaluating the effect of the adrenal damage on the spreading reaction. The diet containing excessive amounts of pantothenate had no influence on the area of spread of hyaluronidase.

The observations reported indicate that in the rat the effect of adrenalectomy on the hyaluronidase spreading reaction is non-specific. Cortisone in large doses does inhibit the intradermal spread of hyaluronidase. The

effect of the dietary situations on the spreading reaction is most probably due to the local influences of nutritional fractions on the skin apparatus of the rat.

Summary. 1. The hyaluronidase spreading reaction was tested in intact, cortisone injected and adrenalectomized rats and the effect of various dietary situations was studied. In intact rats, a diet containing excessive amounts of vit. B_{12} decreased the area of spread significantly. At present, there is no obvious explanation for this observation. 2. Both the administration of cortisone and adrenalectomy decreased the area of spread in rats. Sham operation also decreased the area of spread of hyaluronidase. It is suggested that the effect of adrenalectomy is primarily a non-specific effect due to the operative procedure itself.

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Free Wandering Cells and Cortisone. (20428)

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After Bloom(1) reported that cortisone could produce a rapid regression of mast cell tumors in dogs, we began a study of the effects of cortisone on the mast cell in tissue culture. For reasons which are not clear to us, the rapidly destructive effects on the mast cell obtained by Bloom *in vivo* were only mildly demonstrable *in vitro*. Mast cell activity was inhibited, it is true, but this was only a facet of a broader observation, *i.e.,* cortisone can effectively block the nomadic proclivities of free wandering cells. This agrees with previous observations on leucocytes(2,3) but the fact seems to have been overlooked that not only white blood cells but free wandering cells in general are more sensitive to the hormone than are fibroblasts. All free wandering cell activity can be brought to a stop in cultures in which fibroblastic activity remains relatively undisturbed.

Materials and methods. Coverslips (43 x 50 mm) containing crystals of cortisone (free-alcohol) in amounts ranging from 0.0005 to 0.025 mg were prepared by permitting the hormone to precipitate by evaporation from measured volumes of an alcohol-cortisone solution. On these coverslips fragments of 9-10 day old embryonic chick skin were placed in equal parts of blood plasma and embryonic extract before the medium clotted. Controls contained either no hormone, or cholesterol in amounts similar to the amount of hormone used in the experimental cultures. More than 200 pairs of embryonic skin fragments were grown and observations were taken each day over a period of 4 days. We also studied about 200 pairs of spleen fragments obtained from 16-19 day old chick embryos. Here the technic was varied in that, with both the experimental and control cultures, the medium was permitted to clot before a spleen fragment was placed meticulously, with no lateral motion, upon the surface of the medium. This procedure was followed in order to minimize the spread of cells by mechanical means. The spleen fragments were taken from 16-19 day old embryos because, at the age chosen for the source of skin material, namely, 9-10 days, the spleen is both undesirably small and soft.

Results. The method of introducing the