TABLE III. Effect of Hyaluronidase and PDHA on Hypodermoclysis in Rabbits. 6 rabbits in each series.

	Time for hypodermoclysis (min.)			
	Saline	Hyaluronidase (150 TRU)	Saline	PDHA (150 mg)
Mean	47	19	51	18
± SD %	7.6	10.2	8.4	12.7

TABLE IV. Effect of Hyaluronidase and PDHA on Absorption and Excretion of Urokon.

Treatment group*	Spread, mm² at time of inj.		Appearance in urinary bladder (min.)
Saline + Urokon	Mean	4.4	30
	± SD %	22.7	
150 TRU hyaluronidase + Urokon	Mean	10.4	16.3
	± SD %	16.3	
150 mg PDHA + Urokon	Mean	11.4	16.9
	± SD %	30.7	

* 8 guinea pigs/group.

lyzed hyaluronate was without significant spreading activity.

Hypodermoclysis and absorption of x-ray contrast medium. The ability of PDHA to facilitate a hypodermoclysis of PSS in rabbits is shown in Table III. It will be seen that 150 mg of PDHA was as effective as 150 TRU of the enzyme. In other experiments 50 and 100 mg of PDHA added to the clysis was without facilitating effect. Facilitation of spread and absorption of an x-ray contrast medium in guinea pigs is shown in Table IV. It can be seen that 150 TRU of hyaluronidase or 150 mg of PDHA increased the area of spread and the rate of absorption and appearance of the opaque material in the urinary bladder by twofold.

Summary. 1. Increasing the concentration of hyaluronidase by large units resulted in increased spreading activity until a massive concentration of enzyme was used. 2. Highly polymerized hyaluronic acid obtained from *Streptococcus pyogenes,* bovine vitreous humor, and human umbilical cord had no significant spreading action. 3. Partially depolymerized streptococcal hyaluronic acid had significant spreading action in rabbits, hastened the absorption of a clysis of physiological salt solution in rabbits and facilitated the spread and absorption of an x-ray contrast medium in guinea pigs. 4. Further depolymerization of the streptococcal hyaluronic acid resulted in products that no longer had significant spreading action.

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Topical Protection Against Hyperergic Inflammations by a Preceding Local Inflammation. (20819)

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The hyperergic reaction provoked in the rat by the intraperitoneal injection of fresh egg albumin(1) can be topically inhibited by means of a previously produced local inflammation. This was demonstrated by Selye and his collaborators(2), who stated that the degree of topical protection depends on the intensity of the preceding inflammation and considered the phenomenon as not specific, since various irritative substances inhibit topically the hyperergic reaction produced by the egg albumin injection. As regards the mechanism of this protecting action, the aforementioned authors assert that it is strictly locally conditioned.

Method. Fifty white 4-months-old rats were used, with a body weight of between 100 and 130 g, distributed in 5 groups of 10 rats

each. All rats received in their right leg a subcutaneous injection of 0.25 ml of a 4% solution of peptone in water. The rats of the *first* group were given 24 hours later an intraperitoneal injection of 0.5 ml of a 50% solution of recently obtained egg albumin. The *second* group had the right sciatic nerve severed 20 hours after the peptone injection in their right leg, and 4 hours later received an intraperitoneal injection of 0.5 ml of the albumin solution. The animals of the *third* group were adrenalectomized 18 hours after the first injection, and 6 hours later 0.5 ml of egg albumin was injected. The rats of the *fourth* group were adrenalectomized 6 hours before the peptone injection in their right leg, and 24 hours later they received an intraperitoneal injection of 0.5 ml of albumin. Finally, in those of the *fifth* group the suprarenal glands were removed 6 hours before the first injection. One hour after the peptone injection in their leg they received 5 mg of Merck's cortisone acetate intraperitoneally, and 24 hours later an intraperitoneal injection of 0.5 ml of egg albumin.

The surgical operations—sciatic nerve extirpation and adrenalectomy—were carried through with ether anesthesia and under aseptic conditions. Adrenalectomy was performed by the posterior route, and the suprarenal glands as well as the immediate fat were extirpated. After operation the animals received 1% solution of sodium chloride, but otherwise their nourishment was similar to that of the remaining rats. The animals were observed during a period of 4 hours following the albumin injection, the intensity of the general reaction and the swelling of the injected leg being recorded.

Results. The rats of the *first* group showed within 2 hours following the intraperitoneal albumin injection an intense edema and hyperemia of snout and fore-legs; the left leg was edematous and presented a marked hyperemia, whereas the right leg, which had received the peptone injection, showed no reaction at all (Fig. 1, No. 1). The local protection was evident in 9 rats.

Six of the *second* group of rats, whose right sciatic nerve was cut after the local peptone injection, did not show the protection phenomenon, and in the remaining animals this phenomenon was incomplete, the injected leg presenting a slight edema (Fig. 1, No. 2).

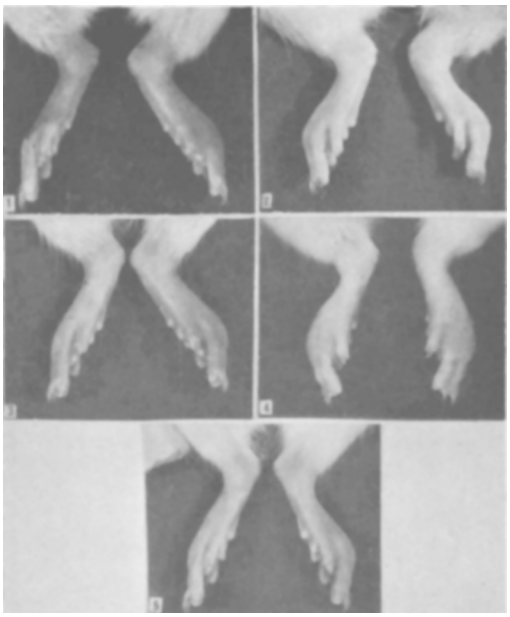

FIG. 1. 1) Injection of 0.25 ml of 4% peptone in right leg. General hyperergic reaction 24 hr later by intraperitoneal injection of 0.5 ml of 50% egg albumin. Right leg protected. 2) Injection of 0.25 ml of peptone in right leg. 20 hr later section of right sciatic nerve, and 4 hr later general reaction by intraperitoneal injection of albumin (0.5 ml at 50%). No protecting effect. 3) 18 hr after the subcutaneous injection of peptone in right leg (0.25 ml) bilateral extirpation of suprarenal glands and 6 hr later peritoneal injection of 0.5 ml of egg albumin. Right leg protected. 4) Bilateral adrenalectomy 6 hr before injection of 0.25 ml of peptone in right leg. 24 hr later general reaction provoked by injection of 0.5 ml of albumin. Absence of the local protecting effect. 5) 6 hr prior to the local peptone injection, bilateral adrenalectomy. One hr after subcutaneous injection of 0.25 ml of peptone in right leg, peritoneal injection of 5 mg of Merck's cortisone acetate. 24 hr later injection of egg albumin (0.5 ml intraperitoneally) to provoke general hyperergic reaction. Right leg protected.

The rats in the *third* group—those adrenalectomized 18 hours following the injection of the locally irritating substance—a phenomenon of local protection against the general reaction provoked by the egg albumin was to be seen (Fig. 1, No. 3).

The animals of the *fourth* group, the suprarenal glands of which were extirpated a few hours prior to the subcutaneous peptone injection in the right leg, did not show the local

protection. The protecting effect was lacking in 9 rats (Fig. 1, No. 4).

In 7 of the *fifth* group rats the protection was quite evident, but in the 3 remaining animals the general reaction which usually is produced by the albumin injection did not appear (Fig. 1, No. 5).

Discussion. The results confirm the protecting effect which the preceding local application of an irritative substance is able to produce against the general hyperergic inflammation provoked by an albumin injection, provided that its action precedes the injection of the antigen by an adequate time interval. In the second place, they show that this topical protection phenomenon may be modified by local and general influences, the last of which depend on the suprarenal glands.

The second of the sciatic nerve of the leg into which peptone was injected reduces or suppresses the protecting effect. Possibly this action is connected with the circulatory changes—hyperemia, increase of the capillary permeability—that follow the section of the nerve.

The effects obtained on the animals belonging to the third, fourth, and fifth groups are more interesting, as they suggest that the mechanism of the phenomenon in question may not be strictly local and may possibly, at least in part, depend on general influences, or more concretely, on the action of the suprarenal glands.

While the rats of the third group, which retained their suprarenal glands, during the period of the local inflammation following the subcutaneous peptone injection, display a very clear topical protection phenomenon, in those belonging to the fourth group, the suprarenal glands of which were extirpated prior to the local application of the irritative substance, the protection is lacking or is very limited. The topical protection reappears in the rats without suprarenal glands provided they receive, a short time after the peptone injection in their right leg, an injection of cortisone.

These experiments appear to establish an interrelation between the presence or absence of the local protection phenomenon and the suprarenal gland or its cortical steroids.

The antiphlogistic action of cortisone is well known, and its effect is partly due to the drug's action on the capillary permeability. Moon and Tershavovec(3) showed that the inflammation secondary to the local application of thermic irritating agents is diminished by treatment with cortisone, which provoked an increase of the capillary resistance, and that in these animals the diapedesis, the capillary hemorrhage and the edema are less frequent. Likewise Benditt(4) reported that cortisone diminishes the capillary permeability and reduces the diffusion area which follows the injection of hyaluronidase. Menkin(5) observed that the extract of the suprarenal cortex suppresses the action of leukotoxine or of the total extract of an inflammatory exudate. Later on, the same author(6) found that some commercial preparations of cortisone increased the capillary permeability. It may be that this paradoxical effect is not directly caused by the cortisone, but by the vehicle in which it is suspended, since the powder of this substance does not produce any increase of the capillary permeability.

Apart from this action on the capillary walls, cortisone diminishes the processes of cellular activity, as has been proved repeatedly. Landauner(7) and Karnofsky and his collaborators(8) demonstrated the inhibiting action of cortisone on the embryo's development. More recently, Menkin(9) has shown that cortisone acetate suppresses to some extent the normal segmentation of the fecundated eggs of *Arbatia punctulata,* the sperm also becoming inactive. The same results were obtained by Rodriguez and the author when studying the effect of cortisone upon the germination and development of some seeds (10).

The conditions of the inflammatory focus are modified considerably in all its stages by the action of these steroids. Michael and Whorton(11) demonstrated that the leukocytic infiltration as well as the local fibrin formation and the degree of edema are modified by cortisone during the acute stage of the inflammation. Likewise, Selye(12) observed by means of the pouch granuloma method that the introduction of small quantities of hydrocortisone directly in the wall of

the bursa diminishes or prevents the appearing of inflammatory alterations.

In view of these results it appears that the subcutaneous injection of peptone and the secondary inflammation produced by it determine the appearance of an alarm reaction. The steroids of suprarenal source which are produced during this reaction may move from the blood to the inflamed tissues due to the increase of capillary permeability which takes place in the inflamed area, accumulating there and modifying the tissue's conditions and the degree of resistance of the capillary wall; they also may reduce the activity of the tissue's cells and perhaps prevent the appearance of some metabolic factor which is important in the development of the inflammatory process. Menkin(13) has proved this with the preparation F, which prevents or inactivates the formation of leukotoxine and of the L.P.F., so that later on, when the second injection is given, the reaction between the antigen—the egg albumin—and the tissue's cell is prevented or rendered difficult, either because the movement of the antigen across the capillary wall is impeded, or because the cellular reactivity is diminished, which might prevent the antigen's reaction with the cell or might render impossible the local production of antibodies. In any case, the result would be an antiphlogistic action depending for the most part on the stimulation of the suprarenal gland.

Summary. 1. The hyperergic reaction provoked in the rat by the intraperitoneal injection of egg albumin can be topically inhibited by a previously produced local inflammation. 2. The topical protection phenomenon can be modified by local and general influences. The section of the sciatic nerve of the injected leg prevents or diminishes the protecting effect. The extirpation of the suprarenal glands prior to the application of the locally irritating substance prevents the topical protection phenomenon, which reappears if the animals receive immediately after the action of the locally irritative agent an intraperitoneal injection of cortisone. 3. The topical protection phenomenon depends partly on the moving, from the blood to the tissues, of the corticosteroids which are freed during the alarm reaction following the local inflammatory irritation; these corticosteroids have an anti-inflammatory action which diminishes or prevents the development of phlogistic alterations in the tissues which previously have suffered an inflammation.

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