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THE EFFECTS OF ADRENALECTOMY AND CORTICOSTERONE ON VASCULAR PERMEABILITY RESPONSES IN THE SKIN OF THE RAT

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Summary.—The vascular permeability response induced in the rat by intracutaneous histamine or serotonin is noticeably influenced by previous adrenalectomy or treatment with corticosterone. Permeability responses were demonstrated by the local exudation of circulating Evans blue at sites of intracutaneous injection of the above permeability factors, the intensity of the responses being assessed by the diameter of the blue lesions as well as by the amount of exuded dye.

In adrenalectomized rats maintained for 72–80 h on 0·9% solution of NaCl, the permeability response to histamine was enhanced about 10-fold, that to serotonin about 5-fold. When rats were given subcutaneous corticosterone, 1·0 mg/animal 1 h before testing, the responses to the same 2 permeability factors were decreased about 10-fold and 5-fold respectively. Corticosterone also decreased the enhanced responses in adrenalectomized rats to levels somewhat below those in mock adrenalectomized controls. The results support the proposal from other work that vascular exudation in experimental inflammation is regulated by an anti-inflammatory factor that accumulates in injured tissues and owes its effect to release of corticosteroids.

MOST OF THE literature on the acute inflammatory response deals with factors that mediate the component vascular reactions and, in particular, the increase of vascular permeability. On the other hand, little information is available concerning the natural control of the permeability response. Evidence has recently been obtained, however, that the exudation of plasma in acute inflammation is regulated by a feed-back mechanism which is induced comparatively early in the inflammatory response (Garcia Leme and Schapoval, 1975). In carrageenin or dextran lesions of the rat's paw, an "anti-inflammatory factor" accumulates in the injured tissues and owes its effects to release of corticosteroids *via* the hypothalamo–pituitary–adrenal axis. The anti-inflammatory factor has been harvested in perfusates of the injured tissues, being obtained from paws $2\frac{1}{4}$–$2\frac{3}{4}$ h after injection of carrageenin, but not $\frac{1}{2}$–1 h after injection.

The effects of the factor have been demonstrated by decrease of the inflammatory response in normal rats given intravenous perfusate before the injection of carrageenin into the paw. However, the inhibition of oedema by the perfusate is abolished by prior adrenalectomy of the receptor animals. The perfusate also elevates the serum level of corticosterone, with concomitant depression of the adrenal's content of ascorbic acid. Catecholamines from the adrenal do not seem to play a substantial role in the phenomenon because the perfusate still induces its effects in adrenal demedullated rats (Garcia Leme and Schapoval, 1975).

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Although histamine and serotonin probably do not play a major role in evoking exudation in acute inflammatory lesions (Wilhelm and Mason, 1960), these amines nevertheless induce a consistent and relatively strong permeability response in the skin of the rat. We have therefore sought further evidence for the role of corticosteroids as regulators of increased vascular permeability by testing the effects of adrenalectomy and of administration of corticosterone on the permeability responses induced by intracutaneous histamine and serotonin in the rat.

MATERIALS AND METHODS

Assessment of vascular permeability responses with Evans blue.—The fur on the dorsal trunk of male Wistar rats (225–275 g body weight) was closely clipped and, immediately before testing, the animals were given intravenous Evans blue, 25 mg/kg as a 2·5% solution in 0·45% NaCl. Permeability factors (PFs) were made up in Ringer–Locke's solution and injected intracutaneously in a standard injection volume of 0·1 ml. Each animal received 8 intracutaneous injections in the skin of the dorsal trunk within the test area indicated by Wilhelm *et al.* (1958). The 8 intracutaneous injections comprised 3 different doses of each of 2 PFs, plus 2 control injections of 0·1 ml Ringer–Locke's solution. The sites of injection of each dose were varied from animal to animal, the rats being kept under light anaesthesia with ether during injection of PFs. Thirty min after the injections, the animals were killed, the whole thickness of the skin reflected from the dorsal trunk and the diameter of the lesions measured on the under surface to the nearest 0·5 mm. The content of exuded dye in the lesions was then estimated by excising the skin sites bearing individual lesions, mincing the skin with scissors and holding the minced skin from each lesion in 5 ml formamide at 37° for 2 days (Mustard *et al.*, 1965; Lykke and Cummings, 1969). The resultant solution was filtered and the optical density of the filtrate assessed at 619 nm on a Vitatron (Holland) UC 100 colorimeter. The concentration of dye in the filtrate was estimated from a standard graph recording the optical density of serial dilutions of a weighed sample of Evans blue in formamide.

Permeability effects were estimated in (1) normal, (2) adrenalectomized, (3) mock adrenalectomized, (4) corticosterone injected and (5) adrenalectomized rats given corticosterone. Dosage-response lines were obtained by plotting mean lesion diameter or mean amount of extracted dye against log dose PF.

Bilateral adrenalectomy.—Under ether anaesthesia, the adrenal glands were removed surgically by a ventral abdominal approach. The adrenalectomized rats were subsequently maintained on 0·9% aqueous solution of NaCl and tested 72–80 h post-operatively. During this post-operative period, functioning accessory adrenocortical tissue does not develop. Controls for the adrenalectomized animals were represented by mock adrenalectomized rats, in which laparotomy, handling of abdominal viscera and closure of the incision of the abdominal wall were performed to a similar extent as for actual adrenalectomy. These animals were also tested 72–80 h post-operatively.

Corticosterone treatment.—Corticosterone was suspended in Ringer–Locke's solution and administered subcutaneously in a dose of 1 mg/rat. Body weight of the rats ranged between 240 and 260 g and corticosterone was given 1 h before intracutaneous injection of PFs.

Drugs.—Evans blue (Searle Scientific Services) was supplied by George T. Gurr, England; histamine acid phosphate by British Drug Houses; 5-hydroxytryptamine creatinine sulphate (serotonin) by May & Baker; corticosterone and formamide by E. Merck AG. Doses of histamine and serotonin are cited as the amounts of base.

RESULTS

PF potency of histamine and serotonin in control rats

Normal and mock adrenalectomized rats served as controls for tests of the effects of corticosterone and adrenalectomy, respectively (see below). It was necessary, therefore, to assess the reactivity of both types of control animals

TABLE.—*Relative PF Potency (ebd or SBD) of Histamine and Serotonin in Normal and Mock Adrenalectomized Rats*

	Histamine		Serotonin		Serot./Hist.	
Treatment	ebd (μg)	SBD (μg)	ebd (μg)	SBD (μg)	ebd	SBD
Normal	3·5	0·4	0·09	0·025	38	16
Mock adrenalectomized	2·4	0·5	0·06	0·011	40	45

to the test PFs. The results were compared in terms (1) an effective blueing dose (ebd)—*i.e.*, the dose in the standard injection volume of 0·1 ml that on the under-surface of the skin induces a mature lesion having a diameter of 9·5 mm and (2) a standard blueing dose (SBD)—*i.e.*, the dose that induces the exudation in the mature lesion of 10 μg Evans blue (Carr and Wilhelm, 1964).

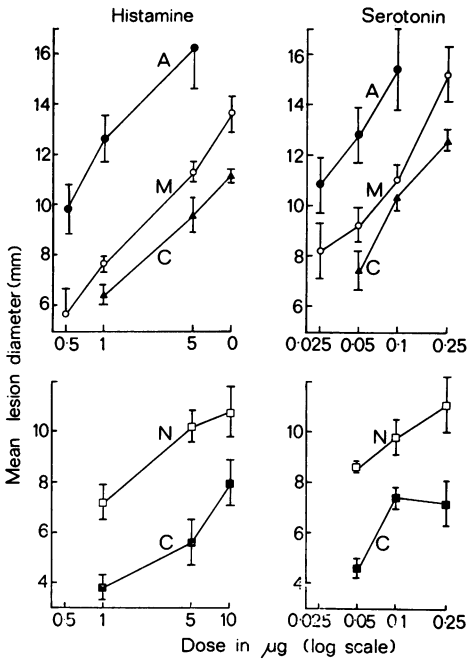

FIG. 1.—Effects of adrenalectomy and corticosterone on the permeability response to histamine and serotonin, estimated by lesion diameter on the under surface of the skin.

Upper blocks: Enhancement of permeability response in adrenalectomized rats (A) compared with mock adrenalectomized controls (M), and reversal of enhancement in adrenalectomized animals by subsequent administration of corticosterone (1 mg/rat; C).

Lower blocks: Decrease of permeability response in rats given corticosterone (1 mg/rat; C), compared with the response in untreated controls (N). Each point corresponds to the mean ± s.e. mean for one lesion in each of 5 rats for the test groups, of one lesion in each of 10 rats for the mock adrenalectomized groups, and of one lesion in each of 6 rats for the untreated groups. Control injections of 0·1 ml of Ringer–Locke's solution induced a mean lesion diameter (mm) ± s.e. mean of 2·1 ± 0·2 (10 lesions) in adrenalectomized rats, 1·5 ± 0·2 (20 lesions) in mock adrenalectomized rats, 1·1 ± 0·5 (10 lesions) in corticosterone injected rats, 1·2 ± 0·2 (10 lesions) in adrenalectomized rats injected with corticosterone, and 1·7 ± 0·4 (12 lesions) in untreated rats.

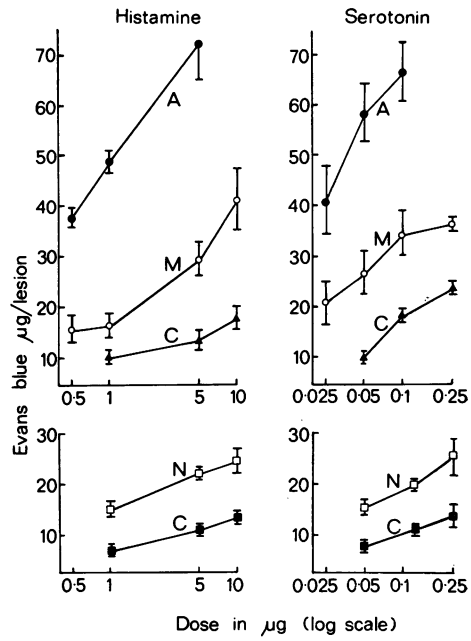

FIG. 2.—Effects of adrenalectomy and corticosterone on the permeability response to histamine and serotonin, estimated by extraction of exuded dye.

Upper blocks: Enhancement of permeability response in adrenalectomized rats (A) compared with the responses in mock adrenalectomized controls (M), and reversal of enhancement in adrenalectomized animals by subsequent administration of corticosterone (1 mg/rat; C).

Lower blocks: Decrease of permeability response in rats given corticosterone (1 mg/rat; C), compared with the response in untreated controls (N). The above results refer to the same lesion as in Fig. 1 and each point similarly corresponds to the mean ± s.e. mean for one lesion in each of 5 rats for the test groups, of one lesion in each of 10 animals for the mock adrenalectomized rats, and of one lesion in each of 6 animals for the untreated rats. Control injections of 0·1 ml of Ringer–Locke's solution induced a mean leakage of Evans blue (μg/lesion) ± s.e. mean of 5·9 ± 1·2 (10 lesions) in adrenalectomized rats, 4·8 ± 0·5 (20 lesions) in mock adrenalectomized rats, 5·5 ± 0·6 (10 lesions) in corticosterone injected rats, 4·0 ± 0·3 (10 lesions) in adrenalectomized rats injected with corticosterone, and 5·3 ± 0·4 (12 lesions) in untreated rats.

As the Table indicates, the results for mock adrenalectomized rats agree quite well with those for normal animals when assessed by lesion-diameter on the under surface of the skin (ebd). In terms of exuded dye (SBD), however, the PF potency of serotonin : histamine is greater in mock adrenalectomized than in normal rats. Nevertheless, this latter result does not detract from the value of mock adrenalectomized animals as appropriate controls because the PF responses are enhanced, not depressed (see below). Both the above methods have been used for the assessment of PF potency to ensure that changes in diameter of the lesions are associated with corresponding variations in intensity of exudation.

Effect of bilateral adrenalectomy on permeability responses to histamine and serotonin

Adrenalectomy considerably increases the PF responses to both histamine and serotonin. The increase in potency, estimated from dosage-response lines recording lesion diameter is approximately 7-fold for histamine and 4-fold for serotonin, compared with values obtained in mock adrenalectomized rats (Fig. 1). Assessed by extraction of dye from the same lesions, the corresponding enhancement of PF activity is about 13-fold for histamine and 6-fold for serotonin (Fig. 2).

Effect of corticosterone in normal rats

Administration of corticosterone (1 mg/rat) decreases the permeability response to both histamine and serotonin, whether the PF effect is assessed by measurement of lesion diameter or extraction of exuded dye. In the case of lesion diameter, the decrease in PF activity is about 8-fold for histamine and 5-fold for serotonin (Fig. 1). Assessed by extraction of dye, the decrease for the same lesions is about 13-fold for histamine and 6-fold for serotonin (Fig. 1).

Effect of corticosterone in adrenalectomized rats

Although the above results suggest strongly that corticosterone antagonizes the permeability response to histamine or serotonin, convincing evidence of this effect requires that the enhancement of the PF response in adrenalectomized rats be reversed by administration of corticosterone. A group of 5 rats was therefore adrenalectomized and subsequently given the standard dose of corticosterone 72–80 h after adrenalectomy. As illustrated in Fig. 1 and 2, corticosterone substantially reverses the PF effects of both amines. In fact, the animals given corticosterone become rather less reactive to histamine and serotonin than the corresponding mock adrenalectomized controls. However, such a result is not surprising since adrenalectomy deprives the animals of their physiological supply of corticosteroids and so might be expected to exaggerate the susceptibility of such animals to the subsequent administration of corticosterone.

DISCUSSION

The present results indicate that the intensity of the permeability response to histamine or serotonin is subject to the influence of adrenal cortical hormones. Corticosterone depresses the permeability effects of the above amines in normal animals, whereas the enhanced PF response in adrenalectomized animals is decreased by corticosterone to levels somewhat lower than in the control animals.

Furthermore, the intensity of the effects of adrenalectomy and corticosterone on the permeability responses to histamine and serotonin has a magnitude similar to that of the same procedures on the permeability response in carrageenin lesions of the rat's paw, as reported by Garcia Leme and Schapoval (1975). It also seems noteworthy that the order of effect on permeability responses has been similar whether the response is short-lived as for histamine and serotonin (Wilhelm

and Mason, 1960) or prolonged as in carrageenin lesions (Garcia Leme and Schapoval, 1975). The present work therefore supports the proposal of Garcia Leme and Schapoval (1975) that the adrenal cortex may well be a prime regulator of increased vascular permeability and the consequent exudation in acute inflammation.

Cortical hormones and adrenalectomy have long been known to modify the vascular reactions comprising the inflammatory response (Germuth, 1956). Cortisone was observed in the 1950s to induce vasoconstriction, resulting in decrease of blood flow and of vascular exudation (Ashton and Cook, 1952; Spain, Molomut and Haber, 1952; Moon and Tershakovic, 1952, 1954; Wyman *et al.*, 1954). Adrenal insufficiency was reported by Zweifach, Shorr and Black (1953) to impair vascular tone, the condition of the capillary bed in adrenalectomized rats being restored to normal by extracts of the adrenal cortex. On the clinical side, the report of Hench *et al.* (1949, 1950) that various clinical and biochemical features of rheumatoid disease are ameliorated by corticosteroids or adrenocorticotrophic hormone, has prompted a considerable volume of subsequent work on that condition.

Even before the 1950s, Ingle (1937) and Noble and Collip (1941) had noted that adrenalectomized rats were unduly susceptible to systemic histamine. In the rat, the secretion of the adrenal cortex is mainly, if not entirely, corticosterone (Bush, 1951). Bilateral adrenalectomy is followed by rapid disappearance of corticoids from the blood, the normal level being decreased by 95% in 4 h (Fortier, 1959). The present results concerning the respective effects of adrenalectomy and corticosterone on intracutaneous histamine in the rat therefore seem to explain the earlier findings of Ingle (1937) and of Noble and Collip (1941).

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REFERENCES

Ashton, N. & Cook, C. (1952) *In vivo* Observations of the Effects of Cortisone Upon the Blood Vessels in Rabbit Ear Chambers. *Br. J. exp. Path.*, **33**, 445.

Bush, E. I. (1951) Paper-chromatographic Study of the Secretion of the Adrenal Cortex in Various Mammalian Species. *J. Physiol. Lond.*, **115**, 12P.

Carr, J. & Wilhelm, D. L. (1964) The Evaluation of Increased Vascular Permeability in the Skin of Guinea-pigs. *Aust. J. exp. Biol. med. Sci.*, **42**, 511.

Fortier, C. (1959) Pituitary ACTH and Plasma Free Corticosteroids Following Bilateral Adrenalectomy in the Rat. *Proc. Soc. exp. Biol. Med.*, **100**, 13.

Garcia Leme, J. & Schapoval, E. E. S. (1975) Stimulation of the Hypothalamo–pituitary–adrenal Axis by Compounds Formed in Inflamed Tissue. *Br. J. Pharmac.*, **53**, 75.

Germuth, F. G., Jr (1956) The Role of Adrenocortical Steroids in Infection, Immunity and Hypersensitivity. *Pharmacol. Rev.*, **8**, 1.

Hench, P. S., Kendall, E. C., Slocumb, C. H. & Polley, H. F. (1949) The Effect of a Hormone of the Adrenal Cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of Pituitary Adrenocorticotrophic Hormone on Rheumatoid Arthritis. *Ann. rheum. Dis.*, **8**, 97.

Hench, P. S., Kendall, E. C., Slocumb, C. H. & Polley, H. F. (1950) Effects of Cortisone Acetate and Pituitary ACTH on Rheumatoid Arthritis, Rheumatic Fever and Certain Other Conditions. *Archs intern. Med.*, **85**, 545.

Ingle, D. J. (1937) Resistance of the Rat to Histamine Shock After Destruction of the Adrenal Medulla. *Am. J. Physiol.*, **118**, 57.

Lykke, A. W. J. & Cummings, R. (1969) Inflammation in Healing. 1. Time-course and Mediation of Exudation in Wound Healing in the Rat. *Br. J. exp. Path.*, **50**, 309.

Moon, V. H. & Tershakovec, G. A. (1952) Influence of Cortisone Upon Acute Inflammation. *Proc. Soc. exp. Biol. Med.*, **79**, 63.

Moon, V. H. & Tershakovec, G. A. (1954) Effect of Cortisone upon Local Capillary Permeability. *Proc. Soc. exp. Biol. Med.*, **85**, 600.

Mustard, J. F., Movat, H. Z., Macmorine, D. R. L. & Senyi, A. (1965) Release of Permeability Factors From Blood Platelet. *Proc. Soc. exp. Biol. Med.*, **119**, 988.

Noble, R. L. & Collip, J. B. (1941) The Response of Normal, Hypophysectomised and Adrenalectomised Rats to Histamine Administration. *Am. J. Physiol.*, **133**, 623.

Spain, D. M., Molomut, N. & Haber, A. (1952) Studies of the Cortisone Effects on the Inflammatory Response. *J. Lab. clin. Med.*, **39**, 383.

Wilhelm, D. L. & Mason, B. (1960) Vascular Permeability Changes in Inflammation: The Role of Endogenous Permeability Factors in Mild Thermal Injury. *Br. J. ex. Path.*, **41**, 487.

Wilhelm, D. L., Mill, P. J., Sparrow, E. M., Mackay, M. E. & Miles, A. A. (1958) Enzyme-like Globulins From Serum Reproducing the

Vascular Phenomena of Inflammation. IV. Activable Permeability Factor and Its Inhibitor in the Serum of the Rat and the Rabbit. *Br. J. exp. Path.*, **39**, 228.

WYMAN, L. C., FULTON, G. P., SCHULMAN, M. H. & SMITH, L. L. (1954) Vasoconstriction in the Cheek Pouch of the Hamster Following Treatment With Cortisone. *Am. J. Physiol.*, **176**, 335.

ZWEIFACH, B. W., SCHORR, E. & BLACK, M. M. (1953) The Influence of the Adrenal Cortex on Behaviour of Terminal Vascular Bed. *Ann. N.Y. Acad. Sci.*, **56**, 626.