Further Studies on Mechanism of Increased Capillary Permeability in Inflammation with the Aid of Cortisone and ACTH. (18859)

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It has been shown that adrenal cortical extract and cortisone suppress the increase in capillary permeability induced by leukotaxine or whole exudate(1,2). The increase in capillary permeability induced by commercial cortisone acetate is referable to the vehicle in which the cortisone is suspended and not to the material itself(3). More recently it has been demonstrated that whereas an alkaline exudate is inhibited from causing an increase in capillary permeability by cortisone(2), neither adrenal cortical extract nor cortisone have such an effect when the inflammatory reaction has attained an acid reaction. This is shown to be due to another factor released with the progress of an acute inflammation(4,5). This factor has been termed *exudin*(4). The combination of leukotaxine in the earlier stage of an inflammation and the liberation of exudin in the later phase of an acute inflammation offer a reasonable explanation for the mechanism of the increased capillary permeability throughout the duration of an acute inflammatory reaction. In early observations it was noted that the increase in capillary permeability induced by testicular extract was suppressed by adrenal cortical extract; however, the flare or spreading reaction was not inhibited(1). These observations have now been repeated with hyaluronidase. From 500 to 900 γ of hyaluronidase have been injected in conjunction with 2.5 mg of cortisone acetate. The injection of hyaluronidase was performed before, after or simultaneously with the cortisone acetate. These studies were made on several rabbits. It was found, for instance, that after injection of cortisone and hyaluronidase mixed together into the skin of the abdomen of rabbits, the subsequent intravascular injection of 5 cc of 1% trypan blue in saline is followed by no reduction in the spreading reaction. There seems at times to be a slight diminution in the intensity of increased capillary permeability as judged by the local accumulation of dye. The increase in capillary permeability often, but not always, follows the injection of such doses of hyaluronidase in the skin of the

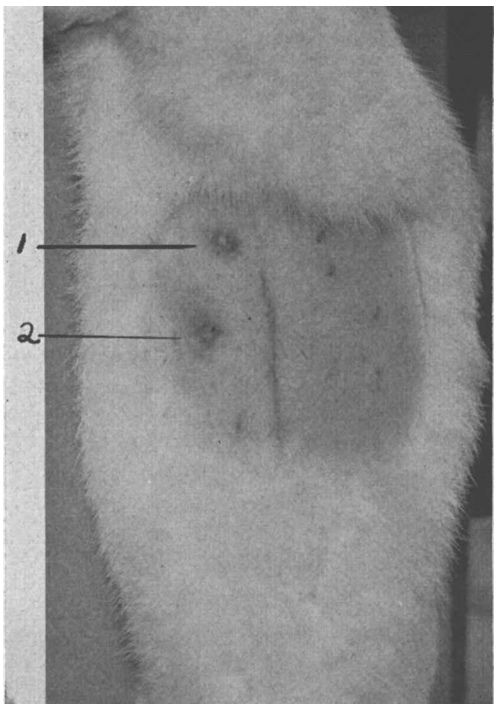

FIG. 1. Rabbit 302—Upper left hand (No. 1) represents effect on capillary permeability following injection of 2.5 mg of commercial cortisone acetate. Photograph was taken 1 hr after intrav. inj. of 5 cc of 1% trypan blue in saline and 1 hr and 13 min after the local inj. of cortisone. Lower left hand area (No. 2) represents combination of 2.5 mg of cortisone and 900 γ of hyaluronidase. Hyaluronidase was injected locally 2 min prior to injection of cortisone. Cortisone failed to abolish spreading reaction caused by hyaluronidase.

1. Menkin, V., *Am. J. Physiol.*, 1940, v129, 691.

2. Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 39.

3. Menkin, V., *Am. J. Physiol.*, 1951, v164, 294.

4. Menkin, V., *Fed. Proc.*, March 1951, v10, No. 1, Part 1.

5. Menkin, V., *Am. J. Physiol.*, September 1951, in press.

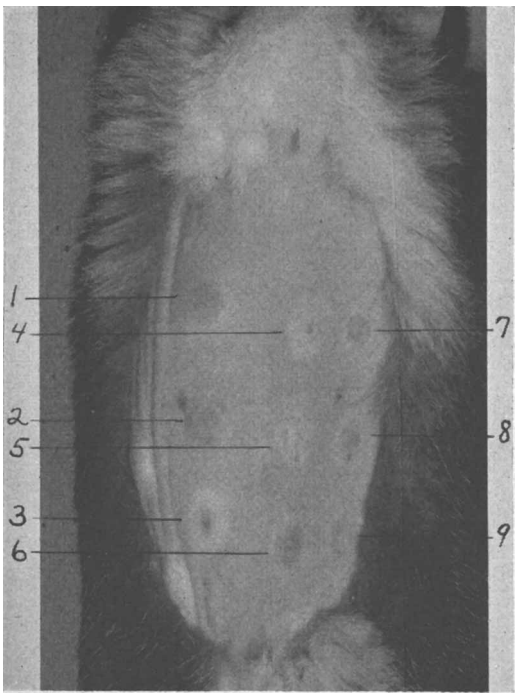

FIG. 2. Rabbit 296—Three rows of skin areas are shown as follows: *Left row*, reading up and down: Area 1—0.5 cc alkaline exudate from dog's pleural cavity. Sample of exudate is at pH 7.6. Note increase in capillary permeability to trypan blue as indicated by the local accumulation of dye. Area 2—0.5 cc of above exudate but converted to about pH 6.0 by addition of 10% lactic acid to sample. Although there is definite degree of necrosis by addition of lactic acid, the local accumulation of dye has occurred. Area 3—0.5 cc of adrenal cortical extract (Upjohn). Accumulation of dye has occurred only in center. *Middle row*, reading up and down: Area 4—0.5 cc from mixture of 0.5 cc of adrenal cortical extract plus 0.5 cc of alkaline exudate as used in Area 1. Note complete suppression of local increased capillary permeability as indicated by absence of dye. Area 5—0.5 cc from mixture of 0.5 cc of adrenal cortical extract plus 0.5 cc of converted exudate to about pH 6.0 as used in Area 2. In spite of acidity of exudative sample, the adrenal cortical material has to a large extent suppressed local accumulation of trypan blue from the circulation. Area 6—0.5 cc from mixture of canine exudate at pH 6.5 plus 0.5 cc saline. Local accumulation of dye, presumably referable to exudin present in acid sample of exudate. *Right row*, reading up and down: Area 7—Same as Area 6, but used only 0.3 cc of mixture. Effect is the same as in 6. Area 8—0.5 cc from mixture of acid exudate used in Areas 6 and 7 plus 0.5 cc of adrenal cortical extract. There is no suppressing effect to local increase in capillary permeability, referable to contained exudin in acid sample of exudate. Contrast this with suppressing effect of adrenal cortical extract utilized in Area 4 with an alkaline exudate, containing presumbly leukotaxine. Area 9—0.5 cc from mixture of acid exudate used in Areas 6, 7, and 8, plus about 700 γ of ACTH in 0.5 cc saline. There is some degree of suppression (*cf.* areas 6 and 9). Whatever dye accumulates in area may be due to some leukotaxine present in acid exudate and against the action of which, as far as only capillary permeability is concerned, ACTH seems impotent.

abdomen(6). The failure by cortisone to reduce the spreading reaction is illustrated in Fig. 1 (Rabbit 302). The details concerning the identification of exudin in inflammatory exudates which have attained an acid level have been described elsewhere(4,5). Exudin in contrast to leukotaxine is not suppressed by cortisone or adrenal cortical extract. This factor seems to be liberated in the final stage of the acute inflammatory reaction and its presence helps to explain the maintenance of capillary seepage during the period of the acute inflammatory reaction.

The acidity of the exudative material seems to contribute little to the effectiveness of exudin. An alkaline exudate can be converted from pH 7.6 to a pH of about 6.0 by the addition of 10% lactic acid, but nevertheless adrenal cortical extract will behave as if one deals with an alkaline exudate, *i.e.* by suppressing its capacity to induce increased capillary permeability. When exudin is present, the extract of the adrenal cortex is incapable of repressing the increase in capillary permeability. This is well illustrated in Fig. 2 (Rabbit 296). Conversely when an acid exudate, containing exudin, is rendered alkaline by the addition of .01 N NaOH, the resulting exudative sample is not repressed by adrenal cortical extract. It therefore seems as if exudin is primarily liberated at a stage which happens to coincide with the developing acidity in the inflamed area. The presence of exudin, in conjunction with the initial production of leukotaxine, offers a reasonable explanation for the increased capillary permeability throughout the length of the inflammatory process. The methods of isolation of exudin have been described elsewhere and will not be reiterated here(4,5).

A few of its biological and chemical properties may, however, be listed. In the first

6. Menkin, V., *Newer Concepts of Inflammation*, C. Thomas, Springfield, Ill., 1950.

place exudin, similarly to leukotaxine, is not to be identified with histamine. Exudin is non-diffusible in contrast to histamine. It fails to produce the same pattern of tissue staining as with histamine(5). Finally, exudin when extracted for histamine by the method of Barsoum and Gaddum(7) fails to contract the isolated segment of guinea pig intestine. Subsequent addition of 1 mg histamine phosphate is accompanied by a marked contraction of the strip of intestine. This indicates that the absence of any refractoriness to the subsequent administration of histamine rules out that exudin is histamine.

The total nitrogen of exudin has been determined by the method of Pregl(8). The results obtained on 7 samples, as measured by Dr. W. Kalnins, may be tabulated as follows:

Nitrogen (%)
10.080
9.230
10.876
9.880
10.974
10.960
10.730
Avg 10.39 % N

The amino nitrogen values of exudin before hydrolysis and after hydrolysis have been determined by the copper method of Albanese and Irby(9). The findings can be listed as follows:

Amino nitrogen	
Before hydrolysis, mg/cc	After hydrolysis, mg/cc
.00336	.00746
.00317	.00746
.02260	.05690
.01306	.02615
.00376	.00933
.00466	.00746
.00460	.00746
.00280	.00653
.00420	.00933
Avg .00691	.01534

7. Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, v85, 1.

8. Pregl, F., *Quantitative Organic Microanalysis*, P. Blakiston's Son and Co., Inc., Philadelphia, 1937.

9. Albanese, A. A. and Irby, V., *J. Biol. Chem.*, 1944, v153, 583.

In brief, the available chemical data do not preclude the possibility that in exudin one is dealing with a dipeptide with a possible prosthetic group attached to it. The Biuret test is positive. However, further chemical studies will, it is hoped, yield more information as to the more precise nature of exudin.

Thus far it has been found that only one substance inhibits the increased capillary permeability caused by exudin. This is ACTH. Not only does ACTH inhibit locally the increased capillary permeability induced by exudin, as described elsewhere(10), but ACTH, in saline intravenously injected immediately prior to the local injection of exudin, likewise represses the increased capillary permeability elicited by the cutaneous injection of exudin in rabbits. The doses of ACTH employed ranged from slightly over 1 mg to 40 mg. Death at times followed the very large doses of ACTH. The data on 9 experiments are assembled in Table I.

It is evident from the results that the preliminary intravenous injection of ACTH suppresses in rabbits, the increased capillary permeability to trypan blue. A clear suppressing effect is detected when 10 mg of ACTH in saline is injected intravenously. This seems to be the threshold dose. When 35-40 mg of ACTH were administered, the lethal effect which sometimes follows seems referable to some type of intoxication. This part of the study requires further study. For instance, the blood flow with such large doses of ACTH has not been measured. When an acid exudate (Table I) is used besides exudin, it is found that intravenous injections of ACTH likewise reduce the increased capillary permeability to trypan blue. Presumably an acid exudate contains exudin, and this may well account for the effect obtained. When an exudate with an alkaline reaction is utilized the inhibiting effect of intravenously injected ACTH is not marked as with an acid exudate (Table I). It is probable that the presence of leukotaxine in an alkaline exudate may explain in part the incomplete suppressing ac-

10. Menkin, V., *Am. J. Physiol.*, September 1951, in press.

TABLE I. Suppressing Effect of ACTH on Local Increased Capillary Permeability Induced by Exudin When ACTH Is Injected Intravenously.

Exp. No.	Dose of ACTH or saline inj. intrav.	Readings on accumulation within about ½ hr after intrav. inj. trypan blue in exudin-treated skin areas: Experimental rabbits	Control rabbits
Exp. 1	1.4 mg ACTH in .9 cc saline	2+‡	
Control	.9 cc saline		3+
Exp. 2*	20 mg ACTH in 2 cc saline	0 in center of area; + to 2+ at periphery	
Control	2 cc saline		4+ at periphery
Exp. 3	20 mg ACTH in 2 cc saline	2+ in one area; trace in another area	
Control	2 cc saline		4+
Exp. 4	20 mg ACTH in 2 cc saline	+ at periphery	
Control	2 cc saline		2+ to 3+
Exp. 5	40 mg ACTH in 4 cc saline	0	
Control	4 cc saline		4+
Exp. 6 (.5 cc canine exudate–pH 7.2)	20 mg ACTH in 2 cc saline	Trace at periphery + to 2+	
Control (.5 cc canine exudate–pH 7.4)	2 cc saline		3+
			3+
Exp. 7† (.5 cc canine exudate–pH 6)	35-40 mg ACTH in ± 4 cc saline	0	
		0	
Control (.5 cc canine exudate–pH 6)	4 cc saline		3+
			4+
Exp. 8† (±.5 cc canine exudate–pH 6)	36 mg ACTH in 3.6 cc saline	0	
		0	
Control (.5 cc canine exudate–pH 6)	3.6 cc saline		2+
			3+
Exp. 9 (.5 cc canine exudate–pH 6)	10 mg ACTH in 1 cc saline	+ to 2+	
		2+	
Control (.5 cc canine exudate–pH 6)	1 cc saline		4+
			5+

* Exudin utilized is in aqueous medium; in other experiments exudin is diluted with physiological saline (1:1) to approach isotonicity.

† These rabbits succumbed about 1 hr or over after the administration of 35-40 mg of ACTH. It is probable that death was referable to the large dose of ACTH injected. In one of those rabbits which was thoroughly autopsied one adrenal could not be found, and the other adrenal appeared as a mass of incongruous material which appeared, in the gross, very hemorrhagic and succulent unlike normal adrenal tissue.

‡ The number of plus signs (+) refers to the extent of trypan blue accumulation into the treated skin areas from the circulating blood.

tivity of intravenously injected ACTH. In other words, cortisone inhibits the increase in capillary permeability caused by leukotaxine, whereas ACTH represses the increase in capillary permeability caused by another component liberated in an inflamed area, namely exudin. The suppressing effect of ACTH on exudin injected intravenously is illustrated in Fig. 3 (Exp. 5, Table I).

The significance of intravenously injected ACTH towards our further comprehension of the favorable effect of this substance in

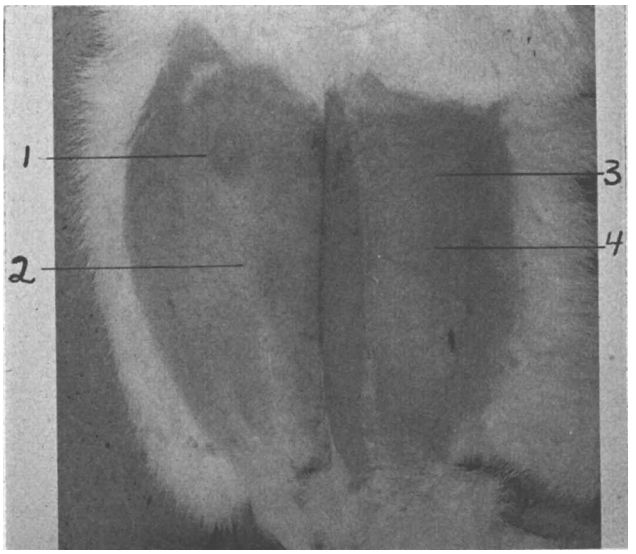

FIG. 3. Rabbits 1076 and 1077 (Exp. 5, Table I)—Two rabbits are placed side by side. *Right hand animal.* Has received 40 mg of ACTH intravenously in 4 cc of saline. 12 min later 0.5 cc of exudin was injected in areas No. 3 and 4 of skin of the abdomen. 6 min later about 5 cc of 1% trypan blue was injected intravenously. Even 25 min later there was no accumulation in exudin-treated skin area. Apparently systemic injection of ACTH suppressed the local accumulation of trypan blue injected intravenously. The animal was still alive 5 days later. *Animal on the left side.* Control rabbit (Rabbit 1076) was injected with 4 cc of saline intravenously. Two min later exudin was injected locally in skin areas No. 1 and 2 of the abdomen. 19 min later about 5 cc of 1% trypan blue in saline was injected intravenously. Two min later, there was local accumulation of the dye. This was very marked in 25-30 min. The photograph of both rabbits was taken about 2 hr later.

various infectious processes is suggestive. It is conceivable that by accumulating in a focus of inflammation from the blood stream, ACTH may reduce the effectiveness of the inflammatory process by mechanisms described in the present paper and elsewhere(11).

Summary and conclusions. Cortisone fails to obliterate the spreading reaction caused by hyaluronidase. This substance seems to inhibit at times somewhat the increased capillary permeability induced by 500 to 900 γ of hyaluronidase. An alkaline exudate or leukotaxine induces an increase in the local capillary permeability. Thus, as shown(1,2), the materials are inhibited by adrenal cortical extract or by cortisone. When, however, the exudate is acid in reaction, the local increased capillary permeability is not repressed by either cortisone or by adrenal cortical extract. This is shown to be referable to another factor liberated in the later stages of an acute inflammatory reaction. This substance is concerned with the mechanism of increased capillary permeability in inflammation. This factor is termed "exudin." Consequently the initial increase in capillary permeability in the development of inflammation is referable to leukotaxine. With the progress of the acute inflammation the pH of the exudate becomes acid and the mechanism of sustained increased capillary permeability is referable to exudin. The degree of acidity does not seem to be the cause of exudin formation. Exudin seems rather to be liberated about concomitantly with the development of an acid reaction in the inflamed area. The nitrogen content of exudin is about 10%. The amino nitrogen values before and after hydrolysis suggest that one is dealing possibly with a dipeptide to which there may be a prosthetic group attached. Further studies, however, will perhaps throw more light on this point. Exudin, tested for the presence of histamine, fails to contract

11. Menkin, V., *Dynamics of Inflammation,* Macmillan Co., New York, 1940.

the strip of guinea pig intestine. When extracted for histamine by the method of Barsoum and Gaddum, there is no evidence that exudin contains any histamine. Its behavior toward cortisone and its indiffusibility indicate that leukotaxine and histamine are dissimilar in nature to exudin. Finally, the intravenous injection of ACTH suppresses the local increase in capillary permeability referable to exudin. The possibility of these findings in regard to a further understanding of the mechanism of ACTH in various infectious conditions are discussed.

Received May 28, 1951. P.S.E.B.M., 1951, v77.

Dermal Spreading of Hyaluronidase as Influenced by Prolonged Local Treatment with Certain Steroid Hormones.* (18860)

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Previous reports from this laboratory have demonstrated the increase in rate and alteration in pattern of dermal spreading by hyaluronidase as influenced by prolonged local (1) and parenteral (2) treatment with adrenocortical extract. As a result of these studies, it was desirable to examine the effects on enzymatic spreading of prolonged local pretreatment with some individual steroids.

Materials and methods. The steroids chosen for investigation were desoxycorticosterone,† 11-dehydro-17-hydroxycorticosterone (cortisone)† and progesterone.† The first 2 were supplied in the free alcohol form and were dissolved in 25% alcohol at a concentration of 1 mg/cc. The progesterone was dissolved in 50% alcohol at the same concentration. Twenty-one litter mate adult male rats of the Long-Evans strain in the weight range of 295 ± 75 g were used. For 5 months 0.1 cc of the desoxycorticosterone solution in 25% alcohol was applied daily to the dorsal cervical region just caudal to the right ear of 6 animals covering an area of approximately 10 sq cm; identical applications of the cortisone solution were made on 6 other animals. Four control animals were treated in a comparable site with 25% alcohol. Three animals were treated in the same manner and dosage with the progesterone preparation and their 2 controls received applications of 50% alcohol. In all 5 groups the left side of each animal was left untreated. Body weights were determined and regrown hair on the neck was clipped at weekly intervals after hair growth patterns had been recorded (3). The technic employed for study of the spreading action of hyaluronidase has been described in detail by Hayes and Reed (4). The hair was clipped closely from the proposed injection sites on each side of the neck and the animals were secured loosely for constancy of position. The animals were quieted by the subcutaneous injection of 0.3 cc of a veterinary solution of sodium pentobarbital (6.5% solution) and were anesthetized lightly with ether only for the duration of the initial injection. The indicator employed was an isotonic solution of hemoglobin. The final injection quantity was 0.05 cc containing 25 units of hyaluronidase.†

* Supported by the Lawrence J. Montgomery Research Fund and a grant from G. D. Searle and Co.

1. Hayes, M. A., Reed, T. G., and Baker, B. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 361.

2. Hayes, M. A., and Baker, B. L., in press.

† The authors wish to express their gratitude to Dr. I. C. Winter, G. D. Searle Co., Chicago, Ill. for the hyaluronidase (Alidase), to Dr. J. M. Carlisle, Merck and Co., Inc., Rahway, N. J., for the 11-dehydro-17-hydroxycorticosterone, to Dr. Edw. Henderson, Schering Corp., Bloomfield, N. J., for the desoxycorticosterone and to Dr. F. E. Houghton, Ciba Pharmaceutical Products, Summit, N. J., for the progesterone.

3. Butcher, E. O., *Anat. Rec.*, 1934, v61, 5.

4. Hayes, M. A. and Reed, T. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 357.