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Further Studies on Effect of Adrenal Cortex Extract and of Various Steroids on Capillary Permeability.*

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The writer has recently demonstrated that the extract of the adrenal cortex tends to inhibit the increased capillary permeability induced by either leukotaxine or an inflammatory exudate.[1] These observations have been interpreted as substantiating further the view that the hormone of the adrenal cortex perhaps plays a significant rôle in the regulation of fluid exchange as manifested by capillary filtration. These studies have been subsequently confirmed by Freed and Lindner.[2] The studies of the writer have primarily concerned themselves with the effect on capillary permeability of the adrenal cortex extract. At the time a footnote merely stated that preliminary observations had been undertaken with percorten, a synthetic preparation of desoxycorticosterone acetate.[1] This material seems to yield results somewhat similar to the effect elicited by the extract of the adrenal cortex. Freed and Lindner have failed to corroborate these preliminary observations on the inhibitory tendency of desoxycorticosterone acetate, although, as stated above, their own experiments with the crude extract of the gland have fully confirmed the writer's observations.[2] Fine and Fischmann have recently reported that desoxycorticosterone acetate does not seem to exert any beneficial effect in shock resulting from hemorrhage.[3] These investigators have been unable to obtain any inhibition of the seepage of trypan blue from the circulation into tissue when desoxycorticosterone acetate (percorten) is injected intravenously instead of introducing the steroid preparation locally. Their findings are not in the least surprising inasmuch as these workers have failed to follow the cutaneous route of injection of the hormone as originally described by the writer.[1] In general, the interpretation of Fine and Fischmann is open to doubt especially in view of their statement that trypan blue is not excreted by the kidneys.[3] The writer has frequently studied the urine of rabbits injected intravenously with trypan blue. The dye can readily be recovered in abundance in the urine.[4]

The various crystalline steroids of the adrenal cortex are soluble in oil. Freed and Lindner[2] merely suspended desoxycorticosterone acetate and corticosterone in physiological saline or in saline-gum acacia solution. It occurred to the writer that their negative results might very well have been referable to the insolubility in water of the preparations injected. However, since adrenal cortex extract is a solution, the results with this material, as obtained by these investigators, have fully confirmed our own original observations.[1] Furthermore, the writer has utilized a preparation of desoxycorticosterone acetate dissolved in sesame oil (percorten), whereas Freed and Lindner used the insoluble suspended material.† The present communication represents the results of observations which support the view that the adrenal cortex extract inhibits increased capillary permeability induced by leukotaxine or exudate. A similar effect is obtained with some of the steroid derivatives

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1 Menkin, V., *Am. J. Physiol.*, 1940, **129**, 691.

2 Freed, S. C., and Lindner, E., *Am. J. Physiol.*, 1941, **134**, 258.

3 Fine, J., and Fischmann, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 98.

4 Menkin, V., unpublished studies.

† The synthetic preparation of desoxycorticosterone acetate, known as percorten, was obtained through the courtesy of Dr. Oppenheimer of the Ciba Pharmaceutical Products, Inc., in Summit, N.J.

TABLE I.
Inhibitory Effect of Various Compounds of the Adrenal Cortex on Induced Increased Capillary Permeability.

Rabbit No.	Accumulation of dye in control areas containing leukotaxine in sesame oil or exudate in sesame oil	Accumulation of dye into skin areas treated with leukotaxine or whole exudate in addition to: Desoxycorticosterone acetate in sesame oil (percorten)	Compound A (Kendall) in sesame oil	Compound B (Kendall) (corticosterone) in sesame oil	Compound E (Kendall) in sesame oil
21-89	++	trace	trace	0	trace to +
21-73	+++	0 throughout; faint trace at periphery	trace in center; + at periphery	trace to + primarily at periphery	++ to +++
21-88*	+		0		0
	++		0	+	0
21-94	+++	+			
3-56	++ to +++	0 throughout; + at periphery			
20-65†	trace to +	0			
20-22‡	++	0			
	+++	0 in center; ++ at periphery			
20-56§	++	0			
	++++	++			

*In this animal the accumulation of dye in the cutaneous areas was negligible but fairly adequate readings were made in the subcutaneous region.

†Final readings made within 17 minutes, whereas in all other experiments the time of observation lasted from a half hour to one hour when the final readings were recorded.

‡Leukotaxine dissolved in saline in this experiment instead of emulsifying with sesame oil as in the other experiments.

§Whole exudate instead of leukotaxine utilized in this experiment.

of the adrenal cortex provided that these compounds are first dissolved in a suitable medium, such as sesame oil. If, however, they are suspended either in saline or serum there follows no inhibitory tendency to increased capillary permeability.

The technique of cutaneous injection and of intravenous trypan blue administration has been described previously.[1] Leukotaxine was isolated from inflammatory exudate.[5] 11-dehydrocorticosterone (Compound A), corticosterone (Compound B), and 17-hydroxy-11-dehydrocorticosterone (Compound E) were obtained through the courtesy of Dr. E. C. Kendall.[6] Percorten, a soluble preparation of desoxycorticosterone acetate in sesame oil, was obtained from Ciba. In accordance with the kind suggestion of Dr. E. C. Kendall, Compounds A, B, and E were brought into solution by adopting the following procedure: Approximately 1 to 2 mg of each fraction was dissolved in a half cm^3 of absolute alcohol. This was followed by the addition of a half cm^3 of sesame oil. The vials with the material were then placed in a vacuum oven and the contained alcohol evaporated at room temperature. About 2.5 mg of leukotaxine or 0.3 cc of exudate was then carefully suspended in the sesame oil containing the steroid fraction. The mixture was injected intracutaneously in the skin of the abdomen of a rabbit, and the experiment carried out as previously described.[1] Cutaneous areas were also injected with a similar amount either of leukotaxine suspended in sesame oil or of exudate emulsified with this medium. These areas served as controls to the ones containing in addition the fractions of the adrenal cortex. 2.5 mg of percorten or desoxycorticosterone acetate dissolved in sesame oil was also employed in a number of experiments.

The results are summarized in Table I. It is quite clear that desoxycorticosterone acetate, Compounds A, B, and E dissolved

[5] Menkin, V., *Dynamics of Inflammation*, Macmillan Company, New York, 1940.

[6] Kendall, E. C., *J. Biol. Chem.*, 1940, **133**, Li (Proc. Am. Soc. of Biol. Chem., Mar., 1940); *Arch. Path.*, 1941, **32**, 474.

TABLE II.
Effect of Adrenal Cortex Extract and of Some Aqueous Insoluble Crystalline Derivatives on Induced Increased Capillary Permeability

Rabbit No.	Accumulation of dye in control areas containing either (1) leukotaxine in saline or serum; or (2) exudate + saline	Accumulation of dye into skin areas treated with leukotaxine or whole exudate in addition to: Extract of adrenal cortex	Compound B (Kendall's corticosterone) suspended in saline	Compound E (Kendall) suspended in saline	Desoxycorticosterone acetate suspended in saline or serum
21-63	+++	0	++ to +++	++ to +++	
21-77	+	0	++	++ to +++	
21-78	+		+		
21-79	+++				+++
20-43*	++				++; ++; ++ (3 areas tested)
24-69	+	0			
21-38†	++++	faint trace			
21-95‡	++ to +++	0			
21-97‡	+++	0			

*Materials suspended in serum instead of physiological saline.
†Extract of adrenal cortex obtained from Wilson Company; in all other experiments the extract was furnished by Dr. E. C. Kendall.
‡Whole exudate employed instead of leukotaxine.

in sesame oil tend to inhibit the marked increase in capillary permeability induced by leukotaxine or exudative material.

On the contrary, desoxycorticosterone acetate crystals‡ suspended in serum or saline; or compounds B and E likewise suspended in saline in concentrations approximating those described above failed to inhibit the enhanced capillary permeability induced by leukotaxine. The results are conveniently summarized in Table II. Furthermore, it is also clear that in contrast to the insoluble steroid preparations, the crude extract of the adrenal cortex, as found previously, markedly inhibits capillary permeability.[1] In general, the effect on the reduced filtration of dye through the capillary wall is definitely more pronounced with the adrenal cortex extract than with the use of crystalline steroids dissolved in oil. (*cf.* Tables I and II).

Conclusions. The extract of the adrenal cortex inhibits the increased capillary permeability induced either by an inflammatory exudate or by leukotaxine, in turn isolated from exudative material. A similar tendency, although generally not quite as pronounced, can be elicited by various steroid fractions such as synthetic desoxycorticosterone acetate (percorten) or Kendall's Compounds A, B, and E derived from the adrenal cortex, provided that these substances prior to assay are dissolved in an oily vehicle. Mere suspension of the steroids in saline or serum fails to reveal any inhibitory activity on capillary permeability.

‡ Obtained through the courtesy of the Research Division of the Schering Corporation, Bloomfield, N.J.