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Adrenal Ascorbic Acid Depletion Without Eosinopenia in Hypophysectomized Rats Injected Intrasplenically with Corticotrophin

By Frederic A. Giere and W. J. Eversole

Several workers (Greenspan *et al.*, 1950; Reinhardt and Li, 1951; Stack-Dunne and Young, 1951) have reported a lack of correlation between the eosinopenic, adrenal ascorbic acid lowering activity, and maintenance of adrenal weights with various adrenocorticotrophic preparations. Reinhardt *et al.* (1951) have critically evaluated assay methods for adrenocorticotrophic hormone (ACTH) and cautioned that differences in rate of absorption, variations in route of administration and dose-time-response relationships may influence the results obtained with different testing procedures. The study reported here was conducted under standardized conditions to determine whether the route of administration would influence the eosinopenic and adrenal ascorbic acid depleting properties of two ACTH preparations.

Hypophysectomized male Sprague-Dawley rats weighing approximately 170 grams were used in groups of eights, 24 to 48 hours post-hypophysectomy. Animals were injected with ACTH either intrasplenically or subcutaneously. The ACTH was dissolved in 0.9% sodium chloride; the volume of injected fluid was 0.2 ml. in all cases. Those animals that were injected with ACTH subcutaneously received saline intrasplenically and those injected with ACTH intrasplenically received saline subcutaneously. This procedure controlled the possibility of changes in eosinophil counts due to mechanical flushing of the spleen. The procedure employed in obtaining the adrenals for ascorbic acid determinations was that of Sayers, Sayers, and Woodbury (1948). Each rat was anesthetized with pentobarbital sodium, one adrenal was removed for ascorbic acid determination and the spleen was exposed for injection. After injection the spleen was returned to the peritoneal cavity and the incision was closed. Additional hypophysectomized animals received injections of ACTH *via* the femoral vein. In all cases the left adrenal was removed just prior to hormone injection and the right adrenal was removed one hour after injection. Ascorbic acid concentration of the adrenals was determined by the method of Roe and Kuether (1943). A sample of tail blood was taken simultaneously with removal of each adrenal. The blood was diluted with phloxine-propylene glycol diluting fluid and eosinophil counts made according to the procedure recommended by Henneman, Wexler, and Westenhaver (1949). The criteria used in determining

the effectiveness of the ACTH preparations were changes in eosinophil counts and adrenal ascorbic acid concentrations.

Table I summarizes the results. There was depletion of adrenal ascorbic acid with all quantities of both ACTH preparations regardless of the site of injection. In experiments with sufficient numbers of cases for statistical analyses the concentration of adrenal ascorbic acid in the "treated" right adrenal was significantly lower than in the control left adrenal ($P<0.01$). Subcutaneous or intravenous administration of ACTH, exception four micrograms given intravenously, resulted in a fall in eosinophils; when four micrograms were given intravenously the fall in eosinophils was negligible. Intrasplenic injection of ACTH at all dosage levels resulted in no significant changes in eosinophil counts.

Cheng, Sayers, Goodman and Swinyard (1949) have reported adrenal ascorbic acid depletion after histamine injection in rats bearing adenohypophysis transplants to the spleen. Our data supports their conclusion that absorption in the spleen or passage through the liver does not alter the adrenal ascorbic acid depleting properties of ACTH. However, our experiments do indicate that the eosinopenic activity of ACTH is modified by passage through the liver and/or spleen. Reinhardt, Hungerford, and Li (1951) have found that there is no obvious close correlation between the eosinopenic activity of either ACTH protein or peptide and the degree of adrenal ascorbic acid lowering activity. In our experiments ACTH, when injected subcutaneously, consistantly caused a drop in eosinophils but did not cause a drop when injected intrasplenically. One possible explanation for such findings is that the spleen and/or the liver modified the ACTH preparations used here in such a fashion as to allow an adrenal ascorbic acid depleting factor to escape into the general circulation but retained or inactivated a fraction involved in lowering the eosinophils. Such an interpretation would be compatible with that of Hungerford, *et al.,* (1952) who, on the basis of their findings, suggested that an eosinopenic factor may be a separate component of ACTH different from an adrenal ascorbic acid depleting factor. Alternatively, it is possible that, in our experiments, a time interval greater than one hour would be necessary for intrasplenically administered ACTH to cause an eosinopenia. Our finding that it requires smaller amounts of intravenously administered ACTH to cause a lowering of the adrenal ascorbic acid than it does to cause an eosinopenia may also have a bearing on the results obtained after intrasplenic administration. It may be that the spleen and liver acted as a filtering device and allowed only that amount of ACTH to reach the general circulation that would affect adrenal ascorbic acid but not eosinophils.

Table 1.

Effects of route of administration on activity of ACTH.

Values are given as mean ± S. E. (IS=Intrasplenic; IV=Intravenous; SQ=Subcutaneous)

Dosage	Method of Administration	No. Rats	Adrenal Ascorbic Acid expressed as mg %		% Change	Eosinophils /cmm		% Change
			Lt. Adr. Before ACTH	Rt. Adr. After ACTH		Before ACTH	After ACTH	
Lot 71-50 R								
9 mg	IS	6	440±21.8	245±17.2	−44	241±30.0	280±39.1	+16
9 mg	SQ	7	397± 7.2	226±10.4	−43	317±33.9	221±49.7	−30
Lot 128-105R								
8 mg	IS	4	431±14.6	271± 8.9	−37	220±43.1	247±47.4	+12
4 mg	IS	1	300	163	−45	133	111	−16
2 mg	IS	2	328± 4.0	185± 5.0	−44	122±11.0	105±17.0	−14
1 mg	IS	4	366±10.2	227±30.7	−38	151±12.2	156±22.4	− 3
16 gamma	IS	5	391± 9.7	237±10.5	−43	349±17.9	341±24.2	− 2
1 mg	IV	3	404± 7.0	231±17.4	−39	117±13.2	92±13.2	−21
16 gamma	IV	7	345±19.9	211±10.1	−39	183±41.1	126±29.5	−31
4 gamma	IV	4	426±13.8	280± 7.6	−34	231±30.8	222±35.1	− 4

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