Effect of ACTH on Permeability of Adrenal Cells to Sugar.*† (25577)

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The view that the mechanism of adrenocorticotropic hormone (ACTH) action upon the adrenal cortex(1), like that of insulin in muscle(2), may involve regulation of cellular permeability processes is being systematically investigated in this laboratory. We briefly reported(3) that D-xylose is excluded from a major fraction of cell water of the adrenal gland in hypophysectomized rats, and that ACTH administration increases D-xylose distribution in adrenal tissue, but not in muscle. Insulin, on the other hand, increases D-xylose distribution in muscle but has no effect in the adrenal. In this paper, detailed results on the effect of ACTH upon adrenal cell permeability to D-xylose, mannitol, sucrose and inulin are presented.

Methods. The studies were conducted using male rats of Sprague-Dawley strain, weighing 120-170 g, with the pituitary either intact or removed 42-48 hr earlier. Animals were anesthetized with avertin (1 ml of 2% solution/100 g body wt, intraperitoneally), and the renal pedicle was ligated close to the hilum using care not to occlude adrenal blood flow. Immediately thereafter, one of the following C^{14}-labeled substances was injected subcutaneously: D-xylose-1-C^{14} (Nat'l. Bureau of Standards), mannitol-1,6-C^{14} (Volk Radiochemicals), sucrose-U-C^{14} and inulin-carboxyl-C^{14} (both from New England Nuclear). Unless otherwise stated, hormonal preparations were injected immediately prior to administration of tracer substance. Corticotropin A (Armour), corticotropin α_b (Li), and β-corticotropin (Bell) were given intravenously (I.V.) in 0.3 ml N/100 HCl-saline; insulin (Iletin, Lilly) intraperitoneally in glucose-saline (using 100 mg glucose/100 g to prevent hypoglycemia), controls for this group received 25 mg glucose/100 g; corticosterone was given in divided dose in 10% ethanol, 50% I.V. and 50% subcutaneously; and bovine growth hormone (Li), I.V. in saline. At varying intervals after administration of radiotracers, the rats were anesthetized with nembutal, blood drawn into a heparinized syringe from the abdominal aorta, and plasma separated immediately. Simultaneously, the animals were exsanguinated and tissues removed, blotted, and weighed. Adrenals were weighed after removal of capsules. In various experiments, samples of diaphragm, gastrocnemius, liver, epididymal fat, and testes were excised. Tracer substances were extracted from tissues by boiling in water for 15 minutes(4,5) and extracts and diluted plasma (1:50) were deionized with the mixed exchange resin Amberlite MB3 (Lamotte Model X) which had been dried at 80° for 20 hr (in control experiments no loss of tracers occurred, including carboxyl labeled inulin). Extracts were planchetted and counted in a flow gas chamber.

Results are presented in terms of volume of distribution of test substance in total tissue, expressed as percent of total tissue wet weight in equilibrium with plasma concentration. Table I presents volumes of distribution of D-xylose in adrenals at 60, 90 and 120 min. after administration to functionally nephrectomized, hypophysectomized or intact rats. Fig. 1 plots individual distribution values shown in Table I against plasma concentration of D-xylose. Over a wide range of plasma levels of D-xylose, distribution values of both intact and hypophysectomized groups remain quite constant within each group. Moreover, an essentially steady equilibrium exists throughout 60′ to 120′ independent of whether the pituitary is intact or removed. These data are consistent with the idea that transfer of a "non-utilizable" sugar into adrenal tissue is by diffusion into a compartment where entry is directly proportional to external concentration. Average volume of distribution of D-xylose in adrenals of intact rats, which

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TABLE I. Volumes of Distribution of D-xylose* in Adrenal Tissue at 60, 90, and 120 Minutes after Tracer Administration in Intact or Hypophysectomized Rats.

Time interval (min.)	Intact pituitary (%)	Hypophysectomized (%)
60	59.0 ± 2.0 (7)	28.4 ± 1.0 (15)
90	61.2 ± 2.2 (22)	33.0 ± 1.1 (19)
120	62.9 ± 2.0 (7)	31.0 ± 3.1 (6)

* D-xylose-1-C^{14}, 800 to 3500 μg/100 g body wt.

had been stressed by the operative procedure, was 59-63% over 60-120 min. covered in Table I. After hypophysectomy, distribution values fell markedly to 28-33%, indicating that xylose is excluded from a large fraction of adrenal tissue in absence of the pituitary.

Table II presents volumes of distribution at 90 min. of various radiotracers in adrenals of functionally nephrectomized hypophysectomized rats treated with various corticotropins, as well as other hormones. An increase in adrenal distribution of D-xylose was achieved in hypophysectomized rats with exogenous ACTH: corticotropin A increased distribution from 30% to 51% ($p = <.001$), corticotropin α_b (Li) to 42% ($p = <.001$) and 36 mU/100 g of Bell's β-corticotropin to 36% ($p = <.05$). Table II also shows that insulin and corticosterone in doses tested had no effect on xylose distribution, despite a concurrent increase with insulin in xylose distribution in diaphragm from 33% ± 3 (S.E.) to 115% ± 46, and in gastrocnemius from 18% ± 1 to 50% ± 5. Li growth hormone given alone (10 μg) or with submaximal dos-

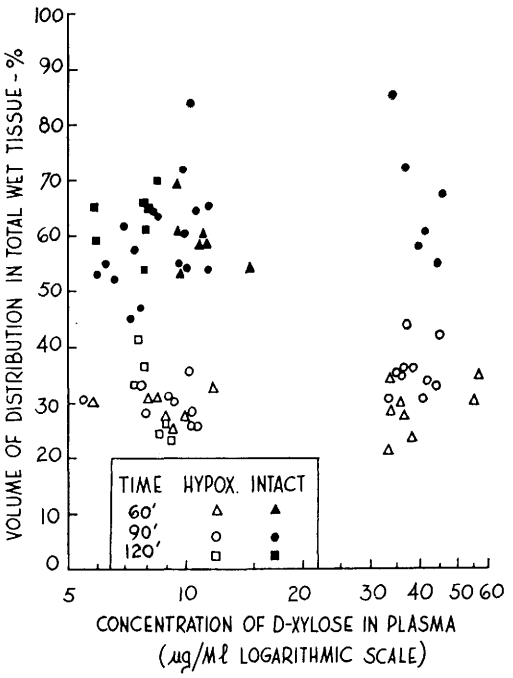

FIG. 1. Volume of distribution at various plasma concentrations of D-xylose in adrenals of functionally nephrectomized hypophysectomized or intact rats 60, 90, and 120 min. after administration.

ages of ACTH (30 μg growth hormone, 36 mU β-corticotropin) did not affect adrenal distribution of xylose. Table II also shows that spaces occupied by sucrose, mannitol, and inulin were not identical, as would be expected if all were true measures of extracellular volume. The inulin space was not affected by ACTH administration nor by factors operating in stressed rats with intact pituitaries. Using

TABLE II. Effect of Various Hormones on Volumes of Distribution of Tracer Substances in Adrenals of Functionally Nephrectomized Rats.

	Treatment (with dosage/100 g body wt)	Vol of distribution* of various radiotracers† in adrenal: D-xylose	Inulin	Sucrose	Mannitol
	Pituitary intact	59.3 ± 2.5 (16)	14.0 ±.4 (9)	18.2 ±2.4 (6)	27.2 ± 2.3 (5)
Hypophysectomized	HCl-saline	30.0 ± 1.0 (9)	14.9 ±.6 (14)	15.3 ±.7 (6)	22.4 ± 1.8 (5)
	Corticotropin A (375 mU)	51.1 ± 3.8 (8)	14.0 ±.1 (6)	20.1 ±.7 (8)	30.6 ± 3.2 (5)
	α_b corticotropin, Li (540 mU)	41.7 ± 2.3 (14)			
	β ACTH, Bell (36 mU)	35.6 ± 2.2 (11)			
	Glucose (25 mg)	26.6 ± 1.2 (5)			
	Insulin (1 U) + glucose (100 mg)	29.2 ± 2.0 (6)	14.6 ±.3 (4)		
	Alcohol	29.5 ± 1.6 (6)			
	Corticosterone (700 μg)	29.0 ± 1.5 (6)			

* Determinations were made 90 min. after administration of tracers and hormones. Values are the mean, stand. error, and No. of observations.

† D-xylose-1-C^{14} (specific activity, 2.26 μc/mg), 800 μg given per 100 g body wt; inulin-carboxyl-C^{14} (S.A. 1.37), 1240 μg; sucrose-U-C^{14} (S.A. 7.41), 202 μg; mannitol-1,6-C^{14} (S.A. 8.47), 165 μg.

inulin as a measure of extracellular volume, corticotropin A increased the percentage of intracellular water occupied by xylose from 28% to 66%.‡ No significant changes in volumes of distribution of xylose or inulin in diaphragm, gastrocnemius, testes, or epididymal fat were noted after hypophysectomy or after ACTH.

Discussion. These studies established that D-xylose is excluded from a large fraction of adrenal cell water in hypophysectomized rats, and that ACTH facilitates xylose entry, specifically into the adrenal cell. Absolute values for xylose distribution in adrenal cortex alone cannot be stated at this time, but considering absence of medullary responses to ACTH by other criteria, it might be expected that ACTH would not affect the medulla in present studies. Demonstration of an ACTH effect on xylose uptake does not require 90 min. interval routinely employed, since ACTH significantly increased distribution of xylose in the adrenal within 20-30 min.§ The time course of response was not pursued further, since at sufficiently brief intervals extremely small increases in availability of a utilizable sugar, like glucose, could produce metabolic results not measurable in terms of xylose distribution.

This response of the adrenal to ACTH is similar to that of skeletal and diaphragmatic muscle to insulin. In absence of insulin, a very limited entry of xylose into muscle cells occurs, suggesting(5,6,7) that a barrier exists which excludes xylose from a major fraction of total intracellular water. If one assumes that all cells within tissues are homogenous in their permeability characteristics, it appears that both insulin and ACTH alter intracellular "barriers" and thus permit entry of D-xylose into much larger compartments in respective target organs. On the other hand, if tissues contain cells with membranes of differing permeabilities, it is possible that some of the cells may be freely permeable to xylose in absence of hormone and ACTH or insulin affect only remainder of cells.

If D-xylose reflects permeability behavior of glucose in the adrenal, as appears to be the case in muscle(2,5,6,7), the significance of these findings to adrenocortical physiology may be widespread. An increase in availability of glucose to adrenocortical cells might be conceived as responsible for many phases of ACTH action, including steroidogenesis, growth, and ascorbic acid release. Without speculative elaboration, the findings demonstrate a specific *in vivo* response of the adrenal to ACTH which must be considered in any explanation of the mechanism of ACTH action. Finally, the similarity of effects on permeability of insulin and ACTH in their respective target organs suggests that these hormones may in this respect have a common mode of action.

Summary. Volumes of distribution of D-xylose and of substances which measure extracellular fluid volume were determined in adrenal and other tissues of nephrectomized rats. Hypophysectomy markedly reduced distribution of D-xylose in intracellular water of the adrenal, and exogenous ACTH increased xylose entry specifically in the adrenal to levels approaching those found in stressed animals with intact pituitaries. Attention is drawn to possible relation of these findings to the mechanism of ACTH action.

‡ Intracellular water was assumed to be the difference between total tissue water (69.8% ± .9 (S.E.), 12 observations) and inulin space.

§ Renal ties and tracer administrations were carried out 60-70 min. before ACTH, so that total interval for tracer equilibration was 90 min.

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