Permeability of Rat Adrenals *in vitro* to D-Xylose in Presence and Absence of ACTH.* (26336)

M. Golden, E. Scully, J. Eichhorn and O. Hechter
Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Previous work *in vivo* has shown that the "non-utilizable" pentose, D-xylose, is excluded from a major fraction of the cell water of the adrenal of hypophysectomized rats, and that administration of adrenocorticotrophic hormone (ACTH) specifically increased distribution of D-xylose in adrenal to a level approaching that found in stressed intact rats(1). These findings suggest that the penetration of sugar into the adrenocortical cell may be the rate-limiting step for glucose metabolism *in vivo,* and raise the possibility that the physiological effects of ACTH may be secondary to its influence on intracellular availability of substrate. In attempting to evaluate the latter possibility, we have examined the effects of ACTH upon D-xylose permeability in sectioned rat adrenals *in vitro,* which exhibit the well-established(2) increase in corticosteroidogenesis following addition of exogenous ACTH. It has been found that sectioned adrenals studied *in vitro* exhibit altered permeability characteristics in that D-xylose rapidly enters a major fraction of adrenal cell water in absence of ACTH; under these *in vitro* conditions, ACTH has no significant effect on sugar permeability but stimulates corticosteroidogenesis.

Methods. Intact or hypophysectomized (2 days) rats of the Sprague-Dawley strain, weighing 150 to 200 g, were employed. Following decapitation, adrenals were removed, bisected, and apportioned for incubation so that each flask contained half of each adrenal from 2 rats, giving a series of paired flasks containing tissue equivalent to 2 adrenals. In most cases, adrenals were preincubated for 1 hr in a gyratory shaker at 38°, under O_2 (5% CO_2), in 3 ml of Kreb-Ringer bicarbonate medium, containing glucose (1 mg/ml). For the final incubation, the preincubation medium was decanted and replaced with fresh medium containing labeled D-xylose and inulin. In all experiments, tracer concentrations were .025 μc/ml of D-xylose-1-C^{14} (National Bureau of Standards, S.A. 2.26 μc/mg) and .03 μc/ml of inulin-carboxyl-C^{14} (New England Nuclear, S.A. 3.0 μc/mg). One of each pair of flasks received 750 mU Corticotrophin A (Armour) in 0.1 ml N/100 HCl-0.9% saline; controls received 0.1 ml of vehicle. After various intervals of incubation ranging from 5 to 120 min, adrenals were removed, blotted in a uniform manner and weighed.

D-xylose and inulin were extracted from the adrenal tissue in a minimum volume of water (.4 ml) by heating in a boiling water bath(1), and the radiotracers from tissue and media were separated by chromatography on #2 Whatman paper for 2 days, using 95% ethanol as a mobile phase. In this system, inulin remains at the starting line and xylose is quantitatively recovered in the overflow flasks. D-xylose and inulin content of incubated adrenals and media were thus determined separately by plating these fractions and counting in a gas flow chamber.

The results with D-xylose and inulin are presented in terms of volume of distribution in total tissue, expressed as percent of total tissue wet weight in equilibrium with the medium (100 x cpm/g tissue per cpm/ml medium). It has been assumed that inulin is a measure of the extracellular compartment, and does not penetrate into the cell water; by measure of total water content of incubated tissues and inulin space, it is therefore possible to calculate the apparent distribution of D-xylose in cell water.

Corticoids in the medium after incubation were determined by the fluorimetric method of Moncloa *et al.*(3).

Results. Table I shows results in terms of (a) volume of distribution of either D-xylose or inulin in total tissue, (b) calculated volume of distribution of D-xylose in intracellu-

* This work has been supported by grants from U. S. Public Health Service, Nat. Science Foundation, and Commonwealth Fund.

TABLE I. Influence of ACTH on Distribution of Tracers and Corticoid Production in Adrenal Tissue of Pituitary Intact and Hypophysectomized Rats *In Vitro.*

Type rat used	Preincubation	Period of incubation (min.)	Vol of distribution of tracers				D-xylose distribution in I.C.W.*		Corticoid (μg/100 mg wet tissue)	
			D-xylose		Inulin					
			Control	ACTH	Control	ACTH	Control	ACTH	Control	ACTH
Pituitary intact	1 hr	5	36 ± 3.6 (6)	41 ± 3.0 (6)	14 ± .7 (6)	17 ± 1.0 (6)	35	40	1.4 ± .2 (6)	3.0 ± .6 (6)
		15	47 ± 2.5 (13)	48 ± 2.3 (9)	22 ± 1.2 (13)	22 ± 1.2 (9)	46	47	1.5 ± .2 (9)	4.0 ± .5 (9)
		30	48 ± 6.7 (4)	55 ± 5.2 (4)	20 ± 3.8 (4)	31 ± 2.3 (4)	49	52	2.8 (2)	8.3 (2)
		60	61 ± 2.0 (18)	61 ± 2.3 (11)	29 ± 3.7 (4)	33 ± 2.3 (4)	67	65	6.3 ±1.0 (9)	18.6 ±4.2 (9)
		120	62 ±11.4 (4)	60 ± 4.1 (4)	28 ± 2.3 (4)	37 ± 3.8 (4)	69	59	5.3 ± .2 (4)	22.9 ± 7.7 (4)
Hypophysectomized	1 "	15	41 ± 3.4 (4)	48 ± 2.7 (4)	19 ± 1.6 (4)	25 ± 1.8 (4)	38	44	1.3 ± .3 (4)	2.4 ± .4 (4)
		60	57 ± 3.6 (4)	69 ± 8.1 (4)	22 ± 1.1 (4)	32 ± 3.1 (4)	63	82	2.1 ± .3 (4)	13.1 ±1.4 (4)
	None	15	62 ± 3.6 (4)	66 ± 2.4 (4)	17 ± 2.9 (4)	16 ± 1.9 (4)	76	83	1.0 ± .1 (4)	1.2 ± .3 (4)
		60	75 ± 1.2 (4)	72 ± 5.1 (4)	19 ± 2.2 (4)	19 ± 1.7 (4)	96	92	1.4 ± .2 (4)	12.1 ± .2 (4)

Values are mean, stand. error, and No. of observations.

* Intracellular water is taken to be the difference between total tissue water (76.6% ± 0.7, 10 observations) and inulin space, assuming that inulin distributes exclusively in the extracellular compartment; ACTH had no detectable effect on water content of incubated adrenals.

lar water, and (c) corticoid production. In the group using adrenals from pituitary-intact rats, D-xylose distribution increases with the interval of incubation up to 60 min, but does not increase further by 120 min. After 5 min incubation, volume of distribution of D-xylose is about two-thirds that at 60 min, indicating a very rapid entry of D-xylose. No significant effect either on rate or final plateau level of D-xylose distribution as a result of ACTH addition was detected. The results further indicate that the inulin space increases with time of incubation, and that ACTH tends to increase the inulin space particularly at 30 to 60 min. A corticosteroidogenic response to ACTH is observed, being clearly evident in 30 min and maximal in 2 hr.

The results on D-xylose and inulin using adrenals from hypophysectomized animals are similar to those in adrenals from pituitary-intact rats, provided that the adrenals of hypophysectomized rats are also preincubated. Without initial preincubation, the D-xylose space is somewhat larger and the inulin space smaller than in preincubated adrenals from either pituitary-intact or hypophysectomized rats. No appreciable effect of preincubation was observed on corticoid production nor on corticoid response to ACTH in adrenals from hypophysectomized rats.

Discussion. These studies establish that sectioned rat adrenals, which respond to added ACTH with increased corticoid production, exhibit marked alterations in permeability relationships relative to those existing *in vivo.* In absence of ACTH, D-xylose is excluded from 70 to 75% of the cell water of the adrenal *in vivo* and ACTH increases the distribution of D-xylose in cell water to almost 70% (1). *In vitro,* however, in absence of added ACTH, D-xylose rapidly enters and distributes in about 70% of the intracellular water of the sectioned adrenal of hypophysectomized or normal rats. Under these circumstances, the finding that *in vitro* addition of ACTH had no significant influence on D-xylose penetration, is not unexpected since the permeability bar-

riers operative *in vivo* do not appear to be present under these *in vitro* conditions. Similarly, inulin space measured *in vitro* is altered from the *in vivo* situation. *In vivo*, inulin space is about 15% of total wet weight of the tissue, while *in vitro* inulin space after preincubation plus one hour incubation is 29% in adrenals from pituitary-intact rats and 22% in those from hypophysectomized rats. Addition of ACTH had no effect on inulin space in glands not preincubated, but tended to increase the inulin space in preincubated glands. With the number of animals employed, the differences in inulin space between control and ACTH are not statistically significant in the series of adrenals from normal animals, but in the group from hypophysectomized rats, preincubated 1 hr, the difference is significant ($P<0.05$). Although we have assumed for purposes of calculating intracellular distribution of D-xylose that the increased inulin space observed as incubation time increases represents an expansion of the extracellular volume, this increase might represent the entry of inulin into some of the adrenal cells. Likewise, the tendency for ACTH to increase inulin space under certain conditions may be interpreted in terms of either alternative.

The alteration of sugar permeability exhibited by bisected rat glands *in vitro* suggests that a large number of cells in the preparations are damaged, whether as a result of relative anoxia suffered by cells in the interior of the tissue section, a deficiency in the medium employed, or to other, as yet unknown, factors involved in the technic. Whatever the basis, it also involves other aspects of cell maintenance, since we have found that intracellular K^+ is lost, and Na^+ is gained by sectioned adrenals *in vitro;* thus, *in vivo,* Na is excluded and intracellular K^+ is about 170 μM/ml cell water, whereas following incubation, the K^+ is reduced to 98 μM/ml cell water, and there is an approximately corresponding gain of Na^+ (unpublished observations). Moreover, *in vitro,* ascorbic acid(4), and even intracellular enzymes are liberated into the medium(5). It is thus apparent that sectioned rat adrenal studied *in vitro* exhibits properties which are significantly different from those which are manifest *in vivo*. The fact that such preparations respond to ACTH with respect to steroidogenesis indicates that the *in vitro* effect of ACTH in this preparation does not appear to be secondary to an increase in substrate availability. Peron and Koritz(6) and Schonbaum *et al.*(5) advanced a similar conclusion from other data. Under physiological conditions, however, where the availability of substrates *is limited* by permeability barriers, the effect of ACTH to modify sugar permeability is probably an important aspect of ACTH action.

Summary. Studies on the permeability properties of bisected rat adrenals, incubated *in vitro,* have revealed marked differences from those which obtain *in vivo*. Thus, in absence of ACTH *in vivo* D-xylose is excluded from a major fraction of the cell water, but *in vitro* D-xylose rapidly penetrates and appears to equilibrate in about 70% of the cell water. *In vivo,* ACTH increases sugar distribution, but under *in vitro* circumstances, where a corticosteroidogenic response is observed, ACTH has no significant influence on rate of penetration or intracellular distribution of D-xylose. ACTH does, however, tend to increase inulin space of sectioned rat adrenal *in vitro*. While these findings suggest that the steroidogenic effect of ACTH *in vitro* is not dependent upon hormonal regulation of sugar availability, the possibility remains that an important aspect of ACTH action *in vivo,* where permeability barriers are operative, involves regulation of sugar permeability.

1. Eichhorn, J., Halkerston, I. D. K., Feinstein, M., Hechter, O., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 515.

2. Saffran, M., Schally, A. V., *Endocrinol.,* 1955, v56, 523.

3. Moncloa, F., Péron, F. G., Dorfman, R. I., *ibid.,* 1959, v65, 717.

4. Saffran, M., Bayliss, M. J., Webb, J. L., *Fed. Proc.,* 1951 v10, 116.

5. Schönbaum, E., Davidson, M., Large, R. E., Casselman, W. G. B., *Canad. J. Biochem. Physiol.,* 1959, v37, 1209.

6. Péron, F. G., Koritz, S. B., *J. Biol. Chem.,* 1958, v233, 256.

Received November 21, 1960. P.S.E.B.M., 1961, v106.