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Effect of Triiodothyronine and Cortisol on Intracellular Penetration of D-xylose. (25056)

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Studies with uncut rat diaphragm preparation demonstrated the *in vitro* effectiveness of insulin in permitting entry of D-xylose into a muscle fiber water space otherwise unavailable to sugar for distribution(1,2,3). To determine whether other hormonal treatments influence sugar penetration in this preparation, the effect of epinephrine, cortisol, and triiodothyronine (TIT) administered *in vitro* and *in vivo* have been examined. Since it was reported that metabolic poisons increase sugar penetration(2), it was of interest to learn if depletion of energy reserves *via* starvation would influence volume of distribution available to sugar.

Methods. The uncut rat diaphragm preparation and technics employed for pentose and sucrose determination were previously described(3). Incubation medium contained 128 meq/l-NaCl, 5.1 meq/l-KCl, 2.72 meq/l-$CaCl_2$, 1.02 meq/l-$MgSO_4$, 20 meq/l-$Na_2HPO_4/NaHPO_4$ buffer system (pH 7.2) and D-xylose at 4 mg/ml ± glucose at 1 mg/ml, or sucrose at 8 mg/ml. For *in vitro* experiments, TIT* was used as sodium salt (dissolved in 0.13 N-NaOH) at final concentration of 6 μg/ml medium. For *in vivo* administration, 300 μg TIT/day/rat were given ip 4 days. Epinephrine HCl was used *in vitro* at concentration of 2 μg/ml. Cortisol† was used *in vitro* at 40 μg/ml in 1% Tween 80 or *in vivo* at dosage regimen of 2 mg/day/rat ip. Insulin‡ was used at 0.4 units/ml (16 μg/ml) *in vitro.* Incubations were carried out in 50 ml medium volume at 38°C with shaking (90 cycles/min. amplitude 8 cm). Following incubation, diaphragm muscle was removed from rib cage, wiped across clean glass plate, weighed, and placed into a tube containing 2 ml distilled H_2O from which xylose or sucrose was extracted and determined. Sugar distribution in tissue was expressed relative to concentration in the medium. All animals were fed *ad lib.* except in experiments where 24 hr or 56 hr starvation period is indicated. Incubations were carried out for 60 and 90 minute periods since previous kinetic studies have shown that equilibration is attained in available water space within 60 minutes.

Results. Table I shows results obtained following *in vitro* or *in vivo* administrations of epinephrine, triiodothyronine, cortisol and/or insulin or starvation. These findings may be summarized as follows:

Triiodothyronine. Addition of TIT to incubation medium had no demonstrable effect upon distribution of D-xylose during 60 or 90 minute incubation. However, following parenteral administration of TIT, distribution of D-xylose is increased over controls. Treatment with TIT produced no change in total tissue water (79.1 ml/100 g ± 0.6) or in the sucrose space which may be assumed a measure of extracellular space. Thus increase in tissue xylose would seem to result from increased intracellular penetration. Furthermore, when diaphragms of TIT treated animals are incubated with insulin *in vitro,* the

* L-Triicdothyronine generously supplied by Dr. A. E. Heming, Smith, Kline & French Laboratory.

† Cortisol generously supplied by Dr. R. H. Silber, Merck Inst. for Ther. Res.

‡ Insulin was generously supplied by Dr. E. Rohrman, Eli Lilly & Co.

TABLE I. Sugar Distribution in Uncut Rat Diaphragm.

Treatment	Xylose, ml/100 g (wet wt)	Sucrose, ml/100 g (wet wt)	Xylose, ml/100 ml intracellular H_2O
Control	37.1 ± .45*(22)†	22.3 ± 1.0*(4)†	26.1
Insulin *(in vitro)*	59.3 ± .78 (29)	22.5 ± 1.1 (4)	64.8
Triiodothyronine *(in vitro)*	37.5 ± 1.6 (6)	22.7 ± .9 (3)	26.1
Triiodothyronine *(in vivo)*	48.1 ± 1.53 (12)	21.5 ± 1.2 (4)	47.0
Idem + insulin *(in vitro)*	64.4 ± .73 (12)	21.9 ± 1.0 (4)	75.0
Epinephrine *(in vitro)*	36.2 ± 1.8 (6)		
Idem + insulin *(in vitro)*	60.1 ± 2.3 (4)		
Cortisol *(in vitro)*	37.4 ± 1.2 (6)		
Cortisol *(in vivo)*	44.4 ± 1.62 (11)	24.4 ± 1.1 (4)	35.3
Idem + insulin *(in vitro)*	63.9 ± 1.33 (12)	24.2 ± 1.0 (4)	70.0
56 hr starvation	48.6 ± 1.6 (10)	23.4 ± 1.2 (4)	44.4
Idem + insulin *(in vitro)*	70.0 ± 1.7 (6)	23.2 ± 1.4 (3)	82.3
24 hr starvation	37.7 ± 1.4 (8)	22.3 ± 1.0 (4)	27.1
Idem + insulin *(in vitro)*	60.6 ± 1.6 (4)	22.6 ± 1.1 (4)	67.0

* Mean, stand. error of mean. † No. of determinations.

intracellular xylose space is greater than observed with insulin in diaphragms from non-treated rats. Thus xylose occupies 65% of total intracellular water in normal diaphragms whereas following TIT treatment, xylose distributes in 75% of cell water. The difference is statistically significant ($p<0.01$).

Cortisol. Cortisol, like TIT, had no influence on distribution of D-xylose when added *in vitro*. Following parenteral injection, an creased distribution of D-xylose was found both in absence and presence of exogenous insulin. Unlike triiodothyronine however, cortisol appears to increase the sucrose space from 22% to 24% of tissue weight. When this larger figure is used to calculate intracellular penetration, an increase from 26% to 35% is found in absence of insulin and 65% to 70% in its presence.

Epinephrine. Epinephrine, *in vitro*, was completely without effect on pentration of D-xylose in presence or absence of added insulin. In view of rapid onset of action which this endocrine factor exhibits, our study does not include parenteral administration of epinephrine since a latent period of action exceeding 90 minutes was not considered a likely possibility.

Starvation. From these results it becomes apparent that either interference with endogenous metabolic pathways by use of metabolic poisons(2), or previous depletion of endogenous "energy-substrate" decreases capacity of intracellular water to exclude sugar and in this way an "insulin-like" effect is obtained. Starvation for 56 hr raised the xylose space from 37.1 ml/100 g in non-fasted controls to 48.6 ml/100 g. In presence of insulin, a distribution of 70.3 ml/100 g was observed which is in excess of the usual 59 ml/100 g found with non-fasted animals. Thus, in effect, starvation treatment produces an almost quantitatively additive penetration of sugar in presence and absence of insulin. Addition of glucose at 1 mg/ml to the incubation medium was without effect on xylose space measured under all conditions employed in these studies.

Discussion. It has been shown that the properties of exclusion which cell water of diaphragm muscle fiber exhibits, can be appreciably altered by a number of conditions apart from and concomitant with insulin treatment. That prolonged starvation readily breaks down such penetration barriers strongly indicates that the system for maintenance of these barriers is energy dependent. This is in good agreement with the work of Randle and Smith using metabolic poisons (2) and with previous findings of intracellular sodium distribution(4). Since both triiodothyronine and cortisol do allow entrance of sugar into an intracellular water space, normally unavailable, the question arises as to whether such action may be involved in the physiological role played by these 2 sub-

stances. If one makes the assumption that sucrose space is a measure of extracellular space (3) then these results show that in the absence of exogenous insulin, triiodothyronine treatment gives sugar access to intracellular water space of some 20 ml/100 ml greater than control values and in the presence of exogenous insulin, an access to water space 10 ml/100 ml greater than that afforded by insulin.

These results with xylose are representative of those with other monosaccharides such as glucose, for the flux of D-xylose which is equivalent to that of D-galactose, closely approximates values obtained for glucose entry as measured by glucose disappearance at physiological 1 mg/ml glucose concentration level(3). However, if a water space which previously excluded glucose or xylose now is penetrable by these sugars, it may now well be penetrable by other suitable substrates which previously were not readily able to gain entrance. If this effect of triiodothyronine and cortisol upon sugar penetration is a physiologically qualitative action, and exogenous treatment merely exaggerates the quantitative values much as exogenous insulin has been shown to do, then one would have an explanation in general terms on cellular level of how a hormone, *e.g.,* insulin, thyroid, or cortisol, may act. This would be to regulate availability of the substrate, whether it be sugar or other, for the various enzymic systems within the intracellular water. However, since other factors which impair cellular energetics also increase available cell water, there is the possibility that the effects of TIT and/or cortisol on sugar penetration merely reflect the variable degree of protein catabolism which they invoke. Furthermore, the specificity of this response on sugar penetration remains to be determined. There is the possibility that the effects observed are merely nonspecific pharmacologic actions and that substances of similar chemical structure might yield similar results. Work is currently under way using chemical analogs of those employed, other steroid hormones and some trophic factors of the anterior hypophyseal lobe.

There remains the question of whether this effect of triiodothyronine or cortisol on sugar penetration is exerted directly upon muscle, or if it is the result of one or more intermediary events which in turn produces the substance capable of acting upon the muscle. Lack of an *in vitro* response with either of these endocrine factors is not too informative since a period of much greater than one hour may be required for the direct effect to be manifested. With epinephrine, however, *in vitro* effects are rapid, and absence of a response here is taken to mean that its anti-insulin action *in vivo* does not in any way involve sugar transfer. Preliminary work of a somewhat different kind with triiodothyronine has demonstrated the effect herein reported to be a direct one in seemingly unequivocal terms. Details of this latter work will be published.

Summary. Distribution of D-xylose in the uncut rat diaphragm was studied *in vitro* under a variety of conditions. It was found that epinephrine, triiodothyronine or cortisol had no effect on distribution of this sugar when administered *in vitro.* Triiodothyronine or cortisol, administered parenterally, increased the space occupied by D-xylose in absence of insulin, and in presence of insulin it resulted in comparatively small but consistent addition to the space occupied when insulin alone was used. A 24-hour starvation period was without effect on sugar distribution whereas a 56-hour period increased the xylose space in absence of insulin and was quantitatively additive in its presence.

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