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Received February 11, 1965. P.S.E.B.M., 1965, v119.

Effect of Insulin on D-Xylose Uptake by the Intact Rat Diaphragm. (30163)

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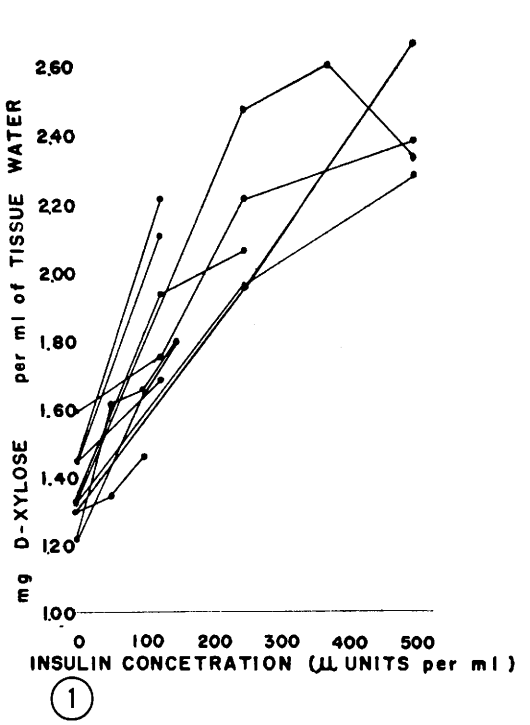
The isolated rat diaphragm has been used extensively for bioassay of insulin-like activity. The finding by Kipnis and Cori(1) and Norman *et al*(2), that insulin increased D-xylose penetration into the rat diaphragm has recently been confirmed by Rieser and Rieser(3) using insulin concentrations of 500,000 microunits per ml in *in vitro* incubation systems. These studies suggested that D-xylose penetration might be used to develop a simple and reliable bioassay technique for insulin activity at low insulin concentrations.

The intact diaphragm preparation described by Kipnis and Cori(1) was used since employment of the whole diaphragm eliminates the less specific and less reliable penetration through cut muscle fibers.

Materials and methods. Wistar rats of both sexes weighing between 100 and 150 g were used. The animals were fasted for 16 to 24 hours before being sacrificed by decapitation. The intact diaphragms together with a ring of the ribs were quickly removed, rinsed in Gey and Gey buffer(4) and gently blotted. The diaphragm preparations were placed in beakers in 30 ml of buffer, pH 7.3-7.4, containing 1.8 mg of glucose, 4.5 mg of D-xylose and 2.0 mg of gelatin per ml. Glucose was used in the buffer to preserve better the viability of the membrane and gelatin was added to prevent adsorption of insulin to glass surfaces. Crystalline zinc insulin* was used to prepare test solutions of known concentrations of insulin with the buffer described above. The insulin solutions were added to the diaphragm preparations just prior to incubation in a Dubnoff apparatus at 37°C for 30 minutes under an atmosphere of 95% O_2, 5% CO_2. After incubation, the diaphragms were washed in buffer, blotted and dissected away from the rib cage. The central segment of muscle was weighed and dried to constant weight for determination of water content. The diaphragm tissue was weighed and homogenized in a mortar with sand and water. Proteins were precipitated with Somogyi reagents and a 1-to-20 dilution of the homogenate was prepared. D-xylose content was determined by the method of Roe and Rice (5). The results were expressed as mg of

* Crystalline zinc insulin was kindly suppied by Dr. Otto Behrens, Eli Lilly Co.



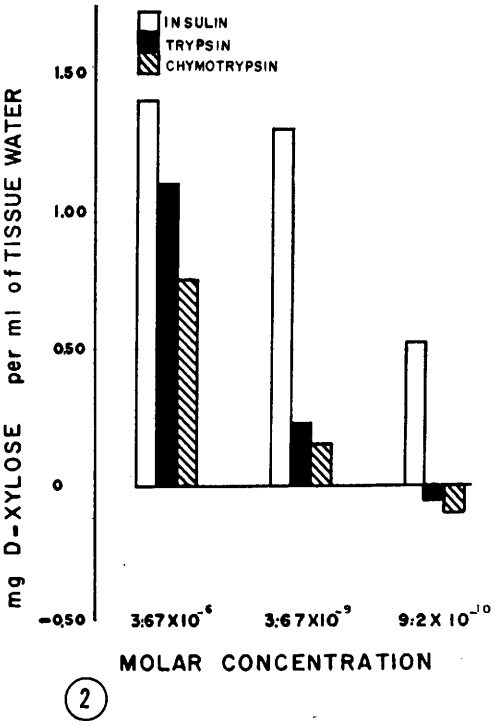


FIG. 1. D-xylose accumulation in intact rat diaphragm preparations after 30 min incubation with indicated concentration of insulin.

FIG. 2. Change in D-xylose content of rat diaphragms from control levels after one hour incubation with indicated compounds. Each block represents the mean of 3 experiments.

D-xylose per ml of tissue water. A value of 77% of the wet weight of the tissue was obtained as the volume of total tissue water. This figure was the mean of 20 different determinations (range 75.8 to 78.4) and was used in final calculation of D-xylose penetration.

Results. D-xylose penetration into intact rat diaphragm preparations was increased in the presence of physiological concentrations of insulin. The results of several experiments are shown in Fig. 1. The D-xylose content of diaphragm was consistently increased above control levels after incubation with from 50 to 500 microunits of insulin per ml of incubation medium. Many changes in experimental procedures were made in an effort to attain greater precision. All failed to eliminate the variability of the response.

Rieser and Rieser(3) recently demonstrated that trypsin and chymotrypsin in a molar concentration equivalent to 500,000 microunits of insulin per ml of incubation medium (3.67×10^{-6} M), promote D-xylose penetration into rat diaphragm preparations. We have confirmed their observation at this concentration but have found that when the molar concentrations of the enzymes were reduced to levels equivalent to the molar concentration of 500 microunits of insulin per ml (3.67×10^{-9} M) and 125 microunits per ml (9.2×10^{-10} M), no significant increase in xylose uptake above control levels was observed (Fig. 2). These results suggested that the increased penetration of the diaphragm in the presence of relatively large amounts of trypsin and chymotrypsin is due to the digestion of the membranes by the proteolytic enzymes. This seemed obvious since the diaphragm tissues appear to be softened after incubation at 37° for one hour. This effect was not noticed at the lower concentrations of the enzymes. We found also that when washed fibrin clots from recalcified plasma were incubated with these 2 enzymes at a molar concentration of $3.67 \times$

10^{-6} in Gey and Gey buffer, complete lysis occurred in 30 minutes and no clot lysis was observed after 24 hours incubation in the lower molar concentrations mentioned above.

Discussion. This study has shown that physiological concentrations of insulin promote D-xylose penetration into intact rat diaphragm preparations *in vitro* and that the relationship between insulin concentration and D-xylose penetration is roughly linear. The response is subject to rather large day-to-day variation and efforts to eliminate such variation have not been successful.

We have demonstrated that the response is specific for insulin when compared with trypsin and chymotrypsin at low molar concentrations. Our observation of a failure of trypsin and chymotrypsin at low concentration to increase the permeability of the diaphragm to D-xylose confirms the point of view(6,7) that the insulin effect upon the penetrability of a non-utilized sugar is specific.

Our findings suggest that D-xylose penetration might be a useful technique for measurement of insulin-like activity if greater precision and reproducibility in the procedure could be obtained.

Summary. Increased D-xylose penetration of the intact rat diaphragm has been demonstrated at insulin concentrations of 50 to 500 microunits per ml, amounts that are within the range of physiological levels. Increased penetrability to D-xylose by preparations containing trypsin and chymotrypsin was observed at relatively high levels of the enzymes but not at molar concentrations equivalent to those of insulin at levels of 125 and 500 microunits per ml. The rates of penetration were approximately linear, but the day-to-day variations of this procedure were too large for a useful bioassay method.

The authors are indebted to Mrs. Jacqueline Rockenbaugh for valuable technical assistance.

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Received February 16, 1965. P.S.E.B.M., 1965, v119.

Stimulation *in vivo* of Renal RNA-Synthesis by Aldosterone.* (30164)

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Karlson(1) has suggested that many hormones act by stimulating the synthesis of specific proteins which in turn are responsible for the physiological actions of the hormones. He has proposed that these hormones act upon chromosomes to expose specific DNA-templates. This results in the synthesis of messenger-RNA which in turn directs the synthesis of protein by the endoplasmic reticulum. Several reports have suggested that aldosterone acts in this manner. Williamson (2) found that actinomycin D, an inhibitor of DNA-directed synthesis of RNA, blocked the antinatruretic action of aldosterone in the rat. Edelman *et al*(3) reported that actinomycin D blocked the action of aldosterone on the transport of sodium by the isolated toad bladder. Crabbé and De Weer(4) also obtained similar results with the isolated toad bladder and skin.

In this report the ability of aldosterone to increase RNA-synthesis *in vivo* has been assessed both by chemical determination of

* Supported in part by U.S.P.H.S. research grant AM-05298 from Nat. Inst. of Arthritis & Metab. Dis.

† U.S.P.H.S. Trainee, grant 5T1 GM-141.