

It also shows that pinguinain is a protein having a small molecular weight.

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Inhibitory Effect of Progesterone on Activity of Human Myometrial Adenosine Triphosphatase

DURING the previous investigations¹ in this laboratory human myometrial strips were exposed to progesterone until all spontaneous activity ceased and the changes in the concentration of adenosine triphosphate (ATP) and phosphocreatine (PC) measured. Until such paralysis had existed for at least 40 min, no significant change in high-energy phosphate bonds (ATP and PC) could be documented. It was, therefore, concluded that in the earlier stages of the myometrial inhibition progesterone might be exerting its effect in the utilization of ATP rather than in its synthesis. As a continuation of the investigations on this problem, the effect of progesterone was then determined on the ATPase activity of the human myometrial strips. This communication deals with the results obtained.

The method of DuBois and Potter² was used to determine the ATPase activity in which human non-pregnant myometrial tissue homogenate was incubated in the presence of calcium chloride, diethyl barbiturate (buffer) and ATP. The amount of inorganic phosphorus liberated in homogenate corresponding to 6 mg and 9 mg wet weight tissue in 15 min at 37° C as determined by the procedure of Rookstein and Herron³ was taken as a measure of ATPase activity. One microgram of phosphorus liberated was interpreted as one unit of ATPase activity. Ten to 300 µg of progesterone dissolved in 30 µl of propylene glycol was added to the homogenate to determine its effect.

The two requirements: (a) that the rate of reaction is linear during the time of incubation; (b) that the rate is directly proportional to the amount of enzyme present in the test system, should be fulfilled if any enzyme assay is to be found satisfactory.

Propylene glycol used as a vehicle (30 µl) did not alter the ATPase activity. Progesterone in concentrations of more than 10 µg (and up to 50 µg tested) per mg wet weight human myometrial tissue was found to inhibit the human myometrial ATPase activity on an average of 27.3 per cent ($P < 0.001$) based on 15 experiments as shown in Table 1.

It is interesting to note that such high concentrations of progesterone were needed to inhibit the human myometrial ATPase activity. It may be that the smaller concentrations of progesterone might inhibit the ATPase

activity when exposed to spontaneously contracting myometrial strip in muscle-bath preparation over a prolonged period of time. Such an investigation is in progress and the results will be reported later.

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Stimulation of Glucose Utilization by Insulin in Human Adipose Tissue Homogenates

To date no effect of insulin on utilization of glucose in a cell-free preparation has been described. Indeed, the apparent ineffectiveness of insulin in cell-free preparations was a major clue to the discovery of the insulin stimulation of trans-membrane transport. We have been examining utilization of glucose in human adipose tissue and have observed a distinct stimulation of utilization of glucose by insulin added to homogenates of omentum.

Omentum was obtained from subjects undergoing abdominal surgery being performed for reasons other than malignancy. Homogenization was achieved within 15 sec, using a 'Vir Tis 23' homogenizer in a Krebs-Ringer bicarbonate buffer¹ gassed with O₂:CO₂(95:5) to pH 7.4. The potassium chloride concentration of the buffer was doubled at the expense of the sodium chloride. A 'defatted' homogenate was obtained by removing the supernatant layer of congealed fat formed during centrifugation in the cold at 500g for 10 min. The homogenate was then supplemented to give final concentrations of 0.001 M ATP, 0.001 M TPN⁺, 0.005 M Mg⁺⁺ and 5.6 mM glucose. Incubation vessels containing 1 ml. of homogenate were incubated for 1 h at 37° C with agitation at 60 cycles/min. Insulin was added to give a concentration of 0.1 unit/ml. homogenate. Glucose was measured by a micro adaptation of the glucose oxidase method of Sunderman and Sunderman².

Table 1. STIMULATION OF GLUCOSE UTILIZATION BY INSULIN IN HUMAN ADIPOSE TISSUE HOMOGENATES

Glucose utilization µg/mg tissue N _g /h	Without insulin 279 ± 75	With insulin 466 ± 248	Δ Insulin 187 ± 105*
Values are means ± S.D. in 11 experiments * P < 0.01.			

As seen in Table 1, insulin produced a 67 per cent increase in glucose disappearance. Though there is indirect evidence of an intracellular action of insulin on both carbohydrate³ and protein metabolism⁴, we believe that this is a direct demonstration of an intracellular insulin effect on utilization of glucose. Previous inability to show such an effect may have been due to the choice of tissue and incubation conditions.

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Table 1

No. of cases	Average ATPase activity in units of human myometrial tissue/mg wet weight*		Average reduction in ATPase activity by progesterone
	Control	Progesterone treated (90-300 µg)	
15	1.98	1.44	0.54 (27.3%)†

* 1 ATPase unit = 1 µg phosphorus liberated.
† t = 7.09.
P < 0.001.