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Studies on Uterine Metabolism. I. Adenosinetriphosphatase Activity of Smooth Muscle.

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The activity of the adenosinetriphosphatase (ATP-ase) enzyme system has been measured in many tissues other than skeletal muscle. DuBois and Potter[1] have indicated that an unidentified smooth muscle yielded, in their tests, about one-third of the ATP-ase activity of striated muscle. We have investigated rather extensively the ATP-ase system in the smooth muscles of the rat, in particular the uterus. ATP was prepared as the dicalcium salt according to Kerr.[2]

The reaction systems were incubated at 37°C for 15 minutes in a final volume of 3 ml, and contained one ml of a tissue homogenate[1] in 0.1 M veronal-HCl buffer of pH 8.4; one ml of a 10^{-3} molar solution of ATP and one ml of veronal-HCl buffer. Magnesium ions were present in the system in a final concentration of 10^{-4} molar.[3] Blanks were run with ATP alone, and also with tissue in the absence of ATP. Addition of other substances was compensated for in volume by a corresponding reduction in the volume of buffer. The liberated phosphate was determined by the method of Fiske and SubbaRow.[4]

Table I expresses the results found for ATP-ase activity of smooth and skeletal muscles of adult Sprague-Dawley rats. These were all used in the metestrus stage as determined by the daily vaginal smear technique. Varying amounts of tissue were used to insure a linear relationship between tissue weight and enzyme concentration.

The larger variations in the smooth muscle determinations were thought to be due to the water content fluctuations which these tissues undergo, depending upon their immediate physiological states. However, calculation of the data on the basis of dry weights yielded similarly extensive variations. It must, therefore, be assumed that these changes arise through some other mechanism.

It is significant, however, that the smooth muscles in these systems exhibit a uniformly higher activity than striated muscle. In later experiments striated muscle was often tested at the same time as smooth muscle from the same animal; the above differences in enzymatic activity were always observed to hold true.

The contractile system of smooth muscle remains considerably more obscure than that of skeletal muscle. If the ATP-ase system of smooth muscle is to follow the same myosin-adenosinetriphosphate changes postulated for striated muscle[5,6] the sensitivities of these systems to chemical mediators such as acetylcholine and epinephrine should be similar. Ziff[7] has reported that acetylcholine, epinephrine and eserine are without effect on the hydrolysis of ATP by myosin, which we assume he obtained from skeletal muscle. We are able to corroborate his findings for striated muscle and extend them to uterine muscle. The concentration of the various mediators used is of the same order of magnitude as that employed to elicit physiological responses *in vitro*. The data are given in Table II.

These variations are not considered significant in the light of changes in enzymatic

1 DuBois, K. P., and Potter, V. R., *J. Biol. Chem.*, 1943, **150**, 185.

2 Kerr, S. E., *J. Biol. Chem.*, 1941, **139**, 121.

3 Singher, H. O., and Meister, A., *J. Biol. Chem.*, 1945, **159**, 491.

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5 Engelhardt, V. A., *Advances in Enzymology*, 1946, **6**, 147.

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7 Ziff, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 249.

TABLE I.
ATP-ase Activity of Smooth and Skeletal Muscle.
Values expressed as μg P liberated in 15 min.

Tissue homogenate	No. of trials	Per mg fresh tissue		Per mg dry tissue	
		Avg	Range	Avg	Range
Skeletal muscle (Sartorius)	9	8.1	(5.8-13.2)	33.7	(25.0- 56.0)
Intestine (1st loop of duodenum)	6	18.0	(14.0-24.2)	61.8	(46.9- 78.0)
Bladder	4	20.9	(16.6-23.2)	81.8	(73.0-103.0)
Uterus	6	20.8	(16.8-24.2)	89.0	(62.6-110.0)

TABLE II.
Influence of Chemical Mediators on ATP-ase Activity of the Uterus.

Substance	Final conc.	Estrus stage	Avg % change in ATP-ase activity of uterus
Acetylcholine	7×10^{-9} M	Metestrus	+1.5
		Diestrus	—4.4
Epinephrine	1×10^{-7} M	Metestrus	+6.3
		Diestrus	+2.4
Eserine	4×10^{-9} M	Diestrus	+1.4

activity of tissues to which the above substances have not been added. As far as these *in vitro* tests go it may be concluded, therefore, that in skeletal and smooth muscle, these mediators do not effect the ATP-ase system *per se*. The activation of ATP-ase by acetylcholine, described by DuBois and Potter[8] was carried out in a system in which the calcium concentration was a factor which is not the case in these experiments.

Consistent changes in the activity of ATP-ase in smooth or skeletal muscle could not be demonstrated with histamine, pitocin or ammonium chloride. Ethyl alcohol (0.7 M) inhibited the system slightly but this is probably due to its protein denaturant properties. Engelhardt,[5] Ziff,[7] and others[9] have indicated that the inhibitory effect of fluoride is only as great as its removal of calcium ions from the medium. In the magnesium activated system we have observed inconsistent effects of fluoride on striated muscle. Uterine muscle ATP-ase, however, is usually inhibited, as shown in Table III.

The ATP-ase activity of the uterus has led us to investigate the effect of the reproductive cycle on such activity. Because of the water shifts it seemed best to calculate

TABLE III.
Effect of 3.3×10^{-3} M Fluoride on ATP-ase System of Smooth Muscle.

Stage of cycle	% change of ATP-ase activity
Estrus	—25.8; —37.8
Metestrus	—15.5
Diestrus	—17.4; —40.7
Proestrus	—18.6

activity on the basis of dry weight of the tissue used. Later experiments have also included per cent nitrogen as a factor. The results from preliminary experiments do not lend themselves to an interpretation of variation with the cyclical changes, as indicated in Table IV.

Representative steroid hormones were added to the incubate in concentrations of between 10^{-4} to 10^{-6} molar. Additions were made in a small amount of alcohol, and alcohol controls run simultaneously. Estrone, estradiol and progesterone were without appreciable effect on the uterus in the metestrus stage; the latter two also had negligible effect during diestrus. In a small number of cases testosterone seemed to inhibit the ATP-ase activity of skeletal muscle; its effect on uterine muscle appeared to be much smaller.

Summary. The ATP-ase activity of smooth muscle is found to be considerably higher than that of striated muscle. It is unaffected by dilute concentrations of chemical mediators. Fluoride apparently has an inhibitory

[8] DuBois, K. P., and Potter, V. R., *J. Biol. Chem.*, 1943, **148**, 451.

[9] Polis, B. D., and Meyerhof, O., *J. Biol. Chem.*, 1947, **169**, 389.

TABLE IV.
ATP-ase of the Uterus Through the Estrus Cycle.
μg Inorganic P Liberated in 15 min per mg Dry Tissue.

Stage	No. of trials	Skeletal muscle Avg	Skeletal muscle Range	Uterus Avg	Uterus Range
Estrus	2	35.5	(35-36)	125.0	(120-130)
Metestrus	9	33.7	(25-56)	93.9	(62-122)
Diestrus	3	50.0	(32-52)	107.3	(63-143)
Proestrus	2	35.5	(17-54)	106.0	(88-140)

effect. Variation in the ATP-ase activity of the uterus could not be correlated with cyclical estrus change. With the possible exception of testosterone, hormones did not appear to affect the *in vitro* systems.

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Production of Essential Fatty Acid Deficiency Symptoms in the Mature Rat.*

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In the course of studies on the effect of vitamin imbalance under various conditions, mature rats were maintained on restricted caloric intake of fat free diets until they lost about 50% of their original weight. In the period of recovery, during which time they were fed the same diets *ad libitum*, various symptoms including those typical of the Burr and Burr syndrome[1] were observed.

Since the original report by Burr and Burr,[2] several workers[3,4,5] have observed the typical skin symptoms of the essential fatty acid deficiency, but in all cases young animals were used. So far as we know, in no case was this deficiency syndrome reported to have been produced in adult rats.

Experimental and results. Mature male rats of the Sprague-Dawley strain averaging about 225 g have been used in these experiments. The rats were placed in individual metal cages with raised screen bottoms at the beginning of the depletion period on the fat free diets. They were weighed and examined weekly or in later experiments twice weekly.

In the first experiment 4 groups of 4 rats each were placed on the 4 diets given in Table I. In addition they all received one drop of haliver oil weekly.

For a period of 2 months each rat received 6 g of food daily. At the end of this period the amount was decreased to 5 g per day. On the 91st day one rat died in each of the groups I, II, and IV (none in the "B + supplement" group). The rats which died weighed 94, 93 and 95 g respectively. On this day all the rats were allowed their respective diets *ad libitum*. The only symptom observed by this time besides the severe emaciation

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