Adenosine triphosphatase activity of intact muscle cells

ROTHSTEIN has shown that enzymes are present at the yeast cell surface[1,2] and at the surface of intestinal mucosal cells[3]. The experiments of LEVINE *et al.*[4] indicating that insulin increases the rate of transport of hexoses across cell membranes, and the phenomenon of insulin binding by muscle described by STADIE and co-workers[5], point to the cell surface as an important structure concerned with the regulation of metabolism. Recently, SHAW AND STADIE[6] have demonstrated the co-existence of two glycolytic systems in the rat diaphragm: a glycogen-synthesizing pathway responsive to insulin and a lactate-synthesizing system which is not affected by insulin. The latter system appears to be at or near the cell surface. Even at the sub-cellular level, when the behavior of the isolated mitochondria is examined, the surface of the mitochondrion exhibits enzymic activity, as shown by LEHNINGER[7], who found that the intact mitochondrion metabolized added reduced diphosphopyridine nucleotide but without accompanying oxidative phosphorylation. When the surface of the mitochondrion was altered, the reduced DPN entered the interior of the particle and oxidative phosphorylation was demonstrated. LEHNINGER concluded that the surface of the mitochondrion contains enzymes which can metabolize added substrate.

We wish to report here experiments which indicate that the surface of the rat diaphragm exhibits adenosine triphosphatase activity. These experiments (Table I) show that the addition of adenosine triphosphate (ATP) to rat diaphragm equilibrated *in vitro* resulted in a rapid hydrolysis, almost complete after 1 hour at 38° C, with practically quantitative recovery of the resulting inorganic phosphate in the solution. The slightly lower values for the decrement of acid-labile phosphate, compared with the increment of inorganic phosphate, may be due in part to a slight production of acid-labile phosphate by the diaphragm in the absence of added ATP. The concentration of the adenylic acid moiety of ATP remained unchanged during the experiment, as shown by ultraviolet absorption measurements at 260 mμ. ATP was hydrolyzed by the diaphragm in nitrogen as well as in oxygen.

TABLE I

HYDROLYSIS OF ADDED ATP IN THE PRESENCE OF RAT DIAPHRAGM*

Expt. No.	*Wt of diaphragm*	*Initial P_i***	*Final P_i***	*Difference*	*Corrected difference****	*Initial acid-labile P§*	*Final acid-labile P§*	*Difference*
	mg	*All values are expressed as micromoles*						
1	132	0.8	5.7	4.9	+ 3.6	9.6	7.4	— 2.2
2	136	0.8	6.3	5.5	+ 4.1	9.6	6.7	— 2.9
3	90	0.8	4.2	3.4	+ 2.9	11.9	8.0	— 3.9
4	292	1.0	10.8	9.8	+ 5.7	11.0	4.6	— 5.4
5	237	1.0	10.3	9.3	+ 6.9	10.0	4.6	— 5.4
6	238	1.0	8.8	7.8	+ 5.4	10.0	5.0	— 5.0
Mean					+ 4.8			— 4.1

* Incubated for 30 minutes in 0.9% NaCl in an atmosphere of oxygen at 38° C. The ATP was a commercial preparation in the form of the barium salt and was converted to the sodium salt before use.

** Inorganic phosphate.

*** Corrected for the production of inorganic phosphate by the control hemidiaphragm incubated in the absence of added ATP. In 6 independent experiments, this averaged 10 micromoles per 30 minutes per gram wet weight of tissue.

§ Measured as the inorganic phosphate liberated after 10 minutes at 100° C in 1 *N* acid.

The possibility that these results were due to the leakage of adenosine triphosphatase from the rat diaphragm during the 30 minute equilibration period was tested by adding the ATP to the medium after removing the diaphragm at the end of 30 minutes and continuing the incubation for another 30 minutes. Under these conditions, there was no hydrolysis of ATP. Inorganic pyrophosphate, β-glycerophosphate, hexose diphosphate and triose phosphate were not hydrolyzed to inorganic phosphate by rat diaphragm. However, hexose diphosphate was split to triose phosphate. In contrast to the experiments with ATP, this activity could be accounted for by the appearance of enzyme in the medium in which the diaphragm was equilibrated. This phenomenon of leaching of aldolase from the diaphragm has been described by ZIERLER[8].

An attempt was made to visualize the site of adenosine triphosphatase activity by incubating the diaphragm with and without added ATP in the presence of 0.001 *M* lead acetate (which did not inhibit the reaction). After washing with water and immersing the tissue in 1% sodium sulfide solution (to convert the precipitate of lead phosphate formed at the site of hydrolysis to the readily visible lead sulfide), the control diaphragm showed deposits of lead sulfide only at the cut edges while the diaphragm incubated in the presence of ATP showed marked deposits of lead sulfide conforming mainly to the outline of the muscle bundles at the surface of the tissue.

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Further studies on the formation of ascorbic acid in plants

Tracer studies[1-5] on the urinary L-ascorbic acid recovered from rats administered ^{14}C-labeled D-glucose have led to the following proposed pathway of conversion:

D-glucose → D-glucuronolactone → L-gulonolactone → L-ascorbic acid.

Non-isotopic experiments[6,7] on animals (normal rat) and plants (cress seedling) with various postulated intermediates led to similar conclusions. The latter studies also proposed an analogous pathway for the participation of D-galactose:

D-galactose → D-galacturonate → L-galactonolactone → L-ascorbic acid.

A study[8] of the conversion of D-glucose-1-^{14}C into L-ascorbic acid in the ripening strawberry led to the discovery that unlike the ^{14}C experiments involving the rat[2,4,5], no inversion occurred in the location of label in the ascorbic acid; that is, in the strawberry, carbon-1 of D-glucose became carbon-1 of L-ascorbic acid, in contrast to the path of conversion in the rat where carbon-1 of D-glucose became carbon-6 of L-ascorbic acid.

In view of the observations[7] relating the pathway of ascorbic acid formation in plants and animals, it became a matter of interest to learn whether the strawberry results represented a unique or possibly an alternative pathway, or whether the observation was representative of the normal path of ascorbic acid formation in plants. For this reason, experiments similar to those employing strawberries fed with D-glucose-1-^{14}C were conducted with cress seedlings (*Lepidum sativum*). In a preliminary study, etiolated seedlings, about 44 hours old, were separated from their testas and partially immersed in a 0.1% D-glucose-1-^{14}C solution (6.1 μc) for 33 hours. After an additional 17 hours in distilled water, the etiolated seedlings were ground up in boiling water, cooled, and centrifuged free of insolubles. The ascorbic acid was recovered as previously described[8]. After three crystallizations from glacial acetic acid, the ascorbic acid contained 470 c.p.m./mmole. Carbon-1 (by decarboxylation) contained 300 c.p.m./mmole as ascorbic acid. Apparently, 64% of the counts were in carbon-1.

In order to confirm this observation, a second experiment was performed. Sterile technique was observed as closely as was practical. The seeds (250) were given a preliminary five-minute soak in saturated calcium hypochlorite followed by several rinses with sterile distilled water. The etiolated seedlings were germinated over a period of 66 hours and then treated (without