Our data lead us to question further the significance of a relationship between morphine tolerance and morphine metabolism in the rat. It appears, rather, that morphine may be a nonspecific inhibitor of drug metabolism by virtue of its endocrine effects.

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Effect of stimulation and catecholamines on glucose-6-phosphate content of intact skeletal muscle*

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RECENT observations of phosphorylase have led to awakened interest in the possible involvement of hexosephosphates in the regulation of contractility. Phosphorylase is the enzyme that catalyzes the reaction: glycogen + inorganic phosphate $\rightleftarrows$ glucose-1-phosphate.[1] As a resultant of altered phosphorylase activity, one may expect to find changes in hexosephosphate levels in tissue, and it has been proposed that such changes may be related to muscle contraction.[2]

Our laboratory[3] has provided evidence that several cardiotonic agents known to increase phosphorylase activity in cardiac muscle do indeed produce increases in glucose-6-phosphate levels in the heart concomitant with their action. Related experiments on skeletal muscle contraction in particular are definitive but fragmentary.

It has been established that electrical stimulation and epinephrine increase both phosphorylase activity and hexosephosphate content of skeletal muscle.[4] Other studies indicate that epinephrine elevates the glucose-6-phosphate content of isolated diaphragm and that exogenously added hexosephosphate improves the contraction of the potassium-depressed diaphragm.[2]

To help clarify the possible role of hexosephosphates in muscle contraction, this report supplements the paucity of data in this area, particularly in studies *in vivo*, and provides some further quantitation for the previously cited basic observations relative to the effects of stimulation and epinephrine on the hexosephosphate content of skeletal muscle.

Male Wistar strain rats (generously donated by Dr. Edward Muntwyler, Department of Biochemistry), weighing approximately 300 g, were anesthetized with 90 mg pentobarbital sodium/kg administered intraperitoneally. The anterior tibial muscles were exposed and placed under a tension of 10 g. A maximal stimulus of 500 μsec duration was applied directly to the muscle for the times and

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frequencies indicated in Fig. 1. In general, the unstimulated resting muscle under tension served as the control for the stimulated contralateral muscle under tension.

For investigating the effect of catecholamines, a muscle, neither stimulated nor under tension, and excised 1 min after the intravenous injection of 0·25 ml saline, served as the control for the contralateral muscle which was removed 1 min after the intravenous injection of catecholamine (calculated as base). At the termination of all experimental periods, the muscles were rapidly excised, dropped into liquid nitrogen, weighed in the frozen state, then placed in ice-cold 0·3 N perchloric acid (4 ml/g tissue) and treated as described in our previous study.[3] Use of Boehringer's purified glucose-6-phosphate dehydrogenase in the spectrophotometric assay for glucose-6-phosphate permitted relatively specific characterization of the hexosephosphate.

Figure 1 illustrates that stimulation for 10 sec at frequencies varying from 1 to 30/sec resulted in a linear increase in the amount of glucose-6-phosphate found in skeletal muscle. No fatigue was evident. Stimulation for 60 sec at the same frequencies gave rise to a still further steep increase in glucose-6-phosphate between rates of 1 and 5/sec with a plateau occurring at the 5 to 30/sec rate. At 30/sec, the muscles displayed fatigue. Although the content of glucose-6-phosphate in the muscles appeared to fall from peak values at this time, the decline was not significant compared to the values at 5 and 10/sec, hence a plateau configuration was assigned.

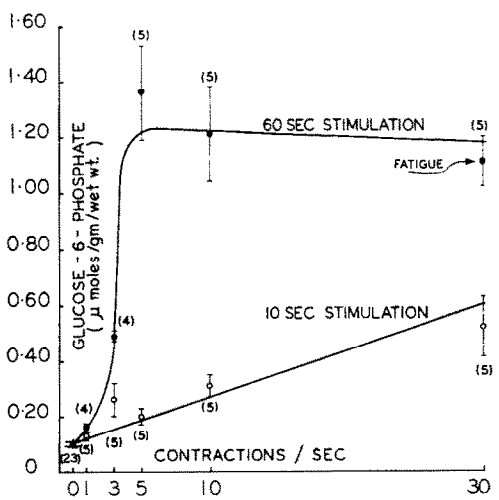

FIG. 1. The effect of stimulation on the glucose-6-phosphate content of the intact anterior tibial muscle of the rat. Each point represents the mean ± S.E. of the number of muscles indicated in parentheses. With the exception of the 1/sec frequency, all points are significantly different from the control (zero frequency = ▲). The point noting fatigue indicates that preparations could not maintain their maximal shortening.

These results are in agreement with previous studies[4-6] which showed that graded increases in phosphorylase activity occurred after increased degrees of skeletal muscle stimulation until the muscle exhibited fatigue, at which time a plateau or fall in enzyme activity ensued. An analogous finding is the apparent leveling off of hexosephosphate content of skeletal muscle when work load is increased.[4]

Table 1 reveals that both epinephrine and isoproterenol, at a dose of 1·0 μg/100 g, significantly increased the glucose-6-phosphate content of the intact anterior tibial muscle of the rat. At an equivalent dose, norepinephrine produced no changes. Inasmuch as epinephrine and norepinephrine were in the L-form and the isoproterenol was available in the DL-form, the ratio of biochemical activities may tentatively be designated as: isoproterenol > epinephrine ≫ norepinephrine. Additional studies with several dose levels would be required to establish this order of potency firmly. In accord with these results, norepinephrine has been shown to have minimal action compared to epinephrine in

increasing phosphorylase activity in skeletal muscle[6, 7] and was found not to be glycogenolytic in that tissue.[8] In further agreement[9] it has been demonstrated that inotropic activity of the catecholamines on potassium-depressed diaphragms is paralleled by changes in tissue glycogen with order of activity for both variables being the same as that reported for glucose-6-phosphate in the present study.

TABLE 1. THE EFFECT OF CATECHOLAMINES ON THE GLUCOSE-6-PHOSPHATE CONTENT OF THE INTACT ANTERIOR TIBIAL MUSCLE OF THE RAT*

	Paired control	*l*-Epinephrine, 1·0 μg/100 g
Mean ± S.E.	0·13 ± 0·04	0·37 ± 0·05
	Difference 0·24; probability 0·02	
	Paired control	*l*-Norepinephrine, 1·0 μg/100 g
Mean ± S.E.	0·14 ± 0·03	0·15 ± 0·02
	Difference 0·01; not significant	
	Paired control	*dl*-Isoproterenol, 1·0 μg/100 g
Mean ± S.E.	0·14 ± 0·02	0·40 ± 0·04
	Difference 0·26; probability 0·01	

* In micromoles per gram wet weight; five pairs of muscle employed for each comparison.

Whether glucose-6-phosphate goes through known metabolic pathways or is directly shunted to muscle contraction through unknown pathways remains to be answered.

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