THE EFFECTS OF PHOSPHATE ADMINISTRATION ON PHOSPHORYLCREATINE, GLYCOGEN, AND LACTATE LEVELS IN ANIMAL MUSCLE

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Although a large body of published work can be found [2, 3, 7, 8] on the problem of the increase in the working capacity of animals following the administration of phosphate, little actual biochemical interpretation of the phenomenon has been offered. While some workers [2, 3] attributed the effect of phosphate to the stimulation of the central nervous system, Embden and co-worker [7] believed the main mechanism of its effect to consist in the stimulation of glucose phosphorylation in muscle directly. In view of the currently accepted importance of the high-energy phosphate compounds in muscular activity, it was felt desirable to establish the effect of phosphate administration on the levels of these compounds in muscle.

The present work deals mainly with the effect of administered phosphate on the muscle level of phosphorylcreatine—one of the most important sources of contractile energy in muscle, in the form of high energy phosphate which can be passed on to ATP. The intensity of glycolysis was also investigated simultaneously by following lactate and glycogen levels.

METHOD

White rats, weighing 130-170 g, were given 30 mg P as orthophosphate per kg intraperitoneally in the form of a 3.8% solution of NaH_2PO_4. Three groups of animals were used, and the phosphate administration was carried out under amytal anesthesia, under anesthesia combined with a nervous block (tying up of n. ischiaticus) or under denervation alone, respectively. The animals were killed by decapitation at various intervals after phosphate administration, the muscle (gastrocnemius) was immediately excised and frozen in liquid air, and portions of the frozen muscle were used for the estimation of phosphorylcreatin (according to A. M. Alekseeva), glycogen (by a micromodification of the Pflüger method) and of lactate (according to Barker and Sommerson).

RESULTS

There were no significant changes in the levels of the compounds under investigation in the muscles of control animals which were given normal saline solution instead of orthophosphate. In control animals the levels of phosphorylcreatine (PC) in muscle were 213-233 mg%, of glycogen—481-533 mg% and of lactic acid—37.3-53 mg%. Administration of phosphate had a specific effect on the levels of all three compounds, the effect being dependent on the time interval between the treatment and assay. Thus, 30 min after phosphate administration (Fig. 1), PC levels fell by 30%. It should be remembered that this drop in PC levels could not have occurred as a result of excessive mobility, since throughout the period the animals were in a state of relative rest and their behavior did not differ from that of the control rats. Within one hour of phosphate administration PC levels returned to normal, but continued rising, exceeding control levels by 58% at two hours. Thereafter the PC content fell gradually, but even six hours from the onset of the experiment, its levels in the muscles of treated animals were 36% above control values. This difference disappeared within 24 hours. The changes in glycogen levels after phosphate administration were less pronounced. Statistical analysis of the data showed a lack of significant effect in most of the time intervals investigated. The only exception was the two-hour interval, where a significant increase in glycogen levels of 18% (Fig. 2) was detected. In

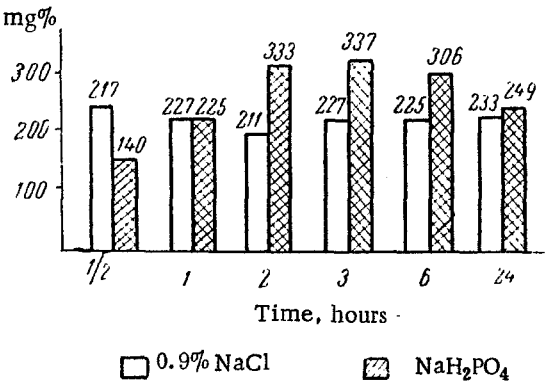

Fig. 1. Phosphorylcreatine levels in muscle after the administration of 0.9% NaCl or NaH_2PO_4.

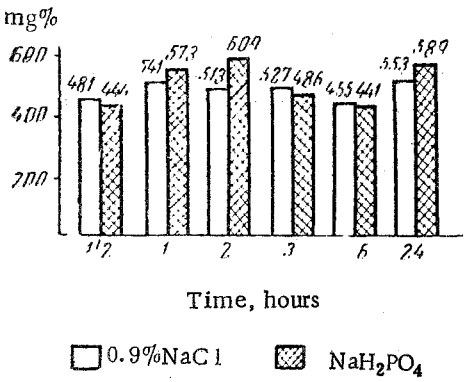

Fig. 2. Glycogen levels in muscle after the administration of 0.9% NaCl or NaH_2PO_4.

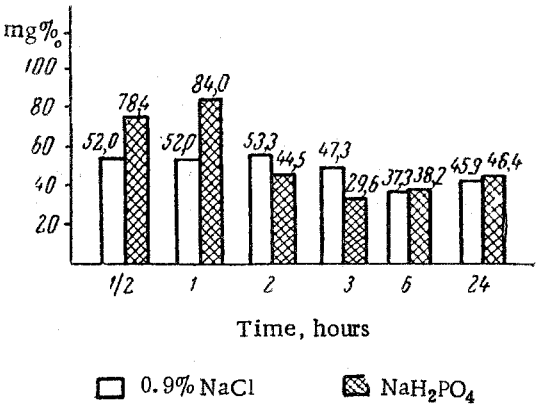

Fig. 3. Lactic acid levels in muscle after the administration of 0.9% NaCl or NaH_2PO_4.

this connection it is interesting to note that Yakovlev [4] found that the change of glycogen content in starved cats given orthophosphate followed a similar time-sequence. Lactic acid assays showed that, after the administration of phosphate, lactate levels rose in the first hour, exceeding control levels by 58-61%, and then fell, returning to normal within two hours (Fig. 3).

Consideration of the over-all result suggests that during the initial period (30 min–1 hour) after phosphate administration the intensity of glycolysis increased, giving rise to the accumulation of lactate in the muscles. This was accompanied by a fall in PC levels. There was no significant simultaneous fall in glycogen levels. It would appear that the increase in muscle lactate had taken place at the expense of the blood glucose, rather than of the muscle glycogen. The possibility of this alternative was demonstrated by Yakovlev [4], who reported a fall in blood glucose levels after phosphate administration. It is characteristic that the changes observed in the present work took place in the absence of any increase in the mobility of the animals. Of greatest interest is the observation that, two hours after phosphate administration, there was a sharp increase in PC content in muscle, and some increase in glycogen levels, both changes favoring the increased potential for subsequent work, which may be the explanation for the increased working capacity following phosphate administration. The present results so far left unanswered the questions: What is the mechanism of phosphate effect on muscle chemistry and, was the total effect of phosphate a direct one on the muscle chemistry, or an indirect one on the central nervous system and mediated through its trophic effect on muscle?

In order to resolve this problem the experiments were repeated using animals in which the action of the higher nervous centers was excluded through the application of amytal anesthesia, or peripheral denervation, or both. In all these experiments the two-hour interval, which previously afforded the greatest degree of change, was employed. The biochemical changes observed were the same as described above, irrespective of whether the experiment was carried out under amytal anaesthesia, or with denervation of the limb, or with a combination of the two (table); phosphorylcreatine levels increased by 50-55%, while glycogen and lactate levels remained at approximately normal values. It was thus demonstrated that PC levels in muscle increased after phosphate administration without the mediation of the higher nervous centers, in anesthetized animals and where the nerve supply to the muscle

TABLE. Phosphorylcreatine, Glycogen and Lactate Levels in the Muscles of Animals, Two Hours After Phosphate Administration (in mg%).

Treatment	PC	Glycogen	Lactic acid
Normal saline	211±24,4	513±42,0	53,3±7,3
Orthophosphate (30 mg P/kg)	333±17,8	609±17,6	44,5±3,9
As above, under amytal anesthesia	328±17,6	602±23,1	49,0±8,0
As above, with anesthesia and denervation	322±15,4	543±17,0	41,2±3,2
As above, with denervation only	360±14,6	556±28,1	46,8±6,7

was disconnected. The results indicated that the effect of phosphate on muscle metabolism is a direct one on the tissue. It is, of course, impossible to exclude any adaptive-trophic effect of the vegetative nervous system but apparently in the present case the effect of the latter was of secondary importance. This assumption is also supported by the work of Veselkina [1], who noted the participation of the somatic nerves in the transmission of the trophic influence of the central nervous system on muscle PC levels. Also, Yakovlev [5] observed, in experiments with isolated muscle, distinct changes in the carbohydrate-phosphorus metabolism of the tissue when the phosphate concentration in the surrounding medium was raised.

It is feasible that one of the factors which limit the relatively low level of PC in muscle is the deficiency of inorganic phosphate. This is suggested by the fact that in muscle only 30-40% of the total creatine present is actually phosphorylated. The increase of the concentration of inorganic phosphate in muscle apparently affects the carbohydrate-phosphorus metabolism of this tissue. It is well known that in muscle the synthesis of high-energy phosphates can be accomplished either by a phosphorylation coupled to oxidation, or during glycolysis. Muscle PC can also be resynthesized by either a transphosphorylation with ATP, or with disphosphoglycerate [6]. The existence of such diverse pathways in muscle makes it difficult to decide unequivocally the nature of phosphate effect observed in the present experiments. One explanation which cannot be excluded is the intensification of oxidation phosphorylation with the subsequent PC synthesis by transphosphorylation from ATP to creatine. Further work is necessary for the fuller elucidation of the phenomenon.

In conclusion, it seems reasonable to advance the suggestion that the main mechanism in the increase of working capacity of muscle by phosphate administration consists in the raising of the levels of energy-rich phosphates in the tissue.

SUMMARY

Within two hours of the intraperitoneal administration of inorganic phosphate (30 mg/kg) to rats, phosphorylcreatine and glycogen levels in muscles increased markedly. Administration of general anaesthesia or the denervation of the lower extremity failed to abolish this effect.

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