Effects of 3-(o-Toloxy)-Propane-1,2-Diol (Mephenesin) on Sodium, Potassium, and Water Content of Frog Skeletal Muscle. (21222)

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The relaxing property of 3-(o-toloxy)-propane-1,2-diol, or Mephenesin, has led in the last 10 years to a revival of interest in this compound. Although reports of Mephenesin effects on muscle and peripheral nerve have been conflicting(1,2), it has been definitely shown that this drug decreases α-excitability of frog muscle(3). The well-established dependence of electrical properties on ion content and exchange suggested a study of sodium and potassium in muscle exposed to Mephenesin. For this study 2 procedures were followed: one consisting of analysis of muscle for sodium and potassium after a period of immersion in drug solution; the other involving the influence of Mephenesin on muscle weight changes resulting from immersion in modified Ringers solution.

Method. Semitendinosus and peroneous muscle pairs were removed from *Rana pipiens* and kept in Ringers solution at 5°C for 4 to 8 hours before the beginning of an experiment. This was found to minimize erratic weight changes often seen in muscles just after their removal from the animal. Ringers solution (R) was of pH 7.3-7.4; its exact composition has been described previously(3). NaCl was reduced to 55.5 mM per liter in hypotonic, sodium-poor Ringers (½ R) and in potassium-rich Ringers (KR), the KCl content being raised to 56.95 mM per liter in the latter case. All solutions then, except ½ R and ½ RD (hypotonic Ringers containing drug) were isotonic. Oxygen was bubbled for 5-10 minutes through all solutions into which the muscles were to be placed. Care was taken in all experiments to minimize trauma to muscles. 1. *Weighing experiments.* Each muscle was blotted on filter paper, weighed on a torsion balance, and placed in a small glass dish containing one of the test solutions. Four solutions were used for immersion in each experiment: R, ½ R or KR, RD, and ½ RD or KRD. Valid comparisons could be made only between paired muscles, and the experiments were arranged accordingly. Each muscle was weighed at intervals over a period of several hours, at the end of which time it was placed again in R. 2. *Sodium and potassium analyses.* After initial weighing, one muscle of a pair was kept in Ringers, while the other was exposed to one of the test solutions. Muscles were allowed to remain at room temperature (18-20°C) in these solutions for various lengths of time, after which they were reweighed and placed in platinum crucibles for ashing. A flame photometer was used to analyze solutions of muscle ash for sodium or potassium. Experimental values were converted to milliequivalents of potassium or sodium per kg initial muscle wet weight, and comparisons were made.

Results. 1. *Weighing experiments.* Table I shows that Mephenesin, even in relatively high concentration, had no effect on the weight increase resulting from immersion in KR. However, 0.3% Mephenesin caused a significant increase in the weight gain of muscle in ½ R. This effect was reversible.

2. *Sodium and potassium.* The changes in potassium and sodium content of muscle brought about by immersion in ½ R in the absence of drug are seen in Table II: there was a slight decrease in potassium, and a large decrease, amounting to about 30% of the normal muscle level, in sodium. It was found that the procedure of repeated handling and weighing of the muscles caused a significant gain of sodium, even in ½ R. Further, in muscles so "handled" Mephenesin seemed to increase the sodium and decrease the potassium content.

Mephenesin, at a concentration of 0.1% in R, produced no significant changes in the sodium and potassium of undisturbed muscles.

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TABLE I. Effects of Mephenesin on Muscle Swelling.

Mephenesin conc. (%)	Exp. procedure	No. of exp.	Mean wt change (%)	Stand. error of mean	Probability
.05	KRD–KR	5	−1.06	± 2.35	.636
	RD–R	5	2.60	± 1.87	.181
.1	KRD–KR	7	−3.37	± 3.18	.289
	RD–R	7	−1.89	± 3.09	.531
	½ RD–½ R	4	− .96	± .94	.300
	RD–R	4	−3.28	± 2.60	.221
.3	KRD–KR	2	− .05	± .71	1.000
	RD–R	2	.40	± .57	.425
	½ RD–½ R	4	21.40	± 1.09	<.001
	RD–R	4	6.98	± 3.74	.097

Max % gain calculated from original wet wt for each muscle. Effects seen increased with time to max at 160 min. Means calculated from differences between paired muscles, one of which was immersed either in hypotonic, sodium-poor Ringers (½ R) or in potassium-rich Ringers (KR), the other in similar solution plus drug. Absence of drug effect in unaltered Ringers indicated by values of (RD–R). Avg % gain of muscle wt in R (22 exp.), 2.80, stand. error of mean, ± 5.89; in ½ R (8 exp.), 27.22, stand. error of mean, ± 3.21; in KR (14 exp.), 20.21, stand. error of mean, ± 5.58.

There was a small, questionably significant loss of potassium from drug-exposed muscles in ½ R. This possibility of a potassium loss was investigated at a higher drug concentration: Table II shows a loss of approximately 20% of the normal cellular potassium produced by 0.3% Mephenesin, both in R and in ½ R.

Discussion. The procedure of isolating and immersing frog muscles in Ringers solutions changes the water and electrolyte balance between fibers and extracellular space. The fact that *in vivo* chloride space values are considerably lower than those calculated from *in vitro* studies is a good indication of this(4,5). A certain amount of independence from such changes can be achieved by considering only differences between muscles of a pair; this was the practice in the present studies.

Muscle swells in a sodium-poor solution (½ R), losing a small amount of potassium and a much larger amount of sodium, around 30% of the normal content. If extracellular fluid is to be in equilibrium with immersion medium, and if 60% of the muscle sodium is found in the extracellular space, then a loss of 30% of the sodium is required from muscle in ½ R. Any potassium loss might represent a tendency for muscle fibers to come into equilibrium with the lowered potassium concentration of the extracellular fluid. Muscle also swells in potassium-rich Ringers (KR), even though isotonicity is maintained by an appropriate decrease of sodium. This indicates a high permeability to potassium. Any agent which could alter permeability to potassium should produce a change in this type of swelling. Shanes(6) found, for example, that cocaine markedly decreased the swelling of muscles in potassium-rich Ringers, and he concluded that potassium permeability had been decreased. In this respect Mephenesin had no effect: the swelling of muscles in KR was not altered. On the other hand, muscles in hypotonic, sodium-poor Ringers definitely swelled more in the presence of Mephenesin than in its absence.

This drug effect on hypotonic swelling is not easily explained. It cannot be interpreted as a decrease in potassium permeability, because Table II shows a greater K loss in Mephenesin (½ RD) than in ½ R. One could speculate that Mephenesin diminished the effect of the sodium pump, thus allowing accumulation of the ion and accompanying water inside the cell. But were this the case, drug-exposed muscles should have swelled in isotonic Ringers solution as well, and this did not occur.

Since the study of swelling involved frequent weighings, the question was raised of changes which might result from such "han-

TABLE II. Effects of Mephenesin on Sodium and Potassium Content of Frog Muscle.

Mephenesin conc. (%)	Exp. procedure	No. of exp.	Mean change (meq/kg muscle)	Stand. error of mean	Probability
No drug	½ R –R	9	K: –7.0	± 3.3	.080
			Na: –17.9	± 5.0	.011
.1	RD–R (not handled)	5	K: 2.4	± 2.5	.392
			Na: –5.1	± 2.1	.104
	RD–R (handled)	6	K: –1.5	± 1.2	.267
			Na: 6.6	± 1.3	.003
	½ RD–½ R (not handled)	5	K: –3.2	± 1.6	.119
			Na: –0.3	± 1.4	.839
	½ RD–½ R (handled)	6	K: –4.8	± 1.8	.046
			Na: 3.1	± 1.3	.063
.3	RD–R	9	K: –14.1	± 3.9	.007
	½ RD–½ R	9	K: –16.6	± 3.6	.002

Mean change arrived at by averaging differences obtained in muscle pairs, one of which had been immersed in Ringers (R) or in hypotonic, sodium-poor Ringers (½ R), the other of which had been exposed to Mephenesin in R or ½ R. Control value was subtracted from experimental (*e.g.*, ½ R–R), so that positive mean change indicates gain by experimental muscle, negative change, loss. Initial wet wt of muscle used in all calculations of meq change/kg muscle.

dling". Muscles disturbed or handled at intervals during the course of a 2- to 3-hour period were seen to gain sodium; muscle weight was also increased. Since in Ringers solution this procedure produced an increase in muscle sodium but no definite change in potassium, it is probable that only extracellular volume increased. Extracellular fluid, being in equilibrium with the immersion fluid, would gain sodium for the muscle by means of volume increase. It is of interest, however, that Mephenesin appeared to intensify this increase in intracellular fluid and sodium (probability: .003).

The weight gains produced by handling confirmed results of similar experiments by Fenn(7). By showing that values for chloride space were uniformly larger whenever muscle weights were larger, Fenn produced good evidence that such weight changes truly represented increases in extracellular fluid.

In Ringers solution no significant potassium change was seen at the end of 2.7 hours in the presence of 0.1% Mephenesin as compared to the control. A small, questionably significant loss of sodium was seen; this amounted to less than 10% of the usual muscle content. In ½ R a similar lack of effect on sodium content was noted. As can be seen from Table II, the loss of sodium in ½ R in the absence of drug amounted to 17.9 meq/kg or roughly 30%; Mephenesin did not alter this loss.

Although there was no statistically significant change in potassium caused by 0.1% Mephenesin in ½ R, the fact that each of 5 muscle pairs showed a small loss (mean: –3.2 meq/kg, probability: .119) suggested further studies at a higher drug concentration. There was a remarkable loss of potassium when muscles were immersed in 0.3% Mephenesin both in R and in ½ R. Muscles in this concentration of drug in ½ R took up water to the extent of almost 25% of initial muscle weight. This is indicated in Table I, in which the possibility of drug osmotic effect has been considered. The cause of such large potassium losses might be attributed to: 1. *Muscle stimulation by drug.* Mephenesin is known to initiate contracture(8), and perhaps this property, manifested over a period of several hours was capable of producing this large potassium loss. Although there was no visible twitching of these muscles, and no length or tension measurements were made, conceivably some shortening did occur. Muscle stimulation generally results in an exchange of potassium for sodium and a gain in intracellular and extracellular water(9). 2. *Release of bound or normally non-diffusible potassium.* Much has been made of the ability of myosin to bind potassium and of the dependence of this

process on pH(10). It is conceivable that any potassium which might be bound in muscle at rest or in the "normal" state becomes free and diffuses out as a result of drug action. The idea that such a release occurs when contraction takes place is far from new(11). 3. *Change in muscle membrane permeability.* In the absence of contraction, this possibility, that the cell is simply made more permeable to potassium, is unlikely. The fact that muscle swelling was not altered by the drug in KR lends improbability to such a notion.

It is possible that more than one mechanism was operative here. If as much as 33% of muscle potassium is bound or non-diffusible (12), then it is possible that some of the 20% loss came from this fraction. Since contraction can be produced by the drug, the changes related to normal function were undoubtedly reflected in the results above (the usually-seen potassium loss, sodium and water gains, and associated permeability changes).

Summary. Mephenesin in concentrations up to 0.3% caused no change in the swelling resulting from immersion in high-potassium Ringers. Mephenesin at a concentration of 0.3%, but not at 0.1%, caused a large, reversible increase in swelling resulting from immersion in sodium-poor, hypotonic Ringers. This relatively high drug concentration also caused a 20% loss of muscle potassium both in regular Ringers and in hypotonic, sodium-poor Ringers. These changes are thought to be related to muscle contraction produced by Mephenesin. Confirming results of similar experiments by Fenn(7), the procedure of disturbing or handling muscles at intervals throughout an experiment was seen to result in muscle weight gain, a small sodium increase, and little alteration of potassium content; this process was intensified by Mephenesin.

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Effect of Air Ions on Succinoxidase Activity of the Rat Adrenal Gland.* (21223)

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The atmosphere contains at all times variable amounts of gaseous molecules that possess a negative or positive charge. The concentration of these "air ions" under normal conditions may vary between 400 and 2000 ion pairs/cc, but under certain conditions an excess of either positively or negatively charged ions may occur. The possible physiologic effect of air ions has interested a number of investigators. In a series of studies carried

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