Effect of Quinidine, Ouabain, and Corticosteroids on Muscle Sodium, Potassium, and Water Content.* (27492)

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The effect of various drugs and physical agents on rat muscle electrolytes and water is being studied in an attempt to explain their actions when administered to humans with the several variants of familial periodic paralysis. Investigation of the effect of external cooling on rat muscle has furnished a possible explanation for the paralytic effect of cold in the hyperkalemic variant of familial periodic paralysis(1,2).

The present report concerns the action on muscle water and electrolyte content of quinidine, ouabain, ACTH, cortisone and triamcinolone (9-alpha fluro-16-alpha hydroxyprednisolone). The effects of quinidine and ouabain, or other agents of the digitalis group, on cardiac muscle electrolytes have been reported, but very limited and contradictory results are available concerning skeletal muscle changes(3,4).

Skeletal myopathies have been produced as toxic reactions in humans and animals given large doses of certain corticosteroids(5,6,7). Excessive digitalis has been reported to have produced parlaysis in a young girl with rheumatic heart disease(8). Two patients with a newly recognized syndrome of periodic paralysis associated with premature ectopic ventricular beats have been seen recently.† In this syndrome digitalis precipitates skeletal paralysis.

Material and methods. White male rats, 140 to 200 g, were employed in groups of 6 in this study. Three to 4 groups were studied per experimental run. Standard rat chow and distilled water were allowed *ad lib*. Control animals were injected with olive oil or received no injections. All injections were given subcutaneously in the back in 0.5 ml volumes. Muscles were removed under pentobarbital anesthesia 4 and 24 hours after the single injections and 24 hours after the last of multiple injections. Quinidine sulfate, 10-30 mg, was administered in propylene glycol, ouabain, 0.2-0.4 mg, cortisone acetate, 2.5-5.0 mg, and triamcinolone, 4 mg in olive oil. ACTH, 2.5-5.0 mg, was administered in 0.9% saline.

The methods of processing and analyzing the muscle have been described(1). In essence, the water content was determined as the difference between wet weight and dry weight after drying to constant weight in an 105°C oven. The fat was extracted with petroleum ether and the redried muscle weighed once again. Nitric acid digests of the tissue were then made. Sodium and potassium concentrations were determined on appropriate dilutions with a flame photometer. Chloride was determined by a Cotlove chloridometer. Serum electrolytes were determined by the same methods without preparatory treatment of the serum.

Chloride in the muscles was assumed to be extracellular. Intracellular sodium and water were calculated as reported previously(2).

Results. The mean concentrations of water and sodium, potassium and chloride are reported in the tables per 100 g of dry fat-free solids (DFFS) and concentrations of intracellular sodium and potassium are also reported per liter of intracellular water. Mean values for all the control animals are given in Table I, Section A. Since there was some variation in control values between experimental runs, each treatment group is compared with the control groups measured simultaneously.

No difference in weight was noted between control animals and animals treated with any of the agents for one day or less. Animals treated with cortisone and ACTH for 3 days or more gained 5-10 g less than the untreated

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† R. Klein, unpublished observation.

animals, and the animals given triamcinolone actually lost 10-30 g. The weight gain of animals given ouabain or quinidine was occasionally slightly less than that of the controls. The weight change of individual animals did not correlate with presence or degree of change in muscle contents. Measurements at 4 hours after single injection of one of the drugs never differed from those 24 hours after a single injection and these results have been combined.

Mean serum values for control animals were sodium—143, chloride—105 and potassium—4.5 meq/l. Concentrations in sera from the treated animals did not differ from these values.

Muscle analyses 4 to 24 hours after single dose of quinidine revealed a significant difference from control values only in the calculated intracellular sodium which was decreased, whether expressed per hundred gram DFFS or per liter of intracellular water ($p < .01$). The mean total sodium per hundred grams DFFS was the lowest of any group except the triamcinolone. The mean potassium per hundred grams DFFS was the highest noted. Neither measurement was significantly different from the simultaneous controls although the differences from the overall group of controls would be significant.

After 3 to 5 days of quinidine treatment, muscle analyses revealed no differences from those of normal control rats save for increased muscle water.

A single injection of ouabain was associated with marked depression of water and potassium contents and concomitant increases in sodium content. When the same dose of ouabain was repeated for 3 to 5 days, these differences disappeared or were no longer statistically significant. Fig. 1 illustrates differences from control values for water, sodium and potassium of animals treated with single or multiple injections of ouabain and quinidine.

Administration of ACTH produced no significant changes in muscle content of electrolytes or water.

The only statistically significant difference from control values after a single injection of cortisone was the decrease in calculated intracellular sodium concentration. On the other hand, when cortisone was given for 3 to 5 days, intracellular sodium content did not differ from the normals but intracellular potassium content was significantly depressed. In addition, muscle water was significantly less than that of control animals.

The effect of triamcinolone did not vary whether the drug was used for one, 2, 3 or 5

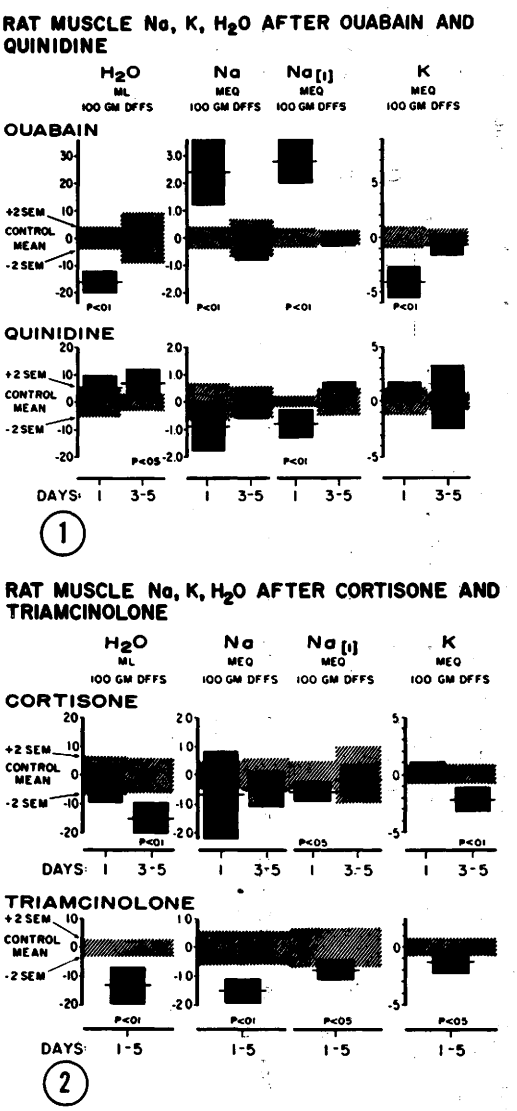

FIG. 1. Shaded area encompasses range of 2 SEM on either side of mean. Results from treated animals are presented in boxes representing means ± 2 SEM. Results are plotted using control mean as a base line.

FIG. 2. Construction is identical with that in Fig. 1.

TABLE I. Mean Concentrations of Water, Sodium, Potassium and Chloride Are Presented per 100 g of Dry Fat-Free Solids and Sodium and Potassium Concentration per Liter of Calculated Intracellular Water.

	H_2O, ml	Na, meq	K, meq	Cl, meq	$Na_{(i)}$, meq	$H_2O_{(i)}$, ml	Na, meq	K, meq
	(DFFS—100 g)						(l $H_2O_{(i)}$)	
A								
Total controls No. of animals	339 ± 1.31 64	9.9 ±.14 64	49.5 ± .24 80	5.8 ±.13 57	2.6 ±.11 57	283 ± 1.34 38	9.4 ± .59 32	175 ± 1.21 37
B								
Control	348 ± 2.00 9	10.5 ±.22 20	50.8 ± .52 20	6.2 ±.23 20	2.4 ±.18 20	284 ± 2.13 9	9.4 ± 1.51 9	180 ± 3.18 9
Ouabain, .2-.4 mg sc, 4-24 hr before sacrifice	332 ± 1.99 10 p <.01	12.9 ±.62 18 p <.01	46.7 ± .71 18 p <.01	5.9 ±.32 18	5.2 ±.42 18 p <.01	271 ± 4.20 10 p =.02	17.5 ± 2.45 10 p <.01	168 ± 1.86 10 p <.01
Control	337 ± 4.3 12	10.0 ±.34 18	48.6 ± .41 18	5.7 ±.22 14	3.0 ±.16 14	286 ± 3.96 8	10.5 ± .81 8	167 ± 2.03 8
Ouabain, .2 mg sc qd × 3-4 days	340 ± 1.99 13	9.8 ±.31 18	47.8 ± .45 23	5.4 ±.13 18	2.9 ±.23 18	289 ± 1.52 15	9.6 ± .89 15	166 ± 2.45 15
C								
Control	336 ± 2.80 12	10.0 ±.33 12	50.0 ± .60 18	5.6 ±.23 11	2.8 ±.10 11	289 ± 4.50 5	9.6 ± .78 5	172 ± 3.76 5
Quinidine, 10-20 mg sc 4-24 hr before sacrifice	341 ± 2.46 13	9.1 ±.46 13	50.9 ± .41 17	5.3 ±.23 13	2.0 ±.23 13 p <.01	292 ± 4.2 9	5.6 ± .90 9 p <.01	175 ± 2.20 9
Control	333 ± 1.68 18	9.7 ±.28 18	48.8 ± .33 17	5.6 ±.23 18	2.5 ±.24 18	281 ± 2.38 17	9.2 ± .58 17	173 ± 1.79 16
Quinidine, 10-30 mg sc qd × 3-5 days	340 ± 2.49 14 p <.05	9.6 ±.25 21	49.2 ±1.40 21	5.2 ±.20 21	2.8 ±.18 21	288 ± 2.24 14	9.4 ± .46 14	170 ± 2.61 14
D								
Control	342 ± 3.27 12	10.3 ±.25 18	50.0 ± .43 18	6.2 ±.25 18	2.3 ±.24 18	282 ± 1.50 12	9.9 ± 1.27 12	174 ± 1.32 12
Cortisone, 5.0 mg sc 4-24 hr before sacrifice	339 ± 3.41 15	9.6 ±.79 14	50.1 ± .50 15	6.2 ±.37 14	1.7 ±.17 13 p <.05	281 ± 1.75 14	6.0 ± .59 13 p <.01	178 ± 1.19 14
Control	337 ± 3.14 22	10.5 ±.28 12	49.8 ± .47 23	5.9 ±.32 12	2.8 ±.50 12	275 ± 1.59 17	10.9 ± 1.41 12	183 ± 2.24 12
Cortisone, 2.5-5.0 mg sc, qd × 3-5 days	322 ± 2.82 21 p <.01	10.0 ±.32 15	47.6 ± .48 21 p <.01	5.7 ±.33 15	2.6 ±.24 15	269 ± 2.48 15 p =.05	9.7 ± .88 15	176 ± 1.26 15 p <.02

TABLE I (continued).

	H_2O, ml	Na, meq	K, meq	Cl, meq	$Na_{(i)}$, meq	$H_2O_{(i)}$, ml	Na, meq	K, meq
	(DFFS—100 g)						(1 $H_2O_{(i)}$)	
E								
Control	337 ± 1.26 (12)	10.2 ± .35 (19)	48.6 ± .51 (19)	5.2 ± .56 (19)	2.8 ± .35 (19)	281 ± 2.08 (6)	9.3 ± 1.86 (6)	176 ± 2.24 (6)
ACTH, 5.0 mg sc 4-24 hr before sacrifice	341 ± 2.66 (15)	10.1 ± .20 (14)	48.7 ± .35 (14)	5.6 ± .07 (7)	2.5 ± .22 (7)	280 ± .76 (7)	8.7 ± .76 (7)	172 ± 1.57 (7)
Control		10.5 ± .28 (19)	49.8 ± .51 (19)	5.9 ± .32 (19)	2.7 ± .50 (19)			
ACTH, 2.5 mg sc qd × 2-4 days		10.4 ± .98 (18)	49.1 ± .94 (23)	5.9 ± .13 (18)	2.7 ± .55 (18)			
F								
Controls	327 ± 1.67 (17)	9.9 ± .28 (12)	49.1 ± .41 (16)	5.2 ± .21 (12)	3.2 ± .34 (12)	270 ± 2.55 (12)	11.7 ± 1.3 (12)	182 ± 3.26 (11)
Triamcinolone, 4 mg sc, qd × 1-5 days	314 ± 3.1 (23)	8.4 ± .20 (17)	47.8 ± .44 (23)	4.6 ± .15 (17)	2.4 ± .15 (16)	261 ± 2.28 (17)	9.3 ± .62 (16)	182 ± .22 (17)
	$p < .01$	$p < .01$	$p < .05$	$p < .05$	$p < .05$	$p < .02$		

In Section B-F, results from groups of treated animals are compared to results from simultaneous control groups. Stand. error of each mean and number of animals are included as well as probability of differences occurring by chance. Intracellular water is calculated assuming an extracellular position for chloride according to the formula:

$$H_2O_{(i)} = H_2O_{(muscle)} - \frac{1000 \times Cl_{(muscle)}}{\frac{Cl_{(serum)}}{.977} \times \frac{100}{H_2O_{(serum)}}}.$$

days. The muscle content of both cations and water was significantly less than that of controls. Fig. 2 illustrates the differences from control values for animals treated with single or multiple injections of cortisone and triamcinolone.

Discussion. The acute effects of ouabain and quinidine were qualitatively predictable from reports of their effects on cardiac muscle but the changes were surprisingly great. Muscle water and potassium were less and sodium more after one injection of ouabain. The effects of quinidine were in the opposite direction though not all were statistically significant. Aikawa *et al.* reported no diminution in muscle water content with digitalis(4). Gertler *et al.* did not comment on this although in their experiment the cardiac water per kilogram of fat-free tissue was less for digitoxin-treated animals than for controls (3). The lack of significant differences from the controls after 3 to 5 days of treatment in this study may have been the result of a compensatory reaction or may have been secondary to drug toxicity. Although obviously sick animals were removed from the study, some of the treated animals gained slightly less weight than did control animals. On the other hand, the dosages roughly approximated those used with other animal species for 7 to 10 days without toxicity and with demonstrable changes in cardiac muscle sodium and potassium.

It would appear that the primary action of cortisone on muscle results in sodium leaving the cell. The reverse of this effect with potassium and water leaving the cell in animals treated 3 to 5 days might be secondary to toxicity from the drug. On the other hand, it could be explained by the separate effects of steroid directly on the muscle membrane and on the kidney. This was suggested by Woodbury to explain the divergent action of DOC and aldosterone in mice(9). Four days treatment with aldosterone produced an elevated muscle potassium content without significant change in sodium. DOC, on the other hand, resulted in an increased intracellular sodium and no change in potassium. Woodbury suggested the possibility that DOC had no direct effect on the muscle and that its renal sodium-retaining effect secondarily raised muscle sodium. This explanation could be adapted to suggest that cortisone acts directly on the muscle membrane to cause sodium to leave (and probably potassium to enter) the cell. This would explain the effect of a single injection. If the renal effect became predominant, the findings after 3 to 5 days of cortisone administration could be explained. This reasoning could also be offered to explain the changing effect of ouabain and quinidine with changing duration of treatment.

Triamcinolone showed no reversal of effect in times and doses employed. Possible failure of more prolonged treatment to accomplish this need not necessarily suggest lack of dichotomy in the action on renal and muscle membranes but might merely indicate continuing predominance of the muscle effect of the very large doses.

In all these experiments there was a tendency for muscle water to vary directly with muscle potassium. Thus potassium concentration per liter of intracellular water tended to deviate less from the control value than potassium concentration per 100 g DFFS with either increasing or decreasing potassium concentration.

Summary. A single injection of ouabain depressed rat muscle water and potassium, and increased its sodium contents. Quinidine acted reciprocally but only the depression of intracellular sodium was statistically significant. Muscle analyses after 3 to 5 daily injections revealed no significant changes from control values. Single injections of cortisone also depressed intracellular sodium, but daily injections for 3 to 5 days were associated with loss of water and potassium from the muscle with a return to normal sodium concentration. These animals when treated for 3 to 5 days gained less weight than did untreated animals. Triamcinolone in single or multiple injections produced marked depressions of muscle water, sodium and potassium. Animals treated with this agent for more than 2 days lost weight.

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Studies on Regulation of Plasma Iron in Dogs with Turpentine Abscesses. (27493)

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Hypoferremia occurs acutely in both man and animals as a result of sterile or pyogenic inflammation(1,2). The drop in plasma iron occurs within the first 24 hours after onset of inflammation and persists for the duration of inflammation. It has been shown that radioactive iron administered to animals with acute inflammation in amounts exceeding the iron binding capacity of the circulating plasma is taken up largely by the reticuloendothelial systems of the liver and spleen (3) as is the iron of senescent red cells(4). The studies by Freireich(4) indicate that during acute inflammation there is a defect in the release of iron from the tissues to the plasma. The mechanisms responsible for these changes in iron metabolism and for the resulting hypoferremia are unknown. This phenomenon—a local inflammatory process acting at a distance upon target tissues—suggested that a humoral factor might mediate the alterations in iron distribution responsible for this hypoferremia.

Preliminary investigations by Cartwright *et al.*(1) suggested that the sterile empyema fluid produced in a dog by intrapleural installation of turpentine induced a lowering of the plasma iron when injected into normal dogs. This report and the above considerations prompted a search for a potent circulating factor in dogs with subcutaneous turpentine abscesses which would be capable of lowering the plasma iron of normal recipient dogs when administered in relatively small volumes.

Materials and methods. Sterile abscesses were produced in mongrel dogs (weighing approximately 11 kg) by subcutaneous injection of 2 ml of turpentine at the base of the scapula. Blood was collected in acid citrate-dextrose solution from normal dogs and from dogs with sterile abscesses, and plasma was separated by centrifugation at 3000 rpm at room temperature. If not used at once, plasma was frozen and stored at −20°C.

The animals were in the fasting state at time of injection of turpentine, collection of blood and infusion of plasma. Bacterial cultures of plasma used for infusion were negative. Recipient animals did not have post infusion pyrexia or other ill effects. Plasma iron levels were determined by the method of Schade *et al.*(5).

Results. Fasting morning serum samples were obtained from 3 normal dogs over a 15-day period and iron levels were determined. The results are shown in Fig. 1. A marked fluctuation in iron level was noted; in one case as much as 200 μg per 100 ml from one day to the next.

In another series of experiments, 45 ml of acute phase plasma (approximately 8% of re-

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