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Anaerobic Uptake of Potassium by Frog Muscle.

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Steinbach[1] has shown that frog muscle loses potassium in exchange for sodium when the muscle is soaked in potassium-free Ringer's solution. He also showed that when potassium depleted muscles are placed in Ringer's solution containing potassium, they regain potassium at the expense of sodium. This uptake of potassium in exchange for sodium requires work which must be supplied by the muscle. It has previously been shown that muscle retains its potassium in Ringer's solution in the absence of oxygen.[2] The present investigation was undertaken to determine the influence of aerobic respiration on the uptake of potassium under the conditions of Steinbach's experiments.

The sartorius, semitendinosus, and tibialis anticus longus muscles from recently caught frogs were dissected and placed in potassium-free Ringer's solution containing M/3000 phosphate buffer, pH 7.4. After one hour the muscles from one frog were transferred to 250 ml of K-free Ringer's solution which was stirred by a current of air and kept in a cold room at 4°C. After about 17 hours the muscles were divided into sets for further treatment. This treatment was of 3 sorts, (a) immediate analysis, (b) analysis after immersion for 4 or 7½ hours in 25 ml of Ringer's solution containing 10 mM K and M/150 phosphate buffer and continuously equilibrated with air, (c) the same as (b) but continuously equilibrated with pure nitrogen starting ½ hour before the muscles were introduced. Paired sets of muscles were used to compare treatments (a) and (b), (b) and (c), and (a) and (c), as shown in Table I. Fenn and Cobb[3] state that muscles lose more potassium in Ringer's solution containing m/3000 phosphate than in M/150 phosphate. One incomplete experiment showed no difference in the uptake of potassium from solutions containing M/3000 and M/150 phosphate buffer.

On removal from the solution, the muscles were individually blotted, weighed on a torsion balance, and placed in small weighing bottles. The bottles were dried over night at 100° and the loss in

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[1] Steinbach, H. B., *J. Biol. Chem.*, 1940, **133**, 695.

[2] Dean, R. B., *J. Cell and Comp. Physiol.*, 1940, **15**, 189.

[3] Fenn, W. O., and Cobb, D. M., *J. Gen. Physiol.*, 1934, **17**, 629.

weight was taken as the water content. On small muscles weighing less than 150 mg this procedure is accurate to within 1%. Chloride was determined by a new micro-diffusion method in which HCl gas is liberated from the dry tissue by concentrated H_2SO_4 and absorbed by NaOH on a piece of filter paper fastened to the lid of the weighing bottle. Chloride was determined on the paper by a modification of the method of Van Slyke.[4] The method and its errors will be described elsewhere. Potassium was determined on the sulphuric acid digest by the method of Wilde.[5] In this way determinations of water, chloride, and potassium could be made on the same tissue. The significance of the results was tested by Students' test, taking a probability that the sets are equal of less than 0.05 as significant.

Table I contains values averaged by sets for the results obtained on 10 frogs during the month of August. K_m and Cl_m are reported in micromols per gram of whole muscle. The fiber water was calculated on the assumption that all the chloride is extracellular and at a concentration of 114 millemolar. K_f represents the concentration of potassium in the fiber water. It is calculated from K_m by deducting the potassium in the extracellular phase which is assumed to have a concentration equal to that of the potassium in the Ringer's solution. A fiber potassium concentration, K_c, has also

TABLE I.
Effect of Oxygen and Nitrogen on Intake of Potassium from a High Potassium Solution.

Set	Gas	Hr in 10 mM K	No. of muscles	Dry wt, mg	H_2O %	K_m	Cl_m	Fiber H_2O %	K_f	K_c	K_d
a	control	0	5	39.6	81	59	26	58	**105**	**105**	**302**
b	O_2	7½	5	39.2	81	69	36	49	**137**	**125**	**355**
a	control	0	8	27.8	81	62	33	52	120	120	**326**
c	N_2	7½	8	26.5	83	66	38	49	128	119	**354**
b	O_2	7½	9	20.3	82	63	50	39	**152**	**141**	**329**
c	N_2	7½	9	21.6	83	62	46	43	**135**	**126**	**346**
b	O_2	4	6	25	81	76	33	52	**139**	**128**	383
c	N_2	4	6	25	82	70	35	51	**128**	**118**	377

Average values for small isolated frog muscles. All concentrations are in micromols per gram. K_m and Cl_m are calculated per gram net weight. K_f is concentration in the water of a chloride free fiber. K_c is concentration in the water of chloride permeable fiber. K_d is amount per gram dry weight. Pairs of values in bold face type are significantly different.

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[4] Van Slyke, D. D., *J. Biol. Chem.*, 1923, **58**, 523.

[5] Wilde, W. S., *J. Biol. Chem.*, 1939, **128**, 309.

been calculated on the assumption that chloride penetrates the fiber and has a concentration in the fiber water equal to the potassium concentration in the Ringer's solution. This assumption follows from the theoretical treatment of Conway and Boyle[6] (see Dean[7]) for fibers that are permeable to chloride as is probably the case when the external postassium is as concentrated as 10 millemolar. A potassium content per gram of dry weight has also been calculated, K_d, allowing for potassium in the extracellular spaces. This was calculated for a chloride-free fiber; values of K_d, calculated on the assumption that the fiber is chloride permeable, are slightly greater in set c. Since the dry weights show no consistent variation between paired sets, they probably are a good indication of the initial weights which were unfortunately not recorded.

The values of K_d, K_c, etc., were calculated individually for each muscle and the results averaged. If the values of these concentrations are calculated from the average values of K_m, Cl_m, and H_2O somewhat different results will be obtained because of the variability of the original data.

On the basis of potassium content K_m, there is no significant difference between the sets. However, the concentration of potassium inside the fibers K_f or K_c, is significantly greater in set b in air than in the initial set a, or the anaerobic set c. There is no significant gain in the concentration of fiber potassium in nitrogen. However, when we consider the amount of potassium in the fiber, K_d, it is apparent that after 7½ hours that the fibers in nitrogen have gained more potassium than either of the other two sets, and after 4 hours the gain is only 2% less in nitrogen than in oxygen. This paradox is explained by the fact that the muscles in nitrogen swell considerably and an increase in fiber size requires an uptake of potassium to keep the concentration constant.

This work shows that muscle fibers can take up potassium against a concentration gradient anaerobically as well as aerobically, and supports the previous work,[2] where it was shown that muscle fibers are permeable to potassium yet maintain their normal potassium concentration in nitrogen just as in oxygen. The fact that the fibers in nitrogen failed to increase their potassium concentration, might be explained by supposing that the fibers were already doing all the work that was possible just to maintain their potassium concentration as the fiber increased in volume.

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[6] Conway, E. J., and Boyle, P. J., *Nature*, 1939, **144**, 709.

[7] Dean, R. B., *Biol. Symposia*, 1940, in press.