Permeability of Frog Skeletal Muscle Cells to Choline

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ABSTRACT Using choline-methyl-C^{14} as a tracer, it has been shown that choline$^+$ penetrates into the cells of resting frog skeletal muscle at a rate similar to that of Na^+, and that it escapes from these cells much more slowly than does Na^+. Some implications of these findings are discussed.

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It is generally believed that choline ions do not penetrate cell membranes, and choline has been used widely as a "non-penetrating" substitute for sodium in experiments with surviving excitable tissues. Recently Koblick (1959) and Kirschner (1960) have shown that choline is capable of diffusing through frog skin at a rate comparable to the passive movement of sodium. The present experiments show that choline penetrates into the cells of resting frog skeletal muscle about as fast as sodium does, and that it escapes much more slowly. The slow entry of choline into the cells should not interfere with its use as a substitute for sodium in brief experiments, since its behavior in this respect is closely similar to sodium. But because it is not removed from the cells at a comparable rate, presumably due to the selectivity of the "sodium pump," choline will be accumulated in the cells on prolonged exposure, and potassium will be displaced, leading to gradual depolarization. In this respect, its action is similar to that of lithium (Keynes and Swan, 1959*b*).

METHODS

Sartorius muscles of *R. pipiens* were suspended on wire frames in bicarbonate-Ringer's solution containing choline-methyl-C^{14} chloride, 3 μc/ml. The concentration of carrier choline was 4.0 m.eq per liter. The solutions were stirred with fine bubbles of 4.4 per cent CO_2 in O_2; bath temperature ranged between 22 and 25°C; weights of individual muscles ranged from 27 to 83 mg. After immersion for 1, 2, or 4 hrs., the muscles were blotted, weighed, and extracted in 1 ml distilled water for more than 2 hrs. The extract was then assayed for C^{14} activity as a layer more than 1 mm thick

under a mylar window gas-flow GM counter. Counting of dried films was considered unfeasible because of their hygroscopic nature. A portion of the soaking solution was diluted to 1 ml and assayed in the same manner. All counts were corrected for background, and tissue radioactivity was expressed as the ratio of corrected counts per gram muscle to corrected counts per milliliter solution. In some experiments (Table

TABLE I

PENETRATION OF CHOLINE-METHYL-C^{14} AND SODIUM^{22} INTO FROG SARTORIUS MUSCLES

Resting muscles, 22–25°C.

(1) Exposure time	(2) Ratio* Muscle counts/*gm.* / Solution counts/*ml.*	(3) Influx at 4.0 m. eq/liter	(4) Influx‡ at 4.0 m. eq/liter
hrs.		*μeq/hr.* × *gm*	*μμeq/sec.* × *cm²*
Choline-Methyl-C^{14}			
1	0.348		
1	0.487		
1	0.278	0.58	0.39
2	0.432		
2	0.378	0.57	0.38
1	0.236		
2	0.653	0.54	0.36
4	0.903		
2	0.553	0.32	0.21
4	0.715		
			Mean 0.34
Sodium^{22}			
1	0.291	—	—
1	0.330	—	—
1	0.342	—	—
1	0.440	—	—

* Braces indicate muscle pairs.

‡ Calculated from column 3 as described in the text.

II), the muscles after immersion were placed in unlabeled Ringer's solution for 1 or 2 hrs. to allow the choline-methyl-C^{14} to diffuse out, and then assayed as above. For comparison with the measurements of choline-methyl-C^{14}, sodium^{22} uptake was measured in several muscles by similar techniques, using Na^{22} concentrations of 0.2 to 2.0 μc/ml bath solution, and assaying the whole muscles and portions of solution in a well-type scintillation counter. The composition of Ringer's solution used was Na^+ 111 m.eq per liter, K^+ 2.0, Ca^{++} 2.0, Mg^{++} 2.0, Cl^- 98.5, HCO_3^- 18.5, with the Na^+ concentrations 4 m.eq per liter less when choline-methyl-C^{14} was present.

RESULTS

Table I documents the progressive penetration of choline-methyl-C^{14} into the soaked muscles. After 4 hrs., the ratio of muscle to bath choline-methyl-C^{14} ("choline space") approaches unity, and is still rising. Comparison of these results with the data for Na^{22} uptake in the lower part of the table shows

TABLE II

CHOLINE-METHYL-C^{14} AND SODIUM^{22} REMAINING IN FROG SARTORIUS AFTER WASHOUT

Resting muscles 22–25°C. The muscles were soaked in radioactive solution for 1 hr., then in non-radioactive solution for 1 or 2 hrs.

(1) Wash time	(2) Ratio Muscle counts/*gm.* / 1st solution counts/*ml.*	(3) Influx at 4.0 m. eq/liter	(4) Influx* at 4.0 m. eq/liter
hr.		*μeq/hr.* × *gm*	*μμeq/sec.* × cm^2
Choline-Methyl-C^{14}			
1	0.171	0.69	0.46
1	0.210	0.84	0.56
1	0.229	0.92	0.62
1	0.208	0.84	0.56
	Mean 0.205		
2	0.188	0.75	0.50
2	0.189	0.75	0.50
	Mean 0.189		0.54
Sodium^{22}			
1	0.008	—	—
1	0.007	—	—
1	0.006	—	—
1	0.007	—	—

* Calculated from column 3 as described in the text.

that in 1 hr. choline penetrates to approximately the same extent as sodium. The active extrusion of sodium by the cells prevents comparison over longer intervals. In eight similar muscles, the mannitol space (1 to 4 hrs.) ranged between 0.184 and 0.227 ml per gm muscle with a mean of 0.204, making it evident that both sodium and choline enter the cells.

If muscles loaded with choline-methyl-C^{14} are immersed in unlabeled Ringer's solution, the extracellular choline is lost rapidly, but little escapes from the cells. Table II gives data for muscles soaked 1 hr. in choline-methyl-

C^{14} Ringer's solution, and then washed for 1 or 2 hrs. in two changes of unlabeled Ringer's solution. More than half the radioactivity taken up remains (compare with Table I). In contrast, almost all the Na^{22} taken up by the muscles in 1 hr. is lost during 1 hr.'s washout. Muscles washed 2 hrs. retained almost as much choline-methyl-C^{14} as muscles washed only 1 hr.

DISCUSSION

From the data in Table II, the influx of choline into the muscle cells was estimated by the principle used by Keynes and Swan (1959 a) to calculate sodium influx. Assuming that all the choline-methyl-C^{14} taken up by the cells remains after 1 or 2 hrs. washing, while all extracellular choline-methyl-C^{14} is removed:

$$\text{Choline influx } (\mu\text{eq per hr.} \times \text{gm}) = \frac{\text{cell radioactivity per gm}}{\text{bath radioactivity per ml}} \times \text{bath } [\text{choline}^+] \ (\mu\text{eq per ml}) \div \text{time of exposure (hr.)}$$

Measured influxes at a bath concentration of 4.0 m.eq per liter choline are given in column 3 of Table II. A second estimate of choline influx was made using the data in Table I for paired muscles soaked for different times. The *increase* in cell/bath radioactivity ratio between 1 and 2 or 2 and 4 hrs. was used in the equation above. These estimates are listed in column 3 of Table I. The values are somewhat lower than those obtained by the first method, possibly due to the increasing internal concentration of choline with prolonged exposure, or perhaps because some extracellular choline was taken up by the cells during the washout period in the first series.

Keynes and Swan (1959 *a*) measured sodium influx into frog sartorius muscles at various external Na^+ concentrations, using lithium to make up the difference between the concentration used and 111 m.eq per liter. At 111 m.eq per liter they reported Na^+ influx to be about $4.3\mu\mu$ eq per sec. $\times$ cm^2 cell surface, and at 4 m.eq per liter (by interpolation of their tabulated data), about $0.30\mu\mu$ eq per sec. $\times$ cm^2. To compare the present measurements of choline influx with the latter figure, I have divided the fluxes in columns 3 of Tables I and II by Keynes and Swan's estimate of the cell surface per gram of frog sartorius (415 cm^2) and by 3600 sec./hr., and listed the values in columns 4 of the tables. The choline influxes in Table I are about the same as their Na^+ influx, those in Table II somewhat greater. According to Keynes and Swan (1959 *a*), sodium influx is not proportional to external Na^+ concentration, being disproportionately large at low concentrations. If the relation between choline influx and external choline concentration should be the

same as for sodium, the influxes of both ions would bear the same relation to each other at 111 m.eq per liter as at 4 m.eq per liter. However, if choline influx is simply proportional to external choline concentration, its influx at 111 m.eq per liter would be about twice as great as that of sodium. In the absence of more detailed information about the relation between choline concentration and choline influx, it seems reasonable to conclude only that the sodium and choline influxes into frog sartorius muscle are of about the same magnitude. If these two ionic fluxes share the same pathway into the cell, it is clear that this pathway shows little discrimination between them.

The outflux of choline$^+$ from the muscle cells, however, is much smaller than the outflux of Na^+. The present data do not permit accurate calculation of choline outflux, but some indication of its small size is given by the observation that muscles washed 2 hrs. in unlabeled Ringer's solution contained only slightly less choline-methyl-C^{14} than those soaked only 1 hr. It follows that the mechanisms which transport Na^+ actively out of the cell discriminate very effectively between Na^+ and choline$^+$.

Since both Li^+ and choline$^+$ have been used as substitutes for Na^+ in artificial saline media for various purposes, it is worth while comparing the extent to which they imitate the properties of Na^+ with respect to its movements through the membrane of frog skeletal muscle cells. For this comparison, the data for Li^+ are taken from Keynes and Swan (1959 *b*). *First*, the influx of Li^+ into the resting cells is one fourth to one half that of Na^+, while that of choline$^+$ is as large as Na^+, or possibly somewhat larger. *Second*, the efflux of Li^+ is one-tenth to one twenty-fifth as fast as that of Na^+, and that of choline$^+$ is also only a small fraction of the Na^+ outflux. *Third*, Li^+ can substitute for Na^+ in maintaining an action potential, while choline$^+$ cannot (Nastuk and Hodgkin 1950; Hagiwara and Watanabe 1955). What the different specificities of the three pathways mean in terms of membrane organization is not presently clear.

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