Potassium Ion: Is the Bulk of Intracellular K^+ Adsorbed?

Abstract. *When a major portion of the intracellular K^+ in frog muscle is reversibly replaced by Na^+, the extra Na^+ gained by the cells does not show the nuclear magnetic resonance signal that free Na^+ does. The data contradict the membrane theory but are in accord with the concept that the bulk of intracellular K^+ is adsorbed.*

According to the membrane theory, the living cell is essentially a sac of aqueous solution; water and solutes in the cell are predominantly in the free state (*1*). The maintenance of a steady amount of a specific solute is usually ascribed to the operation of a "pump" in the cell membrane (*2*). Though it is widely held, the membrane theory is not the only explanation offered (*3, 4*). Thus, the association-induction hypothesis holds that the entire cell is a complex protein-aqueous fixed-charge system (*5–7*) in which water molecules exist as polarized multilayers (*8, 9*). Generally, intracellular solutes are either interstitial—existing in the cell water at concentrations lower than in the medium surrounding the cell, because the entropies of these solutes are lower in the cell water than in the surrounding medium (*5, 8*)—or they are adsorbed on proteins and other macromolecules. This adsorption can be highly specific and may enable the cell to accumulate a solute to a concentration many times higher than that in the medium surrounding the cell (*5, 7, 10*).

Two new techniques make possible the determination of the physical state (free or adsorbed) of the bulk of one intracellular solute, K^+. One of these techniques is the in vitro preservation of living—by the criteria of resting potential, contractility, and K^+ and Na^+ contents (*10, 11*)—frog muscle cells at 25°C for up to 8 days. By lowering the external K^+ concentration while keeping the Na^+ concentration normal, one can replace the bulk of intracellular K^+ with Na^+ (*12*). The cells maintain their functional integrity under these conditions; they promptly return to normal ionic content when returned to a medium with normal K^+ content.

The second technique is the quantitative assay of free Na^+ in intact, uninjured living cells with nuclear magnetic resonance (NMR) spectroscopy. Assay of free Na^+ in 20 percent gelatin gel and in 20 percent bovine serum albumin by either NMR or flame spectrophotometry yields (within ±5 percent) the same result. Thus, the presence of proteins or protein gel per se, at a concentration typical of that in muscle cells, does not significantly alter the NMR signal of Na^+.

The data in Table 1 confirm the existence of an NMR-visible and an NMR-invisible fraction of Na^+ in frog muscle (*13*). Isolated sartorius muscle (from *Rana pipiens* Schreber), kept 3 to 4 days in vitro in a medium containing a normal amount (2.5 mmole/liter) of K^+, shows a small gain in NMR-invisible Na^+ but no change in the total K^+ or free Na^+ content (Table 1). The same muscle, kept 1 to 5 days in media containing low concentrations (0.2 to 0.5 mmole/liter) of K^+, shows extensive changes in ionic composition (Table 2).

Although the changes of total Na^+ vary from sample to sample, the free Na^+ content remains constant (Table 2). The K^+ lost is made up primarily by the NMR-invisible Na^+.

Table 1. Concentrations of sodium and potassium ions in frog muscle. Total K^+ and Na^+ contents determined from hot HCl extracts by flame photometry (*5*, p. 201; *11*); NMR-visible Na^+ determined (on 2-millimeter samples of muscle tissue) with a wide-line spectrometer (Varian) by the method of Cope (*13*); NMR-invisible Na^+ determined as the difference between total Na^+ and NMR-visible Na^+ concentrations.

Muscle type	Duration of in vitro incubation (days)	Total K^+ (μmole/g)	Total Na^+ (μmole/g)	NMR-visible Na^+ (μmole/g)	NMR-invisible Na^+ (μmole/g)	Sum of NMR-invisible Na^+ and total K^+ (μmole/g)
			Freshly isolated			
Sartorius		89.9	23.3	9	14	104
Semitendinosus		81.1	23.3	10	13	94
Tibialis anticus longus		64.3	28.3	12	16	80
Mean		78.4	25.0	10	14	93
			*Preserved**			
Sartorius	4	76.8	33.7	14	20	97
Sartorius	3	83.5	33.5	12	22	106
Sartorius	4	84.9	29.5	11	19	104
Mean		81.7	32.2	12	20	102

* Muscle preserved in a solution containing 2.5 mmole K^+ per liter.

Table 2. Concentrations of sodium and potassium ions in frog muscle preserved in a medium with a low K^+ concentration (0.2 to 0.5 mmole/liter). Determinations made as in Table 1.

Duration of in vitro incubation (days)	Total K^+ (μmole/g)	Total Na^+ (μmole/g)	NMR-visible Na^+ (μmole/g)	NMR-invisible Na^+ (μmole/g)	Sum of NMR-invisible Na^+ and total K^+ (μmole/g)
		Sartorius			
3	37.7	83.6	27	57	95
3	32.4	79.3	29	50	82
5	10.1	112	43	69	79
3	24.2	84.5	26	59	83
2	50.5	76.8	22	55	106
1	53.5	43.5	18	36	80
2	44.5	71.3	25	52	107
2	32.5	69.5	26	44	77
2	37.8	56.9	24	33	71
4	40.8	98.2	28	70	111
		Semitendinosus and tibialis anticus longus			
2	44.0	72.2	26	46	90
1	41.7	48.2	24	24	66
5	38.3	67.9	23	45	83
		*Mean**			
	37.5	74.1	26 ± 1.6*	49	87

* ± Standard error.

The basic tenet of the membrane theory is contradicted in that, in an intact living cell, the Na^+, which has substituted for K^+, is not free but adsorbed. Thus, in agreement with the association-induction hypothesis (*3–5*), most of the intracellular K^+ is adsorbed. When K^+ is low in the external medium, the cell loses its intracellular K^+. The adsorption sites thus vacated are occupied by Na^+ (*5*) which may be expected to be NMR-invisible (*14*); and it was.

GILBERT N. LING
Department of Molecular Biology, Pennsylvania Hospital, Philadelphia 19107

FREEMAN W. COPE
Biochemistry Division, Aerospace Medical Research Laboratory, U.S. Naval Air Development Center, Johnsville, Warminster, Pennsylvania

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Poliovirus Crystals within the Endoplasmic Reticulum of Endothelial and Mononuclear Cells in the Monkey Spinal Cord

Abstract. *The lumbar motor columns of a cynomolgus monkey that had become tetraplegic after experimental infection with a highly virulent strain of type 3 poliovirus were examined by electron microscopy. Crystalline aggregates of poliovirus occurred within the endoplasmic reticulum of endothelial cells as well as of mononuclear inflammatory cells. This finding suggests that the endoplasmic reticulum might be much more involved in poliovirus multiplication than has been previously supposed.*

The findings described here were obtained in an adult cynomolgus monkey that had developed a severe tetraplegia following an intramuscular injection with 2×10^7 TCD_{50} (tissue culture dose, 50 percent effective) of a highly virulent strain of type 3 poliovirus (*1*). Six days after the infection the anterior columns of the lumbar spinal cord were examined by electron microscopy (Zeiss EM 9A), for which conventional fixation and embedding methods (glutaraldehyde, osmium tetroxide, and Epon 812) were used. The remainder of the spinal cord was examined by routine histological methods and by a fluorescent antibody technique.

Histological sections of the lumbar spinal cord showed an almost total loss of motor neurons and a focal-to-diffuse infiltration of the severely damaged anterior horns by inflammatory cells, the predominating types of which were mononuclear elements, including mature macrophages. By the aid of immunohistochemical examinations, the presence of poliovirus antigen could be clearly demonstrated within the walls of intraspinal blood vessels, as well as within the cytoplasm of many mononuclear cells. Electron microscopic study of this process offered interesting additional information. Within the cytoplasm of many endothelial cells, monocytes, histiocytes, and macrophages there occurred aggregates of dense particles of uniform size. These particles appeared spherical or polyhedral in shape, measured about 270 Å in diameter, and were frequently arranged in ordered lattices, the three-dimensional character of which became obvious by their repeated presence in serial sections. Within these ordered lattices the individual particles were usually hexagonally packed but, in some instances, square arrays were also encountered (Fig. 1, a–c).

The size of the individual particles approximates that previously determined for mature polioviruses by different electron microscopic methods (*2–5*). Furthermore, the lattice pattern of the particle aggregates closely resembles that of poliovirus crystals seen in infected tissue culture cells (*5–7*), in cells of infected animal hosts (*8*), and in virus preparations of highest purity (*2, 4*). Finally, the cells containing the crystalline arrays were of the same type as those in which poliovirus antigen was demonstrated by means of the fluorescent antibody technique. Accordingly, there is convincing evidence that the observed particles were indeed poliovirus.

The poliovirus crystals, especially the larger ones, frequently lacked a limiting membrane and hence were embedded in the ground cytoplasm proper. However, smaller crystalline virus aggregates could be found sporadically within membrane-bounded cysts. The limiting membranes of the latter were habitually very delicate and, as a rule, appeared to be blurred or interrupted in many places (Fig. 1, a–c). In some instances, cysts enclosing poliovirus crystals exhibited direct continuity with tubular channels, along the outer surfaces of which typical ribosomes (Fig. 1, b and c) were observed. These tubules, which evidently represented a part of the rough-surfaced endoplasmic reticulum, often converged radially to the virus-containing cysts, thus forming starlike figures (Fig. 1c). They usually included a homogeneous substance of moderate density, but no particles with the appropriate size and shape for mature poliovirus could be detected within them.

At first glance one might be tempted to believe that the poliovirus crystals within endothelial and mononuclear cells were due to endocytosis of virus from the blood or from necrotic neurons, or from both. However, there are two facts incompatible with such an assumption. On the one hand, the described crystalline formations were