Sodium and Potassium Content of Isolated Nuclei

It is well known that the concentration of sodium is low and that of potassium high in the intracellular space of most mammalian tissues. Nevertheless, an appreciable amount of each has been found in preparations of mitochondria isolated by differential centrifugation from homogenates of various tissues, particularly liver[1-4] and kidney[5]. The purpose of the present communication is to report on the association of sodium with another cellular component, the nucleus.

Nuclei prepared from calf thymus cells in 0·25 M sucrose–0·003 M calcium chloride solution[6,7], nuclei from calf thymus, liver and kidney isolated in non-aqueous media[8] and whole tissues similarly treated were kindly provided by Drs. V. G. Allfrey, A. E. Mirsky and S. Osawa, to whom we are greatly indebted. The sodium and potassium content of the isolated nuclei and whole tissues was determined by internal standard flame photometry after digestion with nitric acid.

Thymus nuclei prepared in non-aqueous media contained 238 μequiv. of sodium and 661 μequiv. potassium per gm. dry weight. Since the water content of the nuclei was 83 per cent, the sodium content was calculated to be 40 μequiv. and the potassium content 112 μequiv. per gm. wet weight. The sodium and potassium content of fresh thymus tissue averaged 38 ± 1·5 (s.e.) and 116 ± 1·5 (s.e.) μequiv. per gm. wet weight, respectively, and corresponded closely to the values of 43 μequiv. of sodium and 107 μequiv. of potassium per gm. wet weight found for the whole ground tissue after treatment with non-aqueous media. The similarity of the sodium and potassium concentration in the fresh and treated tissue suggests that there was no significant loss of sodium and potassium from the tissues into the media (petroleum ether, cyclohexane and carbon tetrachloride) during the non-aqueous procedures used for the nuclei.

The electrolyte content of nuclei isolated in sucrose solution was considerably lower, averaging 15 ± 0·8 (s.e.) μequiv. of sodium and 42 ± 2·1 (s.e.) μequiv. of potassium per gm. wet weight (42 experiments). In Table 1 these data are expressed as μequiv. per gm. dry weight. The sodium content of the nuclei isolated in sucrose averaged 87 ± 4·8 μequiv. per gm. dry weight with a range of 40–157 μequiv. per gm. dry weight; the potassium content averaged 244 ± 12·2 μequiv. per gm. dry weight with a range of 141–481 μequiv. per gm. dry weight. If the values obtained for nuclei isolated in non-aqueous media represent the natural cation content of the nucleus, an average of 62·5 per cent (range 17–73 per cent) of the original sodium and potassium is lost from the nucleus by the repeated washings with sucrose solution during the preparation.

The sodium and potassium content of liver and kidney nuclei isolated in non-aqueous media also proved to be approximately the same as that of the whole tissue (Table 1).

Table 1. The Sodium and Potassium Content of Tissues and Nuclei (μequiv. per gm. dry weight)

	Na	K	Na/K
Isolated in non-aqueous media :			
Calf thymus : Whole tissue	257	627	0·41
Nucleus	245	639	0·38
	230	683	0·34
Calf liver : Whole tissue	144	274	0·53
Nucleus	171	322	0·53
Calf kidney : Whole tissue	433	372	1·16
Nucleus	431	410	1·05
Isolated in sucrose solution :			
Calf thymus : Nucleus	86	245	0·35

It is unlikely that sodium and potassium are uniformly distributed throughout the nucleus and cytoplasm. Whole tissue includes a considerable amount of extracellular fluid containing 140 μequiv. of sodium per ml. and only 5 μequiv. of potassium per ml. If the extracellular fluid of the thymus amounts to 10 per cent by weight, there will be 32 μequiv. of sodium and 119 μequiv. of potassium per gm. of cellular mass. Since the cell nuclei occupy 61 per cent of the thymus tissue[8], the content of sodium in the cytoplasm is estimated as 15 μequiv. per gm. and the content of potassium in the cytoplasm as 139 μequiv. per gm. Thus sodium appears to be present in significantly greater amounts in the nucleus than in the cytoplasm at large. The sodium content of the nuclei may have been over-estimated owing to inclusion of connective tissue fragments in the sedimented nuclear fraction. Studies of sodium binding by constituents of this fraction are in progress.

That the sodium and potassium content of the nucleus, or at least of its surrounding cytoplasm, has general functional significance is attested by the finding of Allfrey, Mirsky and Osawa that protein synthesis in isolated nuclei is strikingly dependent upon the presence of sodium ions in the suspending medium[9].

Shinji Itoh*
Irving L. Schwartz

Rockefeller Institute for Medical Research,
New York.
May 25.

* Fellow of the Dazian and Helen Hay Whitney Foundations.

1 Stanbury, S. W., and Mudge, G. H., *Proc. Soc. Exp. Biol. Med.*, **82**, 675 (1953).
2 Mudge, G. H., "Electrolytes in Biological Systems", 112 (Amer. Physiol. Soc., Washington, D.C., 1955).
3 Spector, W. G., *Proc. Roy. Soc.*, B., **141**, 268 (1953).
4 Macfarlane, M. G., and Spencer, A. G., *Biochem. J.*, **54**, 569 (1953).
5 Bartley, W., and Davies, R. E., *Biochem. J.*, **57**, 37 (1954).
6 Stern, H., and Mirsky, A. E., *J. Gen. Physiol.*, **37**, 177 (1953).
7 Allfrey, V. G., *Proc. U.S. Nat. Acad. Sci.*, **40**, 881 (1954).
8 Allfrey, V. G., Stern, H., Mirsky, A. E., and Seatren, H., *J. Gen. Physiol.*, **35**, 529 (1952).
9 Allfrey, V. G., Mirsky, A. E., and Osawa, S., *Nature*, **176**, 1042 (1955).

Ultrastructural Organization of Bone

The high mechanical stability of bone tissue is obtained through the combination of the inorganic component hydroxyapatite with the inelastic fibrous protein collagen. Besides serving as supporting tissue, bone also plays an important part in the ionic homeostasis of the organism. A study of the ultrastructural organization of bone should contribute to a better understanding at the molecular level of these characteristic properties of the osseous tissues.

Through combined electron microscope and X-ray diffraction studies at high and low angles[1-4], the following general picture of the submicroscopic organization of bone can be derived. The fibrous matrix of bone, that is, collagen, appears capable of orienting the hydroxyapatite crystallites in a highly ordered way. Thus the rod- or needle-shaped apatite particles are attached precisely along the collagen periodicity. The X-ray evidence[2,3] derived from the profiles of the wide-angle lines and the low-angle particle scatter, and indicating the presence of rod-shaped apatite particles 40–75 A. wide and about 200 A. long, conforms with the results of electron microscope in-