tween the V_0 and the proton should be the same as between the neutron and the proton, at least as far as both potentials are due to the interaction with π mesons. Under this assumption, the scattering cross-section for the collision of V_0 particles with protons or neutrons is very nearly equal to the proton-neutron scattering cross-section at the same energy. If it should be possible to measure the former cross-sections it would give a clue concerning the validity of the analogy between heavy particle conservation and charge conservation laws.

* The contents of this note have been presented November 10, 1951, to the New Jersey Science Teachers Association.

[1] It is difficult to trace the first statement of this principle. It is clearly contained in the writer's article in *Proc. Am. Philos. Soc.*, **93**, 521 (1949), but may have been recognized about that time also by others, cf. T. Okayama, *Phys. Rev.*, **75**, 308 (1949). C. N. Yang informs me that the purpose of introducing an imaginary character to the reflection properties of certain fermions in the paper of J. Tiomno and C. N. Yang (*Phys. Rev.*, **79**, 495 (1950)) was to explain this principle. Cf. also L. I. Schiff, *Phys. Rev.*, **85**, 374 (1952) and, in particular, P. Jordan, *Z. f. Naturf.*, **7a**, 78 (1952).

[2] Pais, A., "On the *V*-Particle." To appear shortly. Also literature quoted there.

[3] Cf., e.g., the discussion in the article quoted in reference 2.

[4] Cf., e.g., E. P. Wigner and E. Feenberg, *Reports on Progress in Physics*, Vol. VIII, 1942, p. 274, or W. Heitler, *Proc. Roy. Irish Acad.*, **51A**, 33 (1946). Recently K. A. Brueckner gave a very interesting illustration of the principle given in the last reference (*Bull. Am. Phys. Soc.*, **27**, 1, paper Y9 (1952)).

[5] Schwinger, J., *Phys. Rev.*, **78**, 135 (1950) demonstrated possible causes which give apparent, but only apparent, deviations from this equality.

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*ON THE SODIUM AND POTASSIUM BALANCE OF ISOLATED FROG MUSCLES**

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Isolated frog sartorii, so treated as to become enriched in intra-fibral sodium and depleted of potassium have been shown to be able to extrude excess sodium against both chemical and electrical gradients.[1, 2] From various considerations it has been assumed that the potassium uptake under these conditions is due to a passive movement of the ions to reach an electrochemical equilibrium with the extra-fibral potassium. Appropriate calculations make it probable that this is the correct interpretation but it would be desirable to have more direct evidence that potassium uptake is indeed directly dependent upon the outward extrusion of sodium.

The high sodium condition is reached by soaking isolated muscles in K-free salt solution. During this extraction period there is a slow net out-

ward leakage of potassium and an equal net gain of sodium, the sum of the two ions remaining approximately constant for a given muscle. If the potassium depletion is accomplished by substitution of the intra-fibral

TABLE 1

TREATMENT	TIME, MIN.	—Na CONCENTRATION— MUSCLE	FIBERS	Δ FIBERS	—K CONCENTRATION— MUSCLE	FIBERS	Δ FIBERS	FINAL RELATIVE WEIGHT
A								
Extracted:	0	9	8		33	44		
0.11 *M* choline	15	38	11	+3	40	49	+5	98
0.01 *M* Na	0	7	5		30	40		
Recovery:	30	34	5	0	33	40	0	99
0.12 *M* Na	0	8	7		35	47		
0.01 *M* K	90	40	13	+6	41	51	+4	98
	0	7	5		51	68		
	210	36	8	+3	58	73	+5	97
B								
Extracted:	0	63	44		42	56		
0.12 *M* Na	30	15	16	−28	58	74	+18	91
Recovery:	0	62	43		40	53		
0.11 choline	90	8	7	−36	60	76	+23	95
0.01 Na								
0.01 K								
C								
Extracted:	0	32	21		34	45		
0.055 choline	30	24	11	−10	50	62	+17	93
0.065 Na	0	31	20		46	61		
Recovery:	90	23	9	−11	57	72	+11	94
0.055 choline								
0.065 Na								
0.01 K								

Sodium and potassium concentrations in isolated frog sartorii. All muscles were soaked in extraction media as indicated for about 24 hrs. at ca. 5°C. One member of each pair was then analyzed at zero time (start of recovery period), the other member placed for the time indicated in the recovery solution at room temperature (ca. 22°C.). All solutions, extraction and recovery, were buffered at pH 7.2 ± 0.1 with 0.005 *M* sodium phosphate buffer. Muscle concentrations expressed as mmols./kg. final wet weight. For calculating intra-fibral concentrations an extrafibral space of 25% was assumed. Final relative wet weights given as per cent of wet weight at the start of the recovery period (zero time). Each figure represents an average of three separate analyses. "Δ fibers" column gives change in fiber concentration as the difference between the control muscle at zero time and the experimental muscle after recovery. All figures have been rounded out to the nearest millimol.

potassium by an indifferent cation, not actively extruded as is sodium, then the subsequent uptake of potassium during a recovery period in potassium-containing solutions should not take place if the uptake is dependent upon active outward extrusion of, e.g., sodium. On the other

hand, if potassium uptake depends upon any inward secretion of the element, the recovery should take place since there is evidence that chloride ion could enter to maintain electroneutrality.

Experiments are reported here in which muscles have been depleted of both sodium and potassium by soaking in solutions in which potassium is lacking and in which sodium has been substituted in varying degrees by choline. Choline, as choline chloride (General Biochemicals) was used as an indifferent cation since its physical properties are widely different from those of sodium, and hence would not be apt to participate in sodium extrusion mechanisms, and since it has been used widely as an indifferent ion in physiological studies on nerve.[3]

Experimental procedures were as previously reported.[2] Variations in details are noted in table 1 which gives the results pertinent to the present discussion.

Muscles extracted in the cold in K-free, low-Na choline chloride solutions have about the same low potassium concentrations that they would have had had they been extracted in K-free NaCl solutions (compare table 1, A and B). They also show the very low intra-fibral sodium content expected on the assumption that the loss of potassium is compensated for by a gain of choline. No marked changes in weight were observed, hence it can be assumed that the total fiber cation concentration did not change. Such muscles, depleted of both sodium and potassium, show no significant recoveries of potassium when immersed at room temperature in K-containing NaCl recovery solutions. Of perhaps equal importance is the finding that during the recovery treatment the sodium content of the muscles does not rise above a level that can be accounted for by an increase in extrafibral sodium, thus indicating that the fiber surfaces are intact. These findings are completely consistent with the point of view that potassium uptake of K-depleted fibers is dependent upon the active extrusion of another cation, normally sodium.

High concentrations of choline might have toxic effects in general on muscle even though such toxicity is not reflected in the ability of the fibers to maintain a low sodium concentration against a strong chemical gradient (cf. table 1, A). As further tests for possible deleterious effects of the choline, two other series of experiments were carried out. In the one series muscles were extracted in the cold in the usual K-free NaCl extraction medium and then allowed to recover in K-containing choline solutions (table 1, B). In the other series, extractions in the cold were in K-free isotonic mixtures of NaCl and choline chloride with recoveries at room temperature in the same choline NaCl mixtures with added potassium (table 1, C). Muscles depleted of potassium and enriched in sodium to the normal degree are able to regain potassium at a normal rate when intra-fibral sodium is lost (presumably extruded) to the high choline

medium. There are no signs of a toxic effect of choline on the recovery process. Likewise, when extractions and recoveries are carried out in choline-NaCl mixtures, the sodium changes and the coincident potassium shifts (table 1, C) are intermediate between the extremes noted in the other sections of the table.

Unless there are completely hidden special effects of choline, the results demonstrate a dependence of potassium uptake on sodium extrusion. As a tentative generalization, indicated but not proved, it may be suggested that potassium accumulation in cells is a passive process in response to the active extrusion of some other cation, probably sodium in most animal cells.

It is assumed, throughout this discussion, that the muscle fibers during extraction and recovery maintain a reasonably high membrane potential. This assumption is supported by the results reported by Tobias[4] on membrane potentials of K-depleted fibers.

The data of table 1 also give information about maximum rates at which sodium extrusion can be accomplished. A previous estimate of the *minimum* rate gave a figure of about 3 mmol./kg./hr.[2]. From table 1, B it would seem that a half-time for the process of sodium extrusion is about 30 minutes from which it can be calculated that ca. 40 mmols./kg./hr. can be actively extruded under the conditions of these experiments. Other data to be reported elsewhere show that this is indeed a correct figure for the extrusion of sodium into the more normal NaCl-KCl mixtures. This rate of extrusion is very similar to the total *exchange* rate for Na^{24} reported by Levi and Ussing.[5] It can be concluded therefore that it is not necessary to postulate an exchange system other than that represented by the transport system.

During the recovery of muscles from the high sodium condition a considerable amount of energy must be expended. Using the calculations of Levi and Ussing,[5] energy expenditure would amount to around half of the normal resting metabolism. This does not indicate, however, that normal muscle, intact in the body must expend energy at such a high rate to maintain the normal sodium-potassium balance. As reported previously,[1] the time course of potassium loss (and presumably sodium gain) during extraction is very slow. Hence the rate limiting step for normal energy expenditure to maintain ion balance might well be the slow inward ionic leakage.

It would seem that the sodium extrusion system is able to provide a high safety factor perfectly adequate to compensate for the intermittent increase in ionic leakages accompanying activity.

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

[2] Steinbach, H. B., *Am. J. Physiol.*, **167**, 284 (1951).
[3] Lorente de No, R., *J. Cell. & Comp. Physiol.*, **33**, suppl. (1949).
[4] Tobias, J. M., *Ibid.*, **36**, 1 (1950).
[5] Levi, H., and Ussing, H. H., *Acta Physiol. Scand.*, **16**, 232 (1948).

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*TRANSPLANTATION OF LIVING NUCLEI FROM BLASTULA CELLS INTO ENUCLEATED FROGS' EGGS**

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Introduction.—The role of the nucleus in embryonic differentiation has been the subject of investigations dating back to the beginnings of experimental embryology. At first it was supposed by Roux, Weismann and others that differentiation is the result of qualitative nuclear divisions, different blastomeres thereby receiving the different kinds of nuclei which determine their subsequent differentiation. Later on this theory was disproved by numerous experiments showing that, during early cleavage at least, the distribution of the nuclei can be changed at will without altering the pattern of development. The cleavage nuclei have, therefore, been regarded as identical, and differentiation has been ascribed primarily to the well-known localizations in the egg cytoplasm.

This evidence, it should be emphasized, relates only to the early phases of development. During this time it is definitely true that the nuclei in the various blastomeres are equivalent. However, whether they remain equivalent or become differentiated as the various parts of the embryo differentiate has never been tested. The possibility that nuclei might differentiate in response to regional differences in the cytoplasm, and that such nuclear changes might have reciprocal effects on the cytoplasm during cell differentiation, was suggested by Morgan.[1] More recently Schultz[2-4] has discussed the problem more fully, indicating the known cytogenetical mechanisms that could account for nuclear differentiation, and Weisz[5] has reviewed it in relation to ciliate morphogenesis.

Obviously this problem can be solved only by the development of a method for testing directly whether nuclei of differentiating embryonic cells are or are not themselves differentiated. This sort of test could be obtained, as suggested to us several years ago by Schultz, if it were possible to transplant nuclei. Ideally, this type of experiment should be carried out by transplanting the nucleus from an irreversibly differentiated cell into an enucleated egg. The egg cytoplasm when normally nucleated is.