Sodium Transport and Phosphoproteins

In previous work[1] we have shown that liver slices suspended in a medium free of sodium ions show a depressed turn-over of phosphoprotein phosphorus. This effect is reversed by addition of sodium. In these and the experiments shown here, the phosphoproteins have been identified by isolation of radioactive phosphorylserine from acid hydrolysates of the crude proteins. We have found that the phosphoproteins sensitive to Na^+ are non-mitochondrial[1], the mitochondrial phosphoproteins appearing to have different properties[2].

We now find that the effect of Na^+ on phosphoprotein turn-over is strongly influenced by the concentration of externally added Ca^{2+} ions. In Table 1, we show that Ca^{2+} ions tend to inhibit phosphoprotein turn-over as well as that of adenosine triphosphate. Na^+ reverses this effect, but it is clear that as the Ca^{2+} level is reduced the inhibition of uptake of phosphorus-32 falls away, while the system still requires Na^+ for full activity.

Table 1. Effect of Na^+ and Li^+ Ions at Two Ca^{2+} Concentrations

Ca^{2+} level mM	2·8			0·93		
Addition	Choline	Na^+	Li^+	Choline	Na^+	Li^+
ATP, sp.a	97,000	147,000	150,000	155,000	228,000	172,000
Phosphoprotein P, sp.a.	11,700	18,000	12,000	18,400	39,000	17,200

Slices of about 200 mgm. wet weight were suspended in Ringer solution containing choline-chloride instead of sodium chloride. *p*H 7·2 was maintained with sodium bicarbonate, gas phase was 95 per cent oxygen – 5 per cent carbon dioxide. Temperature, 38°. After 10 min. incubation in the medium, which contained phosphorus-32 of specific activity 3×10^8 c.p.m./μmole, Na^+ choline or Li^+ was added to a final concentration of 0·039 M. 15 min. later, trichloroacetic acid (final concentration, 5 per cent) was run into the flasks and the contents homogenized rapidly and chilled.

The effect of Li^+ ions is also shown in Table 1. At high levels of Ca^{2+}, Li^+ causes a marked stimulation of ATP, but has no such effect on phosphoproteins. This experiment is further evidence that the action of Na^+ on the latter compounds is specific.

Cosmos and Harris[3] have shown that calcium ions are gained by frog skeletal muscle on reduction of the external Na^+, and expelled on further addition of Na^+; and have postulated that Na^+ and Ca^{2+} ions compete for a common site (see also Niedergerke and Harris[4]).

The present results strongly support the idea that phosphoproteins are involved in Na^+ transport, and are in keeping with previous suggestions to that effect[5–7].

The evidence for this can be summarized as follows:

(1) Systems deficient in Na^+ show reduced turn-over of phosphoprotein phosphorus;

(2) The addition of Na^+, but not of Li^+, K^+ or of choline reverses this effect;

(3) The level of Ca^{2+} greatly influences the rate of turn-over of phosphoprotein phosphorus and supports the idea of a competition between Na^+ and Ca^{2+} for a common site.

Table 2. Effect of Strophanthin on Phosphoprotein Turn-over

Addition	3×10^{-4} M strophanthin	Specific activity, c.p.m./μmole phosphorus of Phosphoprotein as phosphoserine-P	Increase (per cent)	ATP	Increase (per cent)
Choline	–	479	—	306×10^3	—
Na^+	–	700	46	483×10^3	58
Na^+	+	520	9	460×10^3	51

The conditions were as in Table 1 with 2·8 mM Ca^{2+}. The phosphoproteins were subjected to acid hydrolysis and chromatography to give the activity of phosphorylserine derived from them. Carrier phosphorylserine, 10 μmoles, was added before acid hydrolysis.

(4) Li^+ ions can be shown to stimulate adenosine triphosphate turn-over while having no effect on phosphoprotein turn-over.

(5) Drugs such as strophanthin which inhibit Na^+ transport prevent the effect of Na^+ on phosphoprotein turn-over while having no action on adenosine triphosphate (Table 2).

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Gamma-Aminobutyric Acid and Other Blocking Substances extracted from Crab Muscle

As part of an attempt to identify the transmitter agent released by inhibitory nerves in crustacean muscle we have extracted and purified heat-stable 'inhibitory' substances of a crab muscle and its associated nerve supply. The fibres of the extensor muscle (opener) of the dactyl of the walking leg of *Cancer borealis* are densely innervated by two inhibitory nerve fibres, and by extracting the whole muscle, although adding non-neural elements, we were assured of obtaining the materials stored within the nerve endings. The extracts were assayed on the extensor muscle of the dactyl in the crayfish, *Orconectes virilis*. Only those extracts which imitated the effect produced by neural inhibitory activity (reduction of the excitatory junctional potentials without shifting the resting potential by more than 5 mV.) were considered 'inhibitory' and were further purified.

Fifty-five extensor muscles were dissected free from hypodermis and from the larger bundles of sensory nerves. The muscles were frozen on dry ice, ground to a powder and lyophilized. The resulting powder (1·4 gm.) was extracted three times with 30 ml. of boiling distilled water. The pooled extracts were concentrated by freeze-drying and dialysed against three changes of 100 ml. of distilled water at 4° C. for 32 hr. The physiological activity was confined to the dialysate, which was taken to dryness. 215 mgm. (a half) of this material was dissolved in 0·5 M acetic acid (*p*H 2·5) and fractionated on an Elphor continuous electrophoresis apparatus at 4° C. The extract was applied to the paper at the rate of 1 mgm. of starting material per hour, and the voltage was adjusted so that potassium appeared at one border of the paper and inorganic phosphate near the other. All the fractions were lyophilized, and a portion of each was then dissolved in van Harreveld's solution for assay.

Each active fraction was further purified by ascending paper chromatography as follows. A large amount of the fraction was applied in a long line, near the bottom of a sheet of washed Whatman No. 1 filter paper. After developing with a suitable solvent the paper was dried for a day. Narrow vertical strips were stained with ninhydrin or other colour reagents, and, using these strips as guides, the entire paper was