protein synthesis, and actinomycin, an inhibitor of RNA synthesis (*9*), blocked the insulin-induced increase in hepatic pyruvate kinase activity, it appears that the insulin-induced rise in this enzyme activity is due to synthesis of new enzyme which is dependent on new RNA production.

As a consequence of the foregoing action of insulin on hepatic pyruvate kinase, one would expect a decrease of this enzyme in starvation (when insulin secretion is decreased or absent), and a return of enzyme activity to normal upon refeeding, especially with a high carbohydrate diet (which results in insulin release). Thus, the reported decrease of liver pyruvate kinase in starved rats and the return to normal upon refeeding high carbohydrate diet (*10*) are in accord with our experimental data and may be interpreted on the action of insulin as inducer of hepatic pyruvate kinase activity.

An economy of insulin action is apparent from our data and from other developments in the elucidation of insulin function. Insulin exerts its action in facilitating the entrance of glucose from the blood stream into the peripheral cells, by a process not involving enzyme synthesis (*11*). In contrast, other functions of insulin involve an action on biosynthetic response of functionally related enzymes. Insulin acts as a suppressor (*1*) of the four key gluconeogenic enzymes, glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphoenolpyruvate carboxykinase (*12*) and pyruvate carboxylase (*13*). In contrast, insulin acts as an inducer to glycogen synthetase (*14*). Furthermore, it induces glucokinase (*4–6*) and pyruvate kinase, two of the enzymes studied out of the three strategic glycolytic enzymes. Insulin also induces the key enzymes in fatty acid synthesis (*11*). Thus, insulin acts as a hormone in coordinating metabolic reactions, functioning in lowering the blood sugar, and increasing metabolic utilization and storage. These considerations further emphasize the economy of hormone action in that insulin attacks receptor sites at the source of enzyme production and thus turns on or off the biosynthetic action of whole genetic units governing the production of key rate-limiting enzymes.

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Active Uptake of Sodium by Softshell Turtles (Trionyx spinifer)

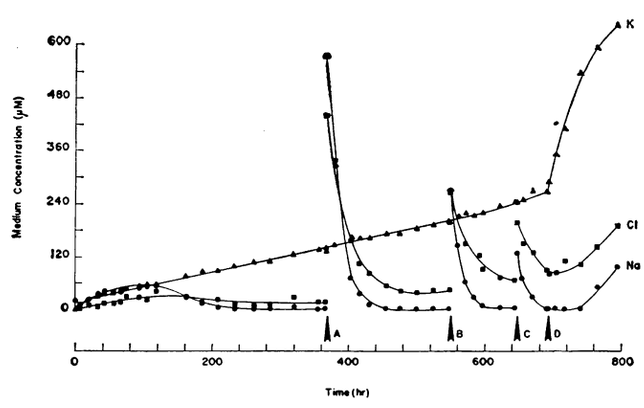
Abstract. *Aquatic softshell turtles* (Trionyx spinifer spinifer) *show a net active uptake of sodium from solutions of this cation as dilute as 5 micromoles per liter. This probably occurs in the pharynx, a site of aquatic respiration. Inhibition of active sodium transport by low temperatures causes an extreme lowering of the sodium concentration in the plasma. Other fresh-water turtles may utilize this mechanism of ionic regulation. The ultrastructure of the pharyngeal villi is similar to that of frog skin and toad bladder.*

Aquatic softshell turtles have the ability to procure oxygen from the water, primarily through the pharynx and cartilaginous plastron (*1*). Water is brought into contact with highly vascularized pharyngeal villi by rhythmic movements of the hyoid apparatus. Although fresh-water reptiles are generally considered to be effectively impermeable to water and salts, the contact of water with respiratory surfaces may create special problems of electrolyte balance. In an initial effort to determine the extent of these problems, sodium balance in the softshell turtle (*Trionyx spinifer spinifer*) was analyzed.

If softshell turtles are to maintain sodium balance, losses of sodium into the environment will have to be made up in the food or by active uptake. To test for active uptake of sodium animals were placed in 2 liters of distilled water in covered aquariums. The concentration of sodium in the medium was measured by flame photometry in order to determine the net sodium influx or efflux. An initial net loss of sodium into the medium was followed by a net uptake until an equilibrium between net sodium influx and efflux occurred (Fig. 1). Initial net sodium loss per gram of body weight for eight turtles was inversely related to body weight. This might be expected since the efflux relative to weight from a large turtle would be less than from a small turtle. Sodium equilibrium was achieved in two turtles in water containing a 5 μM concentration of sodium. The results for one turtle are shown in Fig. 1. The external concentration was increased by adding NaCl at points *A*, *B*, and *C*. After each addition the turtle quickly reduced the concentration to the previous equilibrium value. Net chloride flux was less than net sodium flux (Fig. 1). The failure of chloride to follow sodium particle for particle indicated that other anions, possibly bicarbonate, were participating in the maintenance of the electrical equilibrium. Net potassium efflux was roughly linear for 692 hours, and was not affected by sodium and chloride fluxes.

When ouabain (Sigma) (10 μmole/lit.) was added to the medium (at *D* in Fig. 1), no change occurred in net sodium and chloride flux for 48 hours. For the next 52 hours, until death, net sodium and chloride efflux increased at about the same rate. Net potassium efflux was greatly increased immediately after the addition of ouabain (Fig. 1). A lower concentration (1 μmole/lit.) of ouabain did not significantly affect net sodium, chloride, or potassium fluxes of another turtle. The effect of the 10 μM concentration of ouabain on net sodium flux suggests either that sodium uptake involves active transport, or that renal sodium reabsorption is inhibited.

Net rates of sodium influx were calculated from the rates of sodium uptake following the addition of NaCl



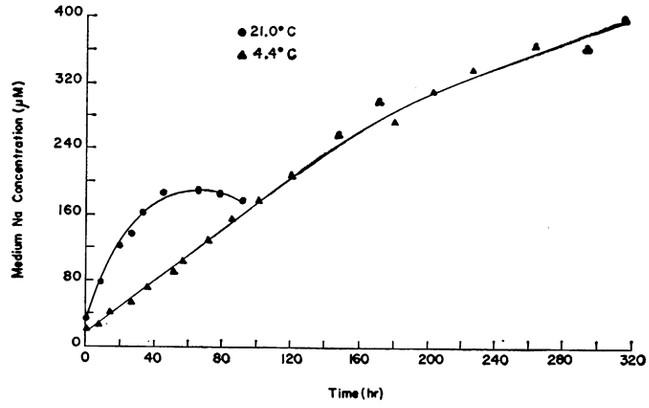


Fig. 1 (left). Net sodium (circles), chloride (squares), and pot assium (triangles) fluxes at 21.0°C in 2 liters of water (initially distilled) for a softshell turtle (*T. s. spinifer*) weighing 240 g. At *A*, *B*, and *C*, NaCl was added to the medium. At *D*, ouabain (10 μmole/lit.) was added to the medium. Fig. 2 (right). The effect of temperature on the net sodium flux of a softshell turtle (*T. s. spinifer*) weighing 430 g in 2 liters of water (initially distilled).

to the medium at points *A*, *B*, and *C* in Fig. 1. These rates varied directly with the initial concentration to which the medium was raised, indicating that there was no saturation of the transport mechanism in this individual at these low concentrations of environmental sodium.

Figure 2 shows the net changes in external sodium concentration for one turtle placed in distilled water at 4.4° and then at 21.0°C. At the higher temperature, sodium influx became greater than efflux at about 60 hours as indicated by the appearance of a maximum in the curve. At 4.4°C sodium efflux exceeded influx, and net efflux remained constant for 150 hours. The gradual change in slope beyond 150 hours at 4.4°C may be due to a decrease in sodium efflux through a change in renal or general permeability or to an increase in sodium influx. In any case, the lower temperature inhibited net sodium influx since no maximum was observed at 4.4°C. Little is known about the specific nature of temperature effects on ion balance in fresh-water lower vertebrates (*2*).

Since softshell turtles hibernate, they might incur sizeable losses of sodium during periods of very low temperatures because active sodium transport is suppressed by cold. To test for this seasonal effect, individuals were kept in simulated hibernation in tanks of running tap water at 7.4° to 15.0°C from September through December, 1964. Hibernating turtles had much lower concentrations of sodium in the plasma than did active animals (Table 1). Sodium concentrations of turtles during the summer, and of turtles kept warm in the laboratory during winter, were similar. Hibernating animals were able to increase the sodium concentration almost to the summer level when placed in tap water at 21.0°C. In turtles which appeared moribund in cold tap water, the sodium concentrations were about one third of those in active animals (Table 1). This extreme lowering of the sodium concentration apparently results from the inhibition of uptake of sodium from the environment and from the renal filtrate by low temperatures. Tolerance of low concentrations of electrolytes in the plasma may be an

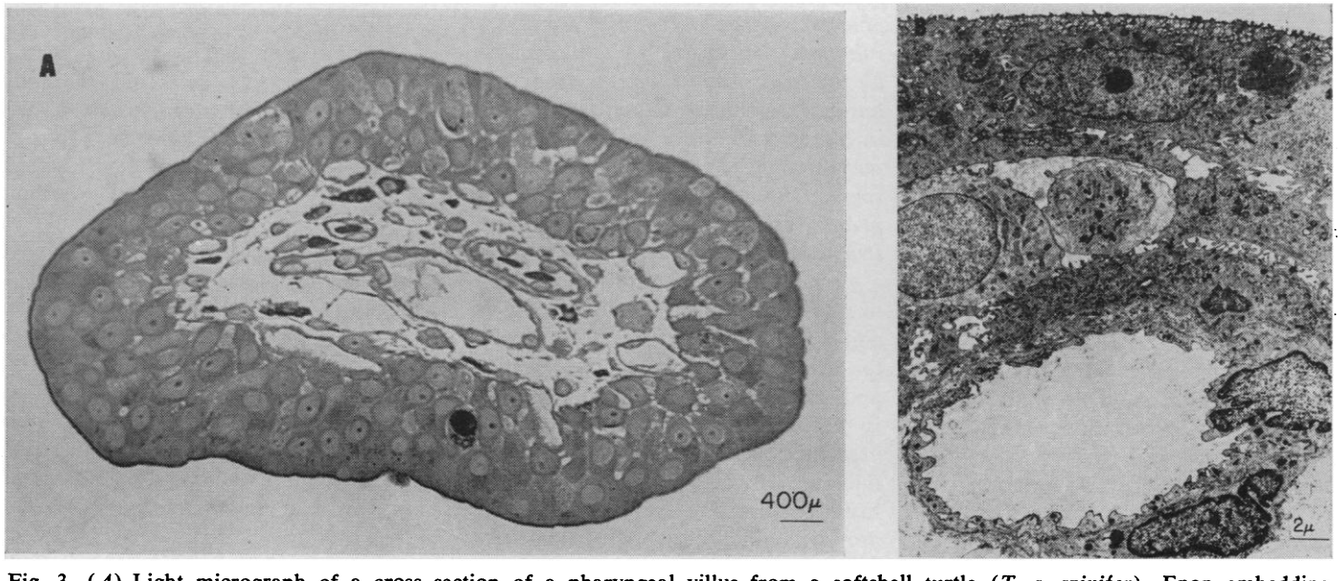


Fig. 3. (*A*) Light micrograph of a cross section of a pharyngeal villus from a softshell turtle (*T. s. spinifer*). Epon embedding medium was removed (*11*) and the section was stained with basic fuchsin (*12*). (*B*) Electron micrograph of the mucosa of a pharyngeal villus with an adjacent capillary from a softshell turtle (*T. s. spinifer*). Stained with uranyl acetate.

important feature of adaptation of these turtles to a fresh-water existence during cold winters. Softshell turtles remained vigorous after the sodium concentration of the plasma decreased by as much as 52 percent.

Our results indicate that softshell turtles have a mechanism by which they can achieve a net uptake of sodium from water containing as little as 5 μmoles of sodium per liter. In the natural habitat of these turtles the sodium concentration of the water varies from 300 to 500 μmoles per liter. Thus the animals are able to offset their sodium losses by taking up sodium from the water, except at low temperatures.

The site of active uptake of sodium in softshell turtles must be a permeable surface in extensive contact with the environmental fluid. In fresh-water frogs and fish, the respiratory surfaces used in water are also the sites of ion uptake. Such surfaces in softshell turtles include the pharynx, plastron, and possibly the cloaca. In *T. s. asper* the pharynx has the dominant role in aquatic respiration (*1*).

To investigate the transport properties of isolated pharynx, plastron, and cloaca we used an Ussing cell (*3*). With reptilian Ringer's solution (*4*) on both sides, each of these tissues exhibited a transmembrane potential in which the outside (mucosa) was negative with respect to the inside (serosa). During the summer, only the pharynx exhibited a sizeable transmembrane current that was apparently associated with cation influx or anion efflux. Electrical responses of the isolated pharynx in relation to the concentration of sodium outside and potassium inside appear qualitatively similar to those obtained with isolated frog skin (*5*). Quantitative comparison of sodium transport in the turtle pharynx with that in the frog skin was not attempted because of difficulties in accurately measuring the surface area of the villus-covered pharynx. The results in vitro, however, show that the pharynx could be carrying on a net uptake of sodium from the environment. Since the cloaca, unlike the pharynx, is not exposed to extensive currents of water, and since it may also participate in the absorption of ions from the gut contents, it probably is not the site of sodium uptake directly from the environment.

The active uptake system for sodium evident in softshell turtles may be a feature in ionic regulation of fresh-water turtles in general. Ion loss across respiratory surfaces may be less important in some forms since the extent of aquatic respiration in fresh-water turtles appears to be variable (*1, 6*). In several fresh-water genera lacking pharyngeal villi we have found potentials and currents in the pharyngeal membranes. In one starved (and presumably electrolyte-depleted) snapping turtle (*Chelydra*), we measured a potential of 70 mv and a current of 57.5 μa/cm^2 across the pharynx at 21.0°C. Similar measurements on the terrestrial turtles *Gopherus polyphemus* and *Terrapene carolina* demonstrated the presence of a pharyngeal potential and current, but the external sodium concentration at which the pharyngeal potential and current reached zero (200 mmole/lit.), was 25 to 500 times higher in these terrestrial turtles than in the fresh-water species. The outside sodium concentration at which the potential and current are zero is important because it marks the boundary below which net cation influx or anion efflux can not proceed. Thus the function of ion transport in the pharynx of terrestrial turtles must be different from that postulated for the pharynx of the softshell turtle.

Table 1. Concentrations of sodium in the plasma of active and hibernating *T. s. spinifer*. Results are expressed as the range; the mean is shown in parentheses.

Condition	No. of turtles	Sodium concentration (mmole/lit.)
Active, summer or winter	6	138.5 to 150.0 (144.1)
Hibernating, healthy	7	69.0 to 98.0 (80.9)
Hibernating, moribund	4	48.6 to 56.6 (52.1)

Anatomical studies of membranes which actively transport sodium have revealed a remarkable diversity of structure (*7*). Light and electron microscopy were employed in preliminary studies of individual pharyngeal villi cut from the dorsal surface of the membrane. The tissue was fixed in Palade's fixative (*8*) and embedded in Epon 812 (*9*). The villi are pleomorphic and consist of a mucosa and a corium containing capillaries of various dimensions (Fig. 3*A*). The mucosa is composed of one to four layers of mixed columnar and cuboidal epithelial cells separated from the corium by a basement membrane. Connective tissue fibrils support the capillaries.

Intercellular spaces are prevalent in sections of villi viewed with the electron microscope (Fig. 3*B*). Numerous microvilli project into these spaces and desmosomes are occasionally present at points of contact between two cells. Desmosomes are generally thought to aid in the support of the tissue rather than to act as continuous barriers to passage of intercellular fluids (*10*). At the peripheral cell surfaces the close association of plasma membranes blocks free passage of substances between the pharyngeal cavity and the intercellular spaces. Microvilli are present on the free surface of the epithelium, although they are smaller than the interstitial projections. The cortical region of the surface epithelium contains numerous vesicles. In the basal region of the epithelium, capillaries are frequently found in close association with the basement membrane. When this occurs, a layer of connective tissue fibrils is interposed between the basement membrane and the capillary endothelium. The villus is structurally similar to certain amphibian membranes in which active sodium transport is known to occur. We believe that the softshell turtle pharynx may prove to be a useful system for the correlation of anatomical and active transport phenomena.

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