Loss of Sodium from the Skin of the Dehydrated Toad, *Bufo marinus*

THE movement of sodium across the skin of frogs and toads has been studied extensively[1]. *Bufo marinus* placed in distilled water loses sodium from the skin at an increased rate when injected with large doses of 'Pitressin'[2]. Increase in concentration of the body fluids as in dehydration potentiates posterior pituitary hormone release in mammals, and the same appears to be the case in amphibians[3]. Therefore it was of interest to see what effect such endogenous release of posterior pituitary hormone had on loss of sodium from the skin.

The loss was measured in normally hydrated toads during a 1-hr. period on three successive days. At the beginning of each measurement they were catheterized, thoroughly washed in distilled water and then placed in 150 ml. of this water for 15 min., rinsed again and finally replaced in 150 ml. of fresh distilled water. At the end of 1 hr. a sample of the fluid bathing the toads was taken and the sodium concentration determined with an EEL-flame photometer. The total loss of sodium was then calculated from the known volume of fluid bathing the toad. Apart from the experimental periods, the toads were kept in tap water. Care in handling ensured that urine was not voided while the measurements were being made. In a second series of experiments the same toads were left without water for 48 hr. at 21° C. and 55 per cent relative humidity during which time they lost 30·0 per cent (S.E. ± 1·25) of their body-weight—a degree of dehydration from which they readily recovered if replaced in water. At the end of this period loss of sodium from the skin was measured as before.

Table 1. Loss of Sodium (mgm./hr.) from the Skin of *B. marinus* under Normal Conditions and during Hydration

Normal			Dehydrated
Day 1	Day 2	Day 3	
0·0516 ± 0·00336	0·0533 ± 0·00391	0·0582 ± 0·01232	0·3352 ± 0·06792

The results are the means ± S.E. in the same group of 15 toads (mean weight, 84·49 ± 2·800 gm.). Normal values all differed significantly from the dehydrated ($P < 0·001$).

The results are given in Table 1. It can be seen that the normal day-to-day rate of loss of sodium is remarkably constant, while in the previously dehydrated toads there is a marked increase in loss of sodium. The rate of loss is about four times as rapid as in *B. marinus* injected with large doses of 'Pitressin'[2]. The discrepancy may be due to a change in the concentration ratio across the skin and/or a change in the properties of the skin itself or to a greater effectiveness of endogenous hormone. In this latter respect it has shown that in *Bufo bufo* uptake of water is not as rapid after even supramaximal doses of posterior pituitary extracts as it is after the animals have been dehydrated[4].

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Bathyscaphoid Squid

Buoyancy equilibrium, or being of the same density as sea water, can confer a great economy of effort upon free-swimming animals and is approached or achieved in a variety of ways. While investigating buoyancy problems during a cruise of the Plymouth laboratory's research vessel *Sarsia* in the southern Bay of Biscay we found a particularly striking adaption in certain mid-water squid. Although some of the oceanic species resembled the coastal forms in being denser than sea water, the majority of the mid-water forms caught were in buoyancy equilibrium. Having neither cuttle-bone nor air sac they must have some other component less dense than sea-water (density = 1·026) to counterbalance the tendency of their proteins to sink.

These mid-water squid had enormous body cavities filled with fluid[1]. When the fluid, and it amounted to two-thirds of the squid s volume, was drained the squid sank; this showed that the buoyancy of the intact animals was given by the low densities of their coelomic fluids. From the volumes of coelomic fluid, the weights of the drained animals under sea water, and the density of that water, it was calculated that the fluid had a density about mid-way between that of distilled and sea water.

Samples of the coelomic fluid were collected from three species of mid-water squid (*Verrilliteuthis hyperborea* (Steenstrup), *Galiteuthis armata* Joubin, and *Helicocranchia pfefferi* Massy). These fluids, odourless but of bitter taste, were stored in a deep freeze and further studied on return to Plymouth.

The fluids from the three species were almost identical in composition and properties. Density-bottle measurements gave densities between 1·010 and 1·012, which accounted quantitatively for the buoyancy equilibria of the squid. Freezing point depressions were close to the value for sea water, showing that buoyancy was not achieved by simple exclusion of solutes. Of the common ions only ammonium can give a solution isotonic with sea water but of the density found. Tests indicated the presence of high concentrations of ammonium, and analyses by a method[2] insensitive to amines revealed about 480 mM ammonium. The sodium concentration was only about 80 mM, and chloride accounted for the necessary anion. (Sea water, which has chloride as its principal anion, has only a trace of ammonia but 490 mM sodium.)

The fluid's acidity, pH 5·2, was doubtless connected with the retention of ammonia in the coelom, for living tissues are permeable to molecular ammonia but relatively impermeable to the ammonium ion which predominates under acid conditions. The acidity accounted for the absence of ammoniacal smell during collection of the samples while the bitter taste of the fluid matched that of an ammonium chloride solution. We have prepared an artificial solution containing sodium and ammonium chlorides in the concentrations found in the coelomic fluid and shown that its density is close to that of the coelomic fluid.

These mid-water squid resemble the protozoan *Noctiluca*[3] in having a high concentration of ammonium ions which preserve tonicity and yet give buoyancy. In 'design' these squid are strikingly similar to the bathyscaphe, relatively recently devised by Prof. Piccard, in that their useful but dense parts are balanced by a large flotation chamber containing a fluid of lower specific gravity.