

part, a measure of the potential dependence of the activity of the electrogenic sodium pump (5, 20).

Finally, although some of our results showing the qualitatively similar action of light and pump activity are steady-state measurements, our evidence suggests that changes in pump activity are also necessary for the production of the graded component of the RP transient. There may, however, be components of the transient which do not involve the pump per se but are secondarily activated by the changes in pump activity induced by light.

In summary, we have presented evidence consistent with the hypothesis that *Limulus* ventral-eye photoreceptors have in their membranes an electrogenic sodium pump, that this pump has some of the characteristics of a high source impedance current generator, that this pump contributes directly to the steady-state membrane potential, and that changes in pump activity underlie the receptor potential evoked by light. Specifically, we suggest that light alters the electrogenicity of the pump, perhaps by affecting the affinities for ions of the pump's sodium or potassium sites (or both) which are thought to lie, respectively, at the inner and outer surfaces of the membrane (12-14). Should our hypothesis prove correct, then the question of how light evokes potential changes in photoreceptors may become the question of how the light-induced conformational changes in rhodopsin molecules, lying at sites in or on the photoreceptor membrane (21), can alter the activity of those presumably nearby membrane molecules which make up the sodium pump machinery.

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Short-Latency Antidiuresis

Following the Initiation of Food Ingestion

Abstract. *A factor associated with the ingestion of food is shown to produce a short-latency antidiuresis. Animals consuming large quantities of a highly palatable solution during a period of food deprivation exhibit an antidiuresis immediately following the initiation of eating. The rapidity of the response raises the possibility of a signaling factor separate from postingestional influences.*

In the present study we investigated the time course of antidiuresis initiated by the ingestion of food in awake unrestrained animals. It is well known that the postprandial phase of food ingestion results in an antidiuresis. A recent report by Stacy and Brook (1) describes this phenomenon in sheep. Earlier, Jacobs (2) reported a decrease in urine production during food ingestion in rats, but the latency of the antidiuresis was not studied.

Experimental subjects were food-deprived animals that overhydrated themselves by consuming large quantities of a highly palatable mixture of 0.125 g of saccharin (sodium-*o*-benzoic sulfimide) and 3.0 g of glucose (S + G solution dissolved in distilled water to a total volume of 100 ml). This technique avoids the complication of release of the antidiuretic hormone associated with the stress imposed by gastric loading. We had demonstrated in an earlier report (3) that when rats have food freely available they ingest the S + G solution during a 24-hour period in amounts equal to 60 percent of their body weight and that food deprivation increases the intake to approximately 90 percent of body weight.

A total of 29 male Holtzman albino rats (80 days old at the start of the experiment; average weight, 300 g) were used. They were housed in individual cages and maintained on 12-

hour cycles of light and dark in a room with a constant temperature of 22°C. The experiment was performed twice, the first time with seven experimental and five control animals and later with nine experimentals and eight controls. Since the results were indistinguishable, they have been combined. Initially, animals were provided with Purina Lab Chow pellets without restriction and the highly palatable solution of S + G. After 4 days of exposure to food and S + G solution the rats were deprived of food for 4 days. Daily measurements of fluid consumption were obtained. Spillage was collected in plastic cylinders mounted under the drinking tubes. Following the 4th day of total food deprivation the subjects were divided into two groups matched for consumption of the S + G solution during the preceding 4 days. The solution was removed and the 16 experimental rats were provided with Purina Lab Chow pellets.

Measurements were made of the volume and concentration of total solids in each urine sample obtained from both groups during the 3 hours following food presentation to the experimental animals. During this period no fluid was available to the animals. Each urine sample was collected in a syringe from a cardboard covered with Saran Wrap placed under the cages and the time of urination was recorded. A num-

ber of assistants kept the animals under constant observation to ensure that all samples were collected immediately after the animals voided. The urine concentration was measured with a TS Meter (4) refractometer to the nearest 0.1 percent of total solids, and volume was determined with a 2-ml syringe to the nearest 0.05 ml (5).

During the 4 days of food deprivation the mean daily consumption of fluid (S+G solution) for the experimental (food) and control (no food) animals was 245 ml. Thus before measurements of urine were started the animals had consumed very large volumes of fluid and were in a constant state of water diuresis which resulted in frequent voiding of a very dilute urine. Immediately after the experimental animals were given the food pellets both the frequency of urine production and the volume of urine decreased (see Fig. 1, top). Among the 16 experimental animals there were only three instances of urination during the first 20 minutes while there were 26 instances among the 13 control animals during this same period (Fig. 1, bottom). The differences in volume and in frequency of voiding persisted for about 130 minutes, and the total volume of urine excreted during the entire 180-minute observation period was significantly lower ($P < .01$, Wilcoxon test) for the fed group than it was for the controls (6). Differences in urine concentration were observed, beginning about 45 minutes after the food was presented and persisting throughout the remaining period of observation (Fig. 1, bottom).

Of primary interest is the short-latency antidiuresis observed in the experimental group. This effect, which appears at maximum strength almost immediately after the introduction of food, cannot be adequately accounted for either in terms of postingestional absorption of the food or on the basis of the dehydrating properties of food in the stomach. Rapidity of response rules out the relevance of postingestional absorption, and if the withdrawal of fluids into the stomach was the sole explanation, a gradual, rather than abrupt, onset of antidiuresis would have been expected. This latter argument would apply as well to the withdrawal of fluids in the mouth as a function of salivation. It would appear that the introduction of food into the mouth or stomach, or both, may provide sensory information that makes it possible for the organism

to prepare for the forthcoming absorption. This conclusion is consistent with the report by Nicolaidis (7), who demonstrated oral regulation of diuresis in rats, hydrated by gastric loading, following the bathing of their mouths with various substances.

In a separate experiment to evaluate

the influence of smell we tested seven animals that were treated in the same way as the present experimental animals except that the food was attached to the outside of the cage. No antidiuresis was observed in these animals. Because animals in the second experiment were very active in trying to

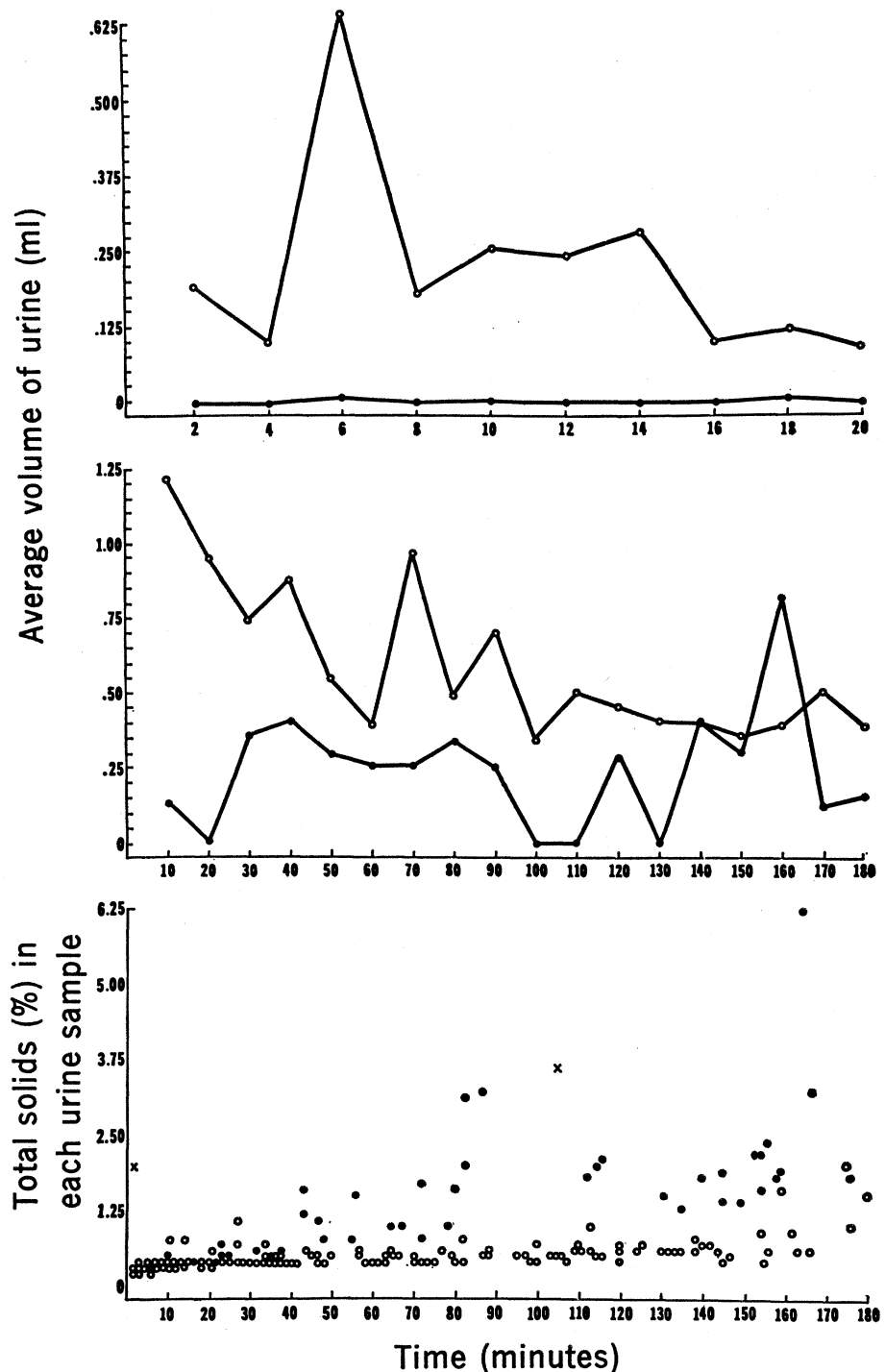


Fig. 1. Average urine volume of experimental (food) and control (no food) animals during the initial 20 minutes (top) after presentation of food to the experimental animals and during the entire 180-minute observation period (middle). The bottom graph depicts the percentage of total solids in each urine sample. The X on the bottom graph indicates the results from the one control animal that had consumed the least amount of the solution during the period of food deprivation. ●, Experimental animals ($N = 16$) that received food; and ○, control animals ($N = 13$) with no food.

reach the food, we have concluded that the short-latency antidiuresis observed in the present study is dependent upon actual ingestion of food, not smell, and does not result from any difference in activity between the groups.

The present study implicates an oropharyngeal- or gastric-signaling factor in the short-latency antidiuresis produced by food ingestion. At this time we are unable to determine whether the antidiuresis results from a direct neural mechanism or whether the antidiuretic hormone is involved. It is not possible to rule out the action of this hormone on the basis of the speed of the response, since Tata and Gauer (8) have demonstrated that the antidiuretic hormone is effective in physiological amounts within 1 to 2 minutes after intravenous injection.

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5. The sensitivity of our experimental technique was demonstrated in experiments in which identically hydrated animals were administered either the short- or long-acting forms of Pitressin. An antidiuretic effect characterized by a decrease in the volume of urine and an increase in its concentration and osmolality was observed in response to injections as small as 5 to 10 microunits per 100 g of body weight.
6. The potency of the antidiuretic effect of food can be seen under special circumstances in which it may be very maladaptive. If animals that have been consuming large quantities of the S + G solution during a prolonged period of food deprivation are given food while the solution is still available, a number of instances of convulsion from water toxicity are produced.
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ance; the gills, if present, were revascularized, and the transplants started to grow. In some transplants pulsations could be observed on the ventral side, indicating that heart tissue was present even though the cut had been made through the heart area. To what extent regeneration of heart tissue occurs after decapitation is not known.

We measured the growth of the transplanted heads on two animals and compared the data with the growth of the host heads and the growth of the heads of intact control larvae from the same batch as the donors (Table 1). The transplants grow relatively faster than their hosts, in some cases resulting in the two heads' approaching each other in size. The transplants apparently have their own growth rate, although they have a common vascular system and are not hormonally separated from their hosts. Moreover, in the first weeks the growth of the transplanted heads is not much slower than that of heads of intact control larvae.

One of the female hosts became sexually mature, mated in a normal manner, and produced normal offspring. Two animals about 21 cm long entered metamorphosis and died before completing it. Normally axolotls seldom metamorphose, but in our experiments this process may have been induced by abnormal amounts of thyroxine. Both host and transplant showed signs of metamorphosis.

In 6 of the 13 successful grafts, reactions could be evoked by tactile stimulation; the same movements later occurred spontaneously. In the best cases the following movements could be observed: (i) movement of the chin; (ii) movement of the gills; (iii) dorsoventral movement of the whole head accompanied by retraction of the eyes; and (iv) complete opening of the mouth. Occasionally a burst of all these movements took place. The reactions were filmed, and two frames from this film are shown in Fig. 1. The average frequencies of these movements were noted during periods of 10 minutes on different days, with intervals of 1 week, starting 3 months after the transplantation (Table 2). The frequency of the movements of the second head could be strongly increased by localized illumination of the eyes through a flexible fiber glass rod connected with a light source. Evidence that the visual pathways were preserved was obtained by direct electrical recording from the

Transplantation of Axolotl Heads

Abstract. *Favorable conditions for organ transplantation exist for some populations of European laboratory axolotls, making transplantations of heads possible. Survival of the transplants is prolonged because homograft reactivity of the host animals is absent. Heads transplanted to the backs of other axolotls grow rapidly and show many reactions characteristic of normal axolotl heads. The behavior of the transplants is independent of that of the host animals.*

Although the presence of first-set homograft reactivity seems to be well established for both anurans (1) and urodeles (2), homograft tolerance is reported in connection with sexual dimorphism (3) and in studies on regeneration in the axolotl (4). However, except for a short report in which tolerance as well as rejection is shown in another population (5), a systematic study of transplantation immunity in the axolotl has not been made.

During a study of regeneration, randomly bred axolotls (*Ambystoma mexicanum*) (6 to 15 cm) accepted from each other transplanted blastemas from regenerating limbs. Similar exchanges of blastemas between animals from different laboratories and between white and dark races were likewise successful. The blastemas grafted to the backs of other axolotls differentiated into limb structures which remained indefinitely attached to their hosts. All experiments were made at a constant temperature

of 20°C. This tolerance is not due to immunological unresponsiveness because the axolotl is competent to reject xenografts; furthermore, it can produce humoral antibodies against various antigenic substances (6).

Feeding axolotl larvae 1.6 to 1.7 cm long were decapitated behind the gills, and each head was transplanted to a wound made previously on the back of an older axolotl (6 to 8 cm). The animals were anesthetized with MS 222 (Sandoz). Of 49 grafts, 13 remained attached to their hosts. The rest dropped off as a result of movements by the host, or died within 2 to 3 days.

In the first 2 days all transplanted heads became very pale, and there were no signs of vascularization. After this critical period the heads became deep red, an indication that circulation had been restored. This color remained until 8 to 9 days after the operation; thereafter it gradually disappeared. The transplants regained a normal appear-