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 oro-pharyngeal- 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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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 appearance; 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 thyroxin. 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

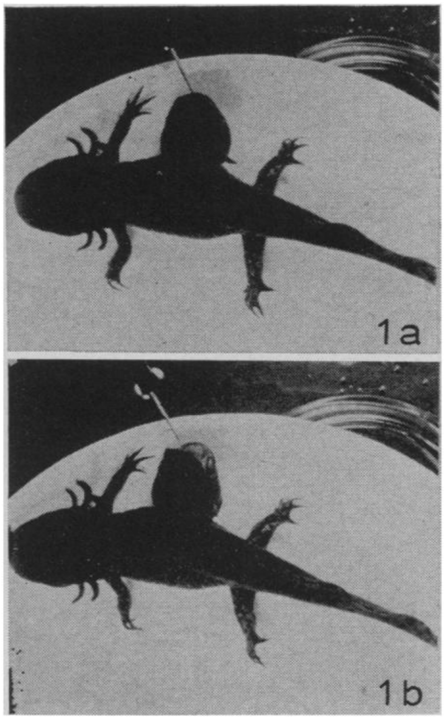

Fig. 1. Two frames from film taken 14 months after transplantation; the first shows the transplant at rest (a), the second its reactions to tactile stimulation (b).

brain of the transplant (7). After a light flash from a stroboscope was applied, a specific evoked response was recorded from the tectum opticum with KCl micropipettes or stainless steel electrodes. The response appeared after a latency of 139 msec and reached a positive peak at 180 msec. In normal axolotls of the same size similar responses with identical time relations were recorded.

Table 1. Growth of head transplants compared with growth of host heads and of intact control larvae.

Time after operation	Diameter (cm) Transplanted head	Head control larva	Host head
	Animal 1		
0 days	0.3	0.3	1.6
12 days	0.4	0.5	1.6
3 weeks	0.6	0.7	1.6
5.5 weeks	0.9	1.0	1.7
7.5 weeks	1.1	1.2	1.8
10 weeks	1.2		2.0
16 weeks	1.5		2.3
49 weeks (killed)	2.1		3.2
	Animal 2		
0 days	0.3	0.3	1.5
4 weeks	1.1	1.3	1.6
10.5 weeks	1.8		2.1
26 weeks	2.7		3.0
39 weeks	2.8		3.3
65 weeks (dead)	2.8		3.4

After the experiments the transplanted heads were removed from the hosts. No connections other than muscles, blood vessels, and skin were observed between host and transplant. Histological sections of the reacting heads were normal, but the sections of the nonreacting transplants revealed abnormalities, mostly hydrocephaly.

It is highly improbable that the reactions of the transplants are mediated by local innervation from the host. No large nerve bundles are present between the base of the transplant and the host. The transplants sometimes moved vigorously while no activity of the host was noticed. Conversely, when the host was feeding the grafted head exhibited no movements at all.

Studies on the movements of heterotopically transplanted limbs in *Ambystoma* show that innervation of these limbs by spinal cord segments which normally do not innervate the limbs does not result in coordinated movements (8), although weak movements seems to be possible. These, however, may be caused by movements of the muscles at the transplantation site (9). In chicks heterotopic limbs completely fail to move when innervated by nerves other than those of extremities, even upon stimulation (10). Furthermore, to postulate influences from the host's nervous system would contradict all evidence for the specificity of reflex pathways located in the normal head itself.

The electrical recordings show that a functional neural pathway is present within the transplant itself and, at least for the retino-tectal response, exclude the alternative of stimulation by way of host innervation.

Heads of dogs have been transplanted by Demikhov and co-workers (11) who connected the blood vessels of the transplant to those of a carrier dog. These transplanted heads also showed many activities such as sleeping and biting, which were not synchronous with the behavior of the hosts. However, the transplants survived between 1 and 4 weeks only, as a consequence of immunological responses against the donor tissue and of surgical imperfections which later became fatal.

The remarkable fact that our head transplants lived without vascularization in the first few days may be due to diffusion of oxygen from the water through the skin, which may have been sufficient for the rather small transplants. Moreover, urodelan brain tissue can regenerate (12), and this suggests that in our case regeneration took place if damage to the brain tissue during the transplantation procedure was limited.

Table 2. Movements of a transplanted head (spontaneous and during illumination). Averages of nine observation periods of 10 minutes on different days.

Movement	Movement (average frequency per 10 minutes) Chin	Gills	Head	Yawn
Spontaneous	15.2	6.2	1.9	0.2
Reactions to illumination	41.2	46.8	21.0	1.6

Ours is not the first attempt to transplant axolotl heads. Kolodziejski in 1933 transplanted axolotl heads, which remained alive for a long time, but showed no movements (13). In these nonmoving heads the brain tissue was abnormal just as it was in the motionless heads in our experiments.

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