Reports

Peculiar Feeding of Amphiuma under Conditions of Enforced Starvation

Abstract. Two *Amphiuma means* confined in aquaria with flowing artesian water for 3 years, with no known supply of food, increased in size. In the latter part of this period they were observed to shed a sheet of skin by a peculiar process and to devour it. This shed skin contained bacteria of the spirilla type, diatoms, and large amounts of unicellular algae, and possibly other organisms. Similar behavior in unstarved specimens of *Cryptobranchus* confined in an aquarium at the University of Alabama has been observed by E. L. Bishop.

The amphibian family Amphiumidae is confined to the southeastern United States. Only one species, *Amphiuma means,* is recognized. This is the largest amphibian on the North American continent, the maximum known length being slightly less than 40 in. The animal possesses two pairs of widely separated, ineffective legs, which have toes but which nevertheless are little more than relic strands of flesh. The eyes are weak; the body is almost perfectly cylindrical. The animal can progress by an eel-like motion on land, and the eggs are laid there in damp places close to water and guarded by the female, but with this exception life is spent in the water. This more or less helpless creature seems to be special prey of certain snakes. In turn, it is known to feed on crawfish, small fishes, insects, and smaller amphibians. These facts have been derived from many sources, but they have been collated and summarized by Oliver (*1*).

In January 1954 one of us (W.E.B.) placed two *Amphiuma means means* in a 5-quart container with 4.5 quarts of water, with only a small hole through which air could pass. The container was kept in a cool, damp place for 17 months, during which time a small amount of water was added to take care of evaporation. At the end of 17 months the animals were shorter and had decreased both in weight and in diameter. They were still vigorous and showed no signs of weakness when excited. *Siren* has been kept for a year under practically the same conditions, except that the water had to be changed about twice a month; otherwise, it became turbid and the animals died. By the end of the year the animals were reduced in size.

On 17 June 1956 two *Amphiuma means means* were put in separate 15-gallon wooden-and-glass aquaria at the Gulf Coast Research Laboratory, with a continuous flow of hydrant water from an artesian well about 300 feet deep. The smaller animal weighed 402 gm and was 691 mm long. Length and weight measurements were made by one of us (W.E.B.) on the smaller animal for the 3 years between June 1956 and June 1959. Measurements on a third specimen were also made during the last year, June to June. The larger animal was not measured. The data show that the animals increased in length and weight, but we wish to withhold them until we can, if possible, repeat the experiment.

This situation aroused considerable argument because the animals were kept under hardware cloth and copper screen wire and were not fed. A small number of encrusting algae grew on the glass of the aquaria, but this was kept fairly well scrubbed off. In discussion as to how these animals survived and grew, suggestions ranged from intussusception to sneak feeding by parties unknown. The former certainly seems to be most improbable in soft artesian water which contains practically nothing. Sneak feeding is also highly improbable for several reasons. This is a marine laboratory, and no one pays much attention to these fresh-water animals except ourselves. The animals are under observation every day and part of every night, and during the school year very few people are about because teaching is done here only in summer. It is highly improbable that these animals could have been fed enough, without detection, to cause them to increase in size. In fact, one of us (G.G.) once took umbrage at the cruel treatment of these helpless beasts and was strongly inclined, as director of the station, to bring the matter to an end, but strong pleadings and insistence on a continuance on the part of the other prevailed. In any case, we have no knowledge that these animals have ever been fed. We have also considered the matter of pure swelling or water uptake, and it has not been ruled out.

This apparent mystery led to increased attention to the *Amphiuma,* which resulted in a most curious observation. Apparently, about once every 3 or 4 days in the summer, at night, the animal peels a thin film of material from its whole body and then swallows it. The process starts back of the gill slits with a dorsal gas bubble. The film breaks around the body and peels back as the animal swims about, rubbing its sides and stomach until the film forms a roll back of the neck. At this point the animal stops swimming and produces a series of rhythmical, wriggling movements with some peristalsis of the whole body, which causes the film to be rolled posteriorly to the tip of the tail, where it peels off. After this, the animal eats the roll. The process has been observed seven times on three different animals. The gas bubble varies in size. The largest were about 1.5 cm in diameter. When the process proceeds properly the peeled film is doughnut-shaped. Occasionally, however, the process does not proceed properly and the film breaks off and rises to the surface with the gas bubble and with only a thin skein attaching it to the animal. However, when this part reaches the tail, the animal takes it and swallows it all to the end, finally engulfing the larger mass. After "shedding," the *Amphiuma* is no longer sleekly brownish-black in color, but is more grayish. Microscopic examination has shown that the film is a mucin-like material containing epithelial cells and bacteria, plus diatoms and unicellular algae in large numbers. Many of the bacteria are spirilla.

The smallest of the three *Amphiuma* was sacrificed after 1 year without being fed. The digestive tract and liver seemed to be small, but there were dark, loose, watery feces in the cloaca.

This shedding process of *Amphiuma* has been called to the attention of most of the students at this laboratory and of several visitors.

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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

Reference

1. J. A. Oliver, *The Natural History of North American Amphibians and Reptiles* (Van Nostrand, New York, 1955), pp. 42, 85, 89, 192, 292.

23 July 1959

Addendum. Since the foregoing report was submitted, there have been some other developments which should be included in this account. On the night of 25 August I noticed that the smaller of the two remaining animals was beginning to shed, with a roll back of its neck. However, this roll did not contain the typical bubble, and apparently the process was not going well. After watching for 1½ hours and seeing no further progress, I left. Next day the animal had the appearance of having shed completely. On 27 August it was found dead. There were no visible causes of death. It was opened at my request by Harry J. Bennett, who has had more experience with the internal organs of *Amphiuma* than anyone else here, because of having searched them for parasites. He thought that the intestine was smaller than usual. The cloaca contained the dark, watery feces noted before. Ovaries were present in a rather flaccid and undeveloped state, but small, yellow, individual ova could be discerned with the naked eye. (This leads to another hypothesis—namely, that the animal could have lived for a very long while on resorbed ovarian material.) The only sign of abnormality was the long urinary bladder greatly distended with liquid. This suggested that possibly the animal had succumbed to some osmoregulatory difficulty. For that reason the larger remaining *Amphiuma* was given a squirt of salt water from the laboratory sea-water system.

On 31 August the larger animal was also found dead. In this instance we have a better idea of the cause of death. Several times these *Amphiuma* had developed little punctate white spots all over the body, which felt hard and calcareous to the touch. They have never been examined microscopically, but we considered them to be fungi. They were controlled by unmeasured doses of potassium permanganate thrown into the water, or by shots of salt water which raised salinity of the aquarium water to 8 per mil; this apparently caused no difficulty to the amphibians. Brode had generally taken care of this matter, and during his absence I apparently let it go too long. The dead animal had a very large atypical swelling in the gill and gular region. It was preserved whole without autopsy. Thus, our observations have, temporarily at least, ended for lack of specimens.

The third development concerns some similar observations on another amphibian. Our colleague in summer teaching, H. T. Boschung, of the department of biology of the University of Alabama, recounted this matter to Everett L. Bishop, Jr., professor of biology at the University of Alabama, who stated that he had made similar observations on *Cryptobranchus,* the hellbender. In a letter to me dated 19 August Bishop stated that two animals were placed in a 75-gallon aquarium on 13 April; the three paragraphs that follow are quoted from his letter.

In the ensuing months I have placed about 50 live crawfish, ranging from an inch and a half to four inches in length, in the aquarium. Practically all of these have been eaten by the hellbenders. However, no significant increase in size has been noted. During the earlier weeks an almost constant laterally rolling motion took place in both specimens. A guess on my part was that this lateral rolling motion was a reflex associated with the respiratory function of the skin folds. . . .

Almost immediately (12 to 15 hours) after the specimens had been placed in water here a shedding of the skin was observed. Although a major portion peeled away from the head and then slipped off the body, there was some apparent random exfoliation. The shed skin had a distinct yellow-greenish appearance. highly suggestive of green algal pigmentation; although we talked about examining for the algae, we failed to do so. The major portion of the shed skin was 'peeled' off the head by a combination of head movements and rubbing against the bottom and sides of the tank. As this film of skin was rolled back below the pectoral limbs a surprisingly sharp flexion of the head permitted the animal to grasp the skin and peel it rapidly from the remainder of the body, engulfing the free part as it did so. The entire process took only about 10 minutes.

Several of us observed this being repeated four or five times since the original shedding. Unfortunately, no accurate records of observation have been kept. My impressions have been, however, that the sheddings have occurred at greater intervals. No one here can recall seeing this process occur since about the middle of June.

Bishop's observations raise the possibility that exfoliation or ecdysis may be present in other tailed amphibia, and also that it may be supplementary to regular feeding.

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10 September 1959

Rhythmic Contraction of Schwann Cells

Abstract. By time-lapse cinematography it was found that Schwann cells in tissue cultures of dorsal root ganglia from newborn rats show a contraction rate of approximately 4 to 18 minutes. This activity is of the order of magnitude reported for oligodendroglia.

In 1937 Canti, Bland, and Russell (*1*) reported pulsatile activity of cells cultivated from an oligodendroglioma, as revealed by time-lapse cinematography. Employing similar methods, Lumsden and Pomerat (*2*) established that oligodendroglial cells from the corpus callosum of the rat exhibited a contraction-expansion cycle of approximately 5 minutes. These findings were extended to include such elements from presumably nonpathological human tissue obtained in the course of lobotomies (*3*) and from oligodendrocytes of neoplastic origin (*4*). Recent studies of oligodendroglia have been summarized (*5*).

Table 1. Rhythmic contraction of Schwann cells from dorsal root ganglia of newborn rats, in vitro.

Culture*	Days in vitro	No. of contractions	Period (min)	Av. rate (min)
1a†	5	8	70	8.75
1b‡	5	None	95	
1c†	5	8	70	8.75
1d†	6	13	230	17.69
2a†	13	16	151	9.43
2b‡	13	17	113	6.64
2c†	13	70	410	5.85
3a†	13	12	70	5.83
3b§	14	21	80	3.81
3c†	14	16	90	5.62
4a†	16	17	120	7.05
4b†	17	12	70	5.83
4c†	17	1	9	9.00

* Letters indicate sequences providing test cells. † Treatment: nutrient. ‡ Treatment: 50 μg of γ-aminobutyric acid per milliliter of nutrient. § Treatment: 100 μg of γ-aminobutyric acid per milliliter of nutrient.

This report is concerned with the behavior of Schwann cells in tissue culture. Dorsal root ganglia obtained from newborn rats were cultivated under dialysis membranes in Rose chambers (*6*); a fluid nutrient consisting of 50 percent Gey's balanced salt solution, 45 percent human ascitic fluid, and 5 percent horse serum, reinforced with a final concentration of glucose at 300 mg/100 ml, was employed.

Phase-contrast, time-lapse cinematographic film records were made of dorsal root ganglia of rats on four cultures incubated from 5 to 17 days at a rate of four frames per minute. Schwann cells exhibited rhythmic contractile activity closely resembling that of oligodendroglial cells derived from the central nervous system.

Since γ-aminobutyric acid was being employed in parallel studies, this substance was added to some cultures of dorsal root ganglia. Table 1 summarizes observations on the contraction rates of Schwann cells obtained in preliminary experiments. On the basis of this limited series, γ-aminobutyric acid is not believed to have exerted a significant effect on cells in this system, but the data establish the phenomenon of rhythmic pulsatile activity for Schwann cells at a rate approximating that reported for oligodendroglia (*7*).

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