

Transepidermal Potential Difference: Development in Anuran Larvae

Abstract. Skin from bullfrog tadpoles does not show the potential difference characteristic of adult frog skin until several days after the emergence of the forelimbs, presumably because the mechanism for active sodium transport is absent or nonfunctional. Exposure to low concentrations of thyroxine accelerates appearance of the transepidermal potential difference at rates which are related to the dosage. This system may provide a new basis for evaluating the importance of specific biochemical mechanisms in active sodium transport.

According to the classical experiments of Ussing and Zerahn (1), the transepidermal potential difference shown by isolated frog skin is the direct result of a specific and active transport of sodium ions from the outside or mucosal surface to the inside or serosal surface. If this hypothesis is correct, and if it also holds for skin from anuran larvae, then young *Rana catesbeiana* tadpoles lack the mechanism for active sodium transport by skin as they do not manifest a transepidermal potential difference until late in metamorphosis (2). This finding is surprising, since one would expect on teleological grounds that tadpoles which live entirely in pond water of low sodium content would have greater need of an epidermal mechanism for sodium conservation than would adult

frogs which are at least partially terrestrial.

The study described herein was undertaken in an effort to pinpoint more closely the time during metamorphosis when ventral skin isolated from tadpoles first demonstrates the capacity to generate a transepidermal potential difference. Large *R. catesbeiana* tadpoles (90 to 140 mm total length) collected locally or purchased from a commercial source (3) during late summer and early fall were kept in distilled water at $22^{\circ} \pm 2^{\circ}\text{C}$ in a room artificially illuminated between the hours of 6 a.m. and 6 p.m. Canned spinach was fed several times weekly and aquarium water was changed every 2 days. As specimens spontaneously reached a desired stage of the metamorphic pattern described by Taylor and Kollros for *R. pipiens* (4), ventral skin was carefully removed and mounted between lucite chambers similar to those described by Ussing and Zerahn (1). Ringer-phosphate solution (amphibian) of the following composition (millimolar): NaCl, 111.2; KCl, 2.68; CaCl₂, 1.36; MgSO₄, 0.41; Na₂HPO₄, 4.16; NaH₂PO₄, 0.84; and glucose, 4.16 (pH 7.4 ± 0.05) was added to both chambers. Simultaneous aeration and circulation of bathing fluid were accomplished by air-lift pumps. Agar bridges leading from each chamber to calomel electrodes permitted measurement of transepidermal potential differences with a Beckman Expandomatic pH meter.

Skin from tadpoles undergoing spon-

Table 1. Transepidermal potential difference of skin from *R. catesbeiana* tadpoles at various stages of spontaneous metamorphosis.

Stage (4)	No. of animals	Difference in potential (mv)
IX to XVII	7	0
XVIII	5	0
XIX	4	0
XX	4	0
XXI	5	1.0 to 15.0
XXII	5	3.5 to 24.0
XXIII	7	4.0 to 21.8
XXIV	5	3.2 to 35.0
XXV (young)	6	17.3 to 56.0
XXV (mature)	5	33.5 to 51.0

taneous metamorphosis did not show a potential difference at the time of forelimb emergence (stage XX, Table 1). However, a few days later at stage XXI, delineated by lateral movement of the angle of the mouth to a point midway between the external nares and the orbit, all specimens tested had transepidermal potential differences, some of which were near the differences recorded for adult frogs. Ventral skin isolated from most tadpoles of stage XXII and beyond could not be distinguished from adult bullfrog skin as far as potential difference was concerned.

It has been known since the study of Gudernatsch (5) that the thyroid gland is intimately involved in the processes of amphibian metamorphosis. Presumably, the secretory products of this gland stimulate maturation of tadpole skin as well as of other tissues and thus may influence development of the mechanism for active sodium transport.

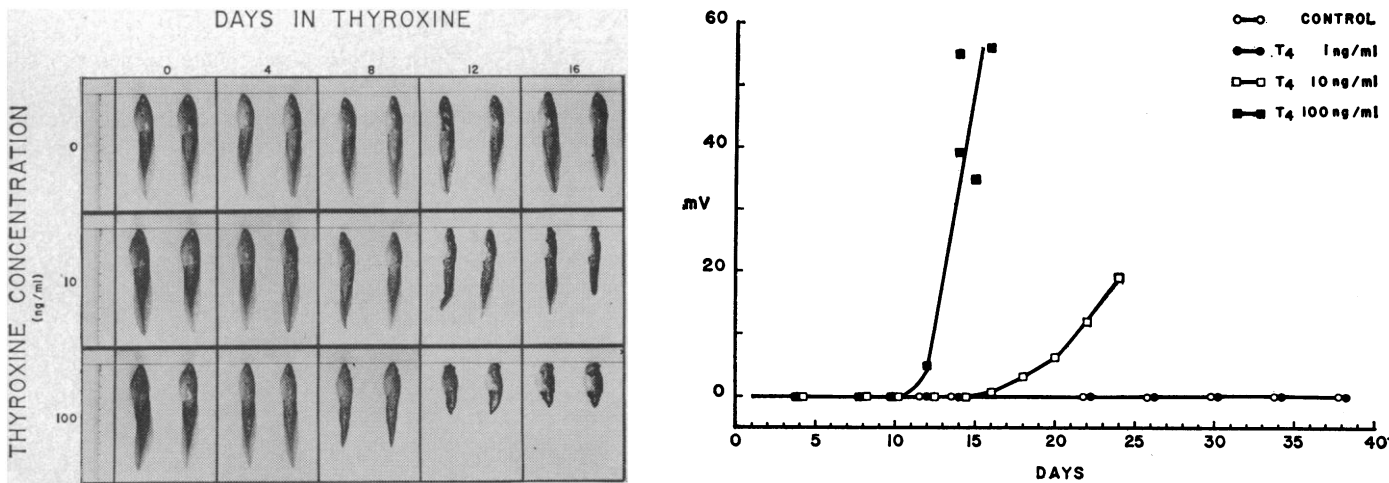


Fig. 1 (left). Progressive effect of continuous exposure to thyroxine on body structure of *R. catesbeiana* tadpoles. The three pairs of tadpoles shown in this figure were from the same group represented in Fig. 2, but were maintained separately in small aquariums and photographed every 4 days as indicated. Marked tail-fin reabsorption and decrease in tail length were apparent by day 8 in animals swimming in water containing 100 ng of thyroxine per milliliter. Similar but less extensive changes were seen by day 12 in animals exposed to lower concentration of hormone. Fig. 2 (right). Time required for development of transepidermal potential in skin from *R. catesbeiana* tadpoles exposed continuously to the three different concentrations of thyroxine shown.

To test this hypothesis young *R. catesbeiana* tadpoles obtained during the winter, all initially between stages V and VIII, were divided into four groups and placed in distilled water containing 0, 1, 10, or 100 ng of thyroxine (T_4) per milliliter. Canned spinach was present in the aquariums approximately 8 hours each day. After the feeding period, the tanks were cleaned and refilled with fresh T_4 solutions. Beginning on day 4, tadpoles from each group were killed at intervals, and sheets of their ventral skin were tested for potential difference as described above.

Although impressive structural changes normally occurring very late in spontaneous metamorphosis were obvious by the 8th and 12th days of exposure to T_4 at concentrations of 100 and 10 ng/ml, respectively (Fig. 1), this treatment did not lead to development of measurable transepidermal potential differences until day 12 with 100 ng/ml and day 18 with 10 ng/ml (Fig. 2). Thereafter, skin potentials increased progressively toward the values measured in adult frog skin (30 to 50 mv). Skin from control tadpoles and those exposed to T_4 at a concentration of 1 ng/ml did not mature sufficiently to generate potential differences within the 38-day period of the experiment.

The results of these experiments leave little doubt that thyroid hormone is instrumental in either establishing or activating the biological machinery essential for active transport of sodium by frog skin. We consider less likely an alternate interpretation of these results, that the potential difference due to active sodium transport is canceled out by the shunting effects of free movement of chloride, or of other anions. This possibility would lead to the conclusion that a relative impermeability to chloride develops late in metamorphosis, thus making manifest the electrical effects of the epidermal sodium pump. In addition, preliminary experiments in which skin from young tadpoles was bathed with chloride-free Ringer solution revealed no potential differences and therefore lend no support to the concept of a simultaneous and equal movement of sodium and chloride.

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References and Notes

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Lizard Reproduction: Refractory Period and Response to Warmth in *Uta stansburiana* Females

Abstract. *Photoperiod does not affect timing of the female reproductive cycle in Uta stansburiana. This lizard is refractory to reproduction until early December. Later, prolonged warmth under laboratory conditions initiates the reproductive cycle in some lizards regardless of photoperiod.*

The influence of photoperiodism on the reproductive cycle of reptiles is complicated by temperature fluctuations which regulate daily activity of ectotherms. Thus, photoperiod experiments on reptiles are difficult to evaluate without knowing the behavior of the animal in nature (1).

Research on reptile reproduction has resulted in three general conclusions: (i) photoperiod provides the primary stimulus for the reproductive cycle in some species (1-3); (ii) temperature usually modifies but seldom, if ever, initiates reproductive response (1, 2, 4, 5); and (iii) a refractory period (as in birds) during which the reproductive cycle cannot be initiated experimentally has not been demonstrated.

The iguanid lizard *Uta stansburiana* in western Texas lays three clutches between March and August; follicles begin to enlarge and undergo vitellogenesis in February or March (6). The population endures cold winters -30°C mean daily minimum for January 1959-62) by burrowing underground, but the lizards do not hibernate, and they are active on sunny days in winter. We have observed their activity for several winters and can predict photoperiod (duration of daily exposure to sunlight) from hourly climatological data (7). Photoperiods esti-

mated in this way are nearly always shorter than observed photoperiods, thus giving a conservative, or minimum, estimate of the actual daily exposure time of lizards to sunlight in nature.

Adult females were kept in terraria (3 m in diameter) in dark rooms for 6 to 9 weeks. Isolated groups of 10 to 20 lizards were exposed to varying photoperiods from fluorescent and heat lamps during the time of day at which the lizards are usually active. To simulate summer conditions, temperature was maintained above 27°C but not kept constant. Food (*Tenebrio* larvae) and water were available at all times. Sand on the floor was not deep enough to permit burrowing. Animals collected from the natural population at the beginning and end of each experiment served as controls.

Observations made under these conditions indicate that the female reproductive cycle of *U. stansburiana* is not timed by photoperiod. Reproductive response as measured by the presence of large yolked follicles was not significant-

Table 1. Reproductive response of female *Uta stansburiana* at 27°C and above. Reproductive females are distinguished by enlarged (>2 mm diameter) yolked follicles. Controls (c) are adult lizards collected from the field at the beginning and end of each experiment. Experimental lizards which lost 0.5 g during the experiment are excluded, because all controls were healthy.

Photo-period (hr/day)	Dates	Lizards	
		No.	No. reproductive
<i>Experiment A</i>			
6(c)*	13 Oct. 1963	10	0
3	13 Oct.-8 Dec.	18	0
6	13 Oct.-8 Dec.	19	0
12	13 Oct.-8 Dec.	20	0
6(c)*	8 Dec. 1963	7	0
<i>Experiment B</i>			
3(c)*	8 & 16 Dec. 1963	10	0
3	16 Dec.-2 Feb.	9	5
3-12†	16 Dec.-2 Feb.	7	4
12	16 Dec.-2 Feb.	6	3
3(c)*	1 Feb. 1964	8	0
<i>Experiment C</i>			
4(c)*	14 Dec. 1964	10	0
<1/2	14 Dec.-1 Feb.	9	6
4(c)*	1 Feb. 1965	10	1
<i>Experiment D</i>			
6(c)*	13 Sept. 1964	12	0
6	22 Sept.-25 Nov.	13	1
6(c)*	29 Nov.	6	0
6	20 Oct.-22 Dec.	16	4
6(c)*	14 Dec. 1964	10	0

* Average minimum, estimated from daily weather records, for the time covered by the experiment. † Photoperiod gradually lengthened from 3 to 12 hours per day.