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STUDIES ON THE STRUCTURE AND FUNCTION OF THE TADPOLE SKIN DURING METAMORPHOSIS*

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The research project carried out during July, 1965 was a preliminary survey of the structure and function of the tadpole skin during metamorphosis. A recent report by Taylor and Barker (Science, 148, 1612, 1965) indicated that the ventral skin of R. catesbeiana tadpoles was unable to carry out active sodium transport during early metamorphosis (stages IX to XX). A few days after foreleg emergence, however, (stage XXI) a potential difference could be measured across the skin, indicating that sodium transport had begun.

Our in vitro studies were carried out on tadpoles of R. clamitans obtained from fresh water ponds on Mt. Desert Island.

1. Potential, resistance and short-circuit current. Employing the lucite chamber technique of Ussing and Zerahn (Acta Physiol. Scand. 23, 110, 1951) we obtained results similar to those of Taylor and Barker. In 13 tadpole skins at various stages of metamorphosis, we observed a potential (10-18 mV) only after the stage of foreleg emergence. The short-circuit current obtained with these later skins was substantial (25-51 $\mu a/cm^2$), showing that a well-developed sodium pump was present. The ohmic resistance of these skins, as well as skins prior to foreleg emergence, was low (240-1200 $ohm.cm^2$), indicating that the skins had a high degree of permeability to ions. Chloride - 36 fluxes measured across several skins were high, consistent with the above observation.

2. Chloride flux. It appeared important to look further into the significance of the absence of a potential in the skins of tadpoles prior to foreleg emergence. Were these skins unable to carry out active sodium transport, or was the absence of potential simply due to coupled sodium and chloride transport? Three experiments were carried out in which cloride fluxes were determined in individual skins, first in one direction (e.g., outside to inside), then in the opposite direction. Although the experiments were not as technically satisfactory as was hoped, there did not appear to be an asymmetry of chloride movement across the skin. Any firm conclusion, however, must await further experiments.

3. Na-K ATP-ase activity. A third phase of the project was an investigation of the biochemical changes that take place in the skin at the time when a potential can be measured across it. As an initial study in this direction, we determined the Na-K activated ATP-ase of early and late skins, with the generous assistance of Dr. Roger Palmer. Homogenates of skins from six groups of frogs were made, and the ATP-ase activity of the supernatant (microsomal) fraction determined after centrifugation (10,000 G), in the presence and absence of 10^{-2} M ouabain. Our results may be tabulated as shown in the chart on the next page.

Na-K ATP-ase activity was not present in the single batch of skins from early metamorphosis (stage XII), and was not present in two of three batches of skins just prior to foreleg emergence (XVIII-XIX). Skins from frogs in late metamorphosis (XXI-XXII) and adult skins showed

*Research supported by N.I.H. (Grant AM-03858).

Stage	No. of skins/batch	Na-K-ATP-ase activity
XII	3	0
XVIII - XIX	3	0
XVIII - XIX	2	0
XVIII - XIX	2	+
XXI - XXII	3	+
Adult	2	+

ATP-ase activity, of the order of 30-40 mMole/hr/kg. The two latter groups of skins were at the stage where a potential could be demonstrated.

Again, the results must be regarded as preliminary. A large number of ATP-ase determinations must be made under a wide variety of experimental conditions, on skins from all stages of metamorphosis, before any conclusions may be drawn. It is possible, for example, that Na-K ATP-ase activity can be demonstrated in a different subcellular fraction of early skins, or that there is a different pH or ionic strength requirement for the ATPase determination in early skins. Nevertheless, the observation is of enough interest to justify further work.

4. Morphology. Finally, a number of skins of tadpoles at different stages of metamosphosis were fixed in osmium tetroxide and embedded in epon, for electron microscopic examination at my home institution. It is possible that we may see distinct morphologic changes that parallel the physiologic changes in the skin.

1965 #20

THE CONTROL OF INTESTINAL MOTILITY IN THE SPINY DOGFISH*

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The effects of serotonin, methylcholine, epinephrine, and bradykinin on the motor function of the gastro-intestinal tract of the spiny dogfish were studied. Changes in motor performance following nervous decentralization were also noted.

Preliminary interpretation of the data suggests an absence of chemoreceptors for serotonin, epinephrine and gradykinin in the gastro-intestinal as seen in mammals. Therefore, gastro-intestinal motility in the spiny dogfish is controled entirely through the C.N.S.

If confirmed, these results are of considerable phylogenetic importance that could give insight into aberrations of intestinal functions in man that are not at present understood.

*Research supported by Surgical Research Fund, Columbia, University.