Prolactin and Tadpole Growth.* (32493)

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Growth of premetamorphic tadpoles of *Rana catesbeiana* can be stimulated by administration of ovine prolactin, as judged by increase in body weight and in tail length(1). This finding has since been confirmed in both *Rana catesbeiana*(2) and *Alytes obstetricans* (3,4). In the latter species the prolactin growth effect was seen in both intact and hypophysectomized animals, indicating that the prolactin influence is not pituitary-mediated. That this stimulatory effect of prolactin in larval amphibians might exist was indicated first by Etkin and Lehrer(5), who autotransplanted the adenohypophyses of *R. catesbeiana* tadpoles to the tail region and obtained gigantism but no metamorphosis. It does not appear that this effect can be ascribed to pituitary STH, inasmuch as bovine STH in our hands has produced no appreciable growth in tadpoles that could not be accounted for by a minimal contamination with prolactin(1). The data of Enemar and von Mecklenburg(6) on the transplantation of pituitaries from various tetrapods into *Rana clamitans* tadpoles can also be interpreted as reflecting prolactin content, rather than STH content, although there is apparently some evidence against this interpretation(7).

The present study was undertaken to extend our earlier observations, which led us to suggest that prolactin might be a larval growth hormone in amphibians(1,8), to determine whether prolactin administration could antagonize the metamorphosis-favoring effect of thyroxin. In this situation, thyroxin stimulates resorption (decrease in size) of the tail and prolactin stimulates its growth (increase in size). These few morphological observations are preliminary to *in vitro* biochemical studies now being pursued in our laboratories.

Materials and methods. Second-year tadpoles of *Rana catesbeiana*, weighing between 10 and 20 g were taken from a holding pond on the Berkeley campus. Each tadpole was placed in a separate plastic box, painted black, containing 600 ml of pond water, and were maintained at about 21°C. One-half teaspoonful of canned spinach was provided as food, and it and the aquarium water were changed every second day. One week was allowed for acclimatization, during which time the tadpoles received daily handling and sham injections. The tadpoles were weighed, and their tail lengths were measured with calipers from the posterior margin of the vent to the tip. Tail depths (heights) were also measured at a standard locus midway along the tail to the nearest 0.1 mm in most experiments. These measurements were taken at the beginning and at the end of an injection period. Animals were injected intraperitoneally *via* a channel through the ventrolateral tail musculature (to prevent leakage), beginning about 0.5 cm posterior to the vent, with 0.9% NaCl, ovine prolactin (Li L2841B) in saline, or bovine somatotropin (NIH B5) in saline, in volumes of 0.05 ml daily. Amounts of hormone administered ranged from 1 to 10 μg per day, as stated in Table I. In some experiments 2 or 10 μg DL-thyroxin per 100 ml were dissolved in the pond water, so that tadpoles were exposed to either 12 or 60 μg of thyroxin per aquarium during the injection period.

Results. From Table I it can be seen that prolactin at all doses used caused an increase in tail length in the absence of exogenous thyroxin and that STH was slightly effective in two experiments (no. 2 and 4). When tail height (depth) was used as a criterion—and we now consider this a more reliable morphologic indicator than tail length in view of occasional damage to the tail tip—prolactin

* We are indebted to Mrs. Diane Dunlop for assistance in these experiments, to the National Science Foundation (Grant GB-2484) and Nat. Inst. Health (Grants AM-11161 and GM-13882) for research support, to the Nat. Inst. Health Endocrine Study Section for growth hormone, and to Professor C. H. Li for prolactin.

TABLE I. Response of Hindleg and Tail to Prolactin and STH in the Presence or Absence of Thyroxin ($m \pm SE_m$).

Exp No.	Daily dose of prolactin or STH	Thyroxin concentration (μg/100 ml)	No. of tadpoles/group	Hindleg change (mm)			Tail length change (mm)			Tail height change (mm)		
				CONT.	STH	PROL.	CONT.	STH	PROL.	CONT.	STH	PROL.
1	10	0	8	0	+.8±.3	0	+1.6±.5	+2.3±.5	+4.4±.7*	—	—	—
2	5	0	10	0	+1.2±.5	0	0	+1.6±.6	+4.6±.8*	—	—	—
3	2	0	10	0	0	0	0	0	+1.2±.8	0	0	+1.8±.7
4	2	0	10	0	0	0	−1.1±.4	+.9±.5*	+1.7±.6*	0	0	+1.6±.4
5	1	0	10	0	0	0	+1.2±.2	+1.6±.7	+3.7±.3*	0	0	+1.6±.6
6	1	0	7	0	0	0	+1.0±.5	0	+2.8±.3*	0	+.9±.4	+1.6±.1
7	5	2	7	+1.8±.5	+2.7±.3	+1.9±.4	+.9±.7	−.7±.2†	+3.5±.8†	−.4±.4	−.4±.4	+1.6±.1*
8	10	10	8	+3.1±.5	+4.3±.6	+3.4±.9	−3.0±.8	−4.1±1.6	+.6±.6*	−2.7±.5	−3.0±.8	−.8±.3*
9	1	10	8	+1.6±.3	+2.6±.5	+.8±.3	−1.3±.4	+.5±.7†	+1.9±1.0*	−.8±.4	−.2±.5	+.1±.3*
10	1	10	9	+1.7±.3	+2.7±.5	+1.4±.5	−2.8±.4	−3.6±.9	+1.8±1.0*	−2.1±.3	−2.4±.7	−.2±.4*

0 no measurable change.
* Significantly different from control or from STH when control is 0, $p < .01$
† Significantly different from control, $.01 < p < .025$

again showed a consistent stimulating effect (Exp. 3-6).

Under conditions where thyroxin was present in the aquarium water in quantities adequate to stimulate significant hindleg growth in all control groups (Exp. 7-10), it also resulted in decreased tail length and tail depth, statistically significant in all groups except Exp. 7. The data suggest that STH may accentuate hindleg growth, possibly due to contamination with STH; STH-treated animals showed significantly more leg growth than prolactin-treated animals in Exp. 9 and 10. In all groups prolactin counteracted the tail-resorbing influence of the added thyroxin, whereas STH did not show this antagonism except for a small effect on tail length in Exp. 9.

Discussion. The metamorphosis-inducing hormone, thyroxin, favors development of adult structures and degeneration of larval structures. The data obtained in these experiments support the concept that prolactin may favor growth of larval structures, such as the tail, and thus act antagonistically to thyroxin. Etkin and Gona(9-11) have obtained more dramatic data of this kind and have succeeded in maintaining premetamorphic tadpoles (*R. catesbeiana*) with large tails as a result of prolactin treatment, while normal control tadpoles had undergone complete metamorphosis and had resorbed their tails entirely.

Etkin and Gona(11) are convinced that the prolactin acts at least in part at the level of the thyroid and is goitrogenic. It is not yet possible to state finally where the prolactin is acting—although the hypothalamic and hypophysial levels would seem to be excluded as obligatory targets by the results of Rémy and Bounhiol on hypophysectomized *Alytes* tadpoles(3,4). Studies in organ-culture now in progress should help decide the issue of direct interaction of prolactin and thyroxin at the tissue level.

Elaboration of Etkin's concept of positive feedback in the differentiation of the hypophysis-thyroid axis in amphibian development(12) leads to the converse possibility in regard to prolactin secretion in larvae (Fig. 1). Etkin suggests that thyroid hormone

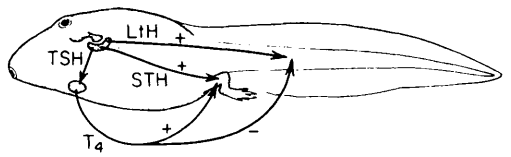

FIG. 1. Diagrammatic representation of the possible interaction of the TSH-thyroxin axis, prolactin (LtH) and growth hormone (STH) in the larval development of frogs. − = inhibition; + = stimulation.

secretion is responsible for the maturation of median eminence-portal system-adenohypophysis connections, resulting in hypothalamic stimulation of TSH secretion and the eventual attainment of levels of thyroid hormone necessary to accomplish metamorphic climax. Conversely, the adenohypophysis can be visualized as secreting prolactin autonomously in earlier larval life, inasmuch as evidence indicates that prolactin secretion by the amphibian hypophysis, as in the case of most mammalian hypophyses studied, is normally under inhibitory control of the hypothalamus (cf. 13); as median eminence development proceeds, prolactin secretion will be increasingly suppressed, to reach minimal levels at the time of metamorphic climax. While the TSH control system is maturing, the control of STH secretion may also be maturing; however, the role of STH at the time of metamorphic climax (in stimulating development of limbs—adult structures—for example) has yet to be assessed. In our hands, STH does not appear to be significant in larval growth.

At the moment we would propose that prolactin be viewed as the hormone of fundamental importance in larval development, which acts in balanced opposition to thyroxin as the hormone basic to eventual adult development. In urodele development, thyroxin is responsible for "land drive"—the emergence of an adult terrestrial eft, and prolactin for "water drive"—the second metamorphosis into an adult aquatic phase(14). The "antagonism" between thyroxin and prolactin in urodeles thus seems to be analogous to that visualized in the anuran tadpole. Indeed, it appears that prolactin may be inhibitory to the first metamorphosis from the aquatic larva to the terrestrial eft in urodeles. Accordingly, it could conceivably also play a role in maintaining the neotenic condition in salamanders such as *Necturus*. However, these possibilities have yet to be examined.

There is a striking analogy between this bihormonal system controlling amphibian growth and development and that operative in insects, wherein neotenin (juvenile hormone) favors larval or nymphal development and ecdyson favors adult development. Normal development toward emergence of the adult condition is based upon the interaction of these two factors. It is also of interest that in the adult stage of some insects, neotenin takes on a second role as a gonadotropin, a role ascribed to prolactin in regard to its luteotropic activity at least in rats, mice and ferrets among mammals(15). The possible influence of prolactin in embryonic and fetal growth in other vertebrates appears well worthy of investigation(cf. 16).

Summary. Mammalian prolactin inhibits thyroxin-induced resorption of the tail in *Rana catesbeiana* tadpoles. Mammalian growth hormone does not show this effect. The possible importance of prolactin as a larval growth hormone in amphibians is emphasized.

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Received July 27, 1967. P.S.E.B.M., 1967, v126.

Isolation of Mouse Lymphocytes for Immunologic Studies by Thoracic Duct Cannulation.* (32494)

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The lymphocyte has been shown to participate in transfer of immunologic memory (1), graft versus host reactions(2), homograft reactions(3), and antibody synthesis(4). Cannulation of the thoracic duct of mice provides the investigator with a pure population of lymphocytes from a highly inbred animal. The technique for collection of lymph from small laboratory animals was originally described by Bollman, Cain and Grindlay (5) in 1948. Gesner and Gowans(6) adapted the method for use in mice; Boak and Woodruff(7) changed the technique by using a surgical adhesive, methyl 2-cyanoacrylate, for cannula fixation. Using these techniques considerable difficulty, in terms of cannula blockade and animal survival, was encountered. This paper will describe several modifications, including the use of a new surgical adhesive, isobutyl cyanoacrylate monomer, that have led to an improved operative success rate.

Materials. The animals used were BALB/cAnN male mice, approximately 25-30 g, obtained from the Rodent and Rabbit Production Section, National Institutes of Health. Sodium pentobarbital* at a dosage of 0.06 mg/g body weight provided satisfactory anesthesia for one hour. The cannula was a 20 cm segment of PE10 polyethylene tubing† (inner diameter 0.028 cm, outer diameter 0.061 cm). One end of the cannula was shaped into an inverted U by placing a fine metal wire inside, bending to the desired form, and then plunging the cannula into boiling water for 5 seconds. After a 2-minute cooling period, the wire stylet was removed leaving the cannula properly molded. The cannula tip was beveled with a scalpel. Crystalline heparin‡ was used for anticoagulation. Methyl, butyl, and isobutyl cyanoacrylate monomers§ were the surgical adhesives used for cannula fixation.

Technique and results. One hour prior to the induction of anesthesia the mouse was given 0.1 ml of corn oil, orally. Following an intraperitoneal injection of pentobarbital the mouse was tied into a modified left lateral position and 1.5 ml of saline was given subcutaneously in the scapular regions. A left subcostal incision was made and the thoracic duct was approached transperitoneally. Abdominal contents and the left kidney were retracted, providing access to the duct, seen as a glistening white structure lying posterior to the aorta (Fig. 1a). The duct, from its origin at the cysterna chyla to its entry into the chest through the aortic hiatus of the diaphragm, was bluntly dissected. A 6-0 silk suture was positioned in the left posterior abdominal musculature, adjacent to the duct at a level several millimeters below the diaphragm (Fig. 1a, 1b). The cannula, brought into the operative field by passage through a #19 needle positioned in the left flank several centimeters caudad to the incision, was flushed with heparinized saline (5 mg/ml); it was positioned with the curved portion lying beneath the left diaphragmatic crus and the beveled tip, parallel to the duct, pointing in a caudad direction. The duct was put on traction and the beveled cannula tip passed through the lateral duct wall. The silk suture was tied around the cannula, where it en-

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† Clay-Adams, Inc., New York.

‡ Hynson, Westcott, and Dunning, Inc., Baltimore, Md.

§ Ethicon, Inc., Somerville, N. J.