marrow elicited a very low degree and duration of protection. Rat marrow permitted 55% of the irradiated mice to survive beyond the longest survival of the untreated controls. The duration of protection however was short, only 6% at 60 days. Irradiated mice protected with marrow from an F_1 hybrid of the irradiated strain showed a high degree and long duration of tolerance of skin homografts from the F_1 hybrid or from the other parent strain. Irradiated mice protected with foreign strain mouse marrow showed a high degree and long duration of tolerance for skin homografts from the same foreign strain. Irradiated mice protected with marrow from the same strain did not tolerate skin homografts from a foreign strain or from an F_1 hybrid.

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I^{131} and P^{32} Accumulation in Anuran Thyroid Gland in Normal and Accelerated Metamorphosis.* (22583)

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The application of radioactive tracers to studies of amphibian development, though infrequent, reveals that the tadpole thyroid can concentrate radioactive iodine and that this process may be influenced by thyroid stimulating hormone (TSH). Employing autoradiographic and histologic methods, Gorbman and Evans(1) demonstrated that radioiodine accumulation in the developing thyroid of Hyla larvae began soon after folliculation appeared in the gland. Autoradiographs became progressively darker as the thyroid increased in size and the colloid increased in amount. Dent and Hunt(2) found marked localization of I^{131} in autoradiograms of the thyroid from Hyla, Bufo, and Rana larvae, with no diminution in thyroidal radioactivity observed throughout metamorphic and post-metamorphic processes. Money, Lucas, and Rawson (3) measured the turnover of I^{131} in normal, thiouracil, and TSH treated *Rana pipiens* tadpoles. Radioactivity of the area of tissue bearing the thyroid ("block dissection") approximated 10-20% of the administered radioactivity. No substantial difference was found between metamorphosing and non-metamorphosing tadpoles. Surprisingly, the administration of TSH usually decreased rather than increased thyroidal I^{131} collection. In the main, the results of these experiments with radioactive tracers do not conform to those

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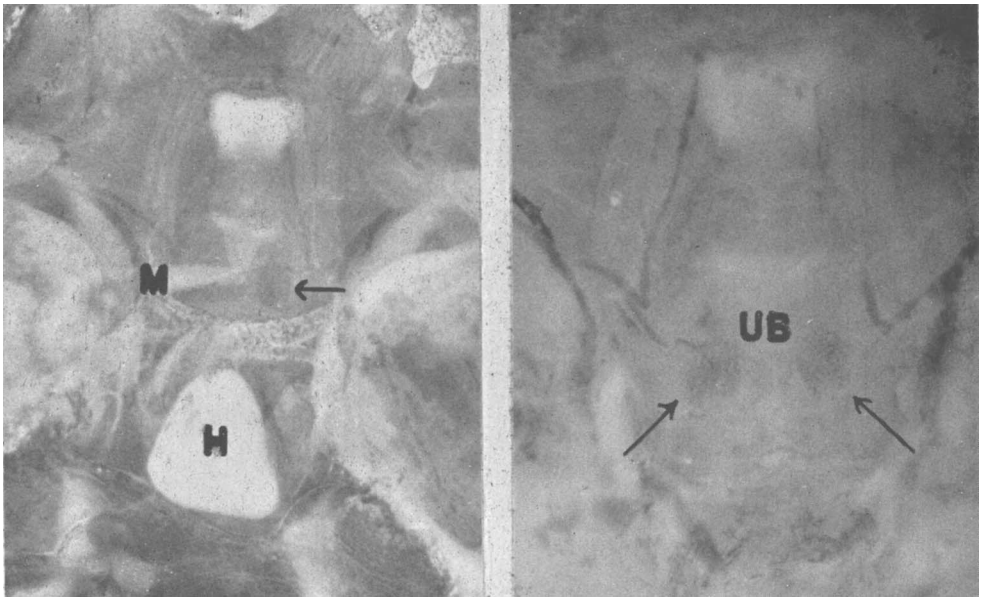

FIG. 1. Photograph (× 12) of freshly dissected hypobranchial region of a young tadpole. Transverse hyoglossus muscle (M) has been removed to expose one thyroid lobe (arrow). Ventral aortic roots and heart (H) have been left *in situ*. (Anatomic relations of developing anuran thyroid have been described(6).)

FIG. 2. Photograph (× 18) of hyoid area, with glossal muscles and heart removed. Thyroid lobes are shown (arrows), one on each side of urobranchial cartilage (UB). Vascularization and folliculation of the glands are recorded.

obtained from earlier morphologic and physiologic studies. The sequence of growth and histologic changes which characterize the thyroid throughout anuran metamorphosis are known to depend importantly on adequate thyrotrophic hormone function in the developing tadpole and can be precociously induced with exogenous thyrotrophin(4-9). The present study attempts to provide a more critical evaluation of functional changes in the larval thyroid by direct measurement of radioactivity in the gland isolated from Rana tadpoles after the injection of P^{32} and I^{131}. The avidity of the thyroid for these radioactive isotopes will be compared in normal metamorphosis and for accelerated development induced with exogenous TSH.

Materials and methods. *Rana clamitans* larvae were obtained from the field in various stages of hindlimb development during the winter, fall, and spring seasons. (This species is partially neotenic; metamorphosis of the tadpole is deferred for approximately one year.) The tadpoles were maintained at laboratory temperature (20-23°C) in aerated aquaria containing spring water and ample amounts of sphagnum moss as the only source of food. During the injection period, larvae were kept in shallow enamel pans and food was withheld. All injections were made into the body cavity by a method routinely used for bioassay(10). Tadpoles were given 0.5-1.0 μc of I^{131} or P^{32} in 0.1 ml of carrier free solution† 3 to 4 hours prior to sacrifice. Thyrotrophin,‡ (Parke, Davis preparation and the U.S.P. Reference Standard), was administered in .05-.1 ml of acid saline. Tadpoles received the last injection of TSH 23-25 hours before measurement of radioactivity in the thyroid. At sacrifice (decapitation), the hypobranchial region and adjacent pericardial sac were exposed (Fig. 1) by removal of in-

† Radioactive isotopes were obtained from Oak Ridge National Laboratory, Tenn.

‡ We express our thanks to Dr. W. Donaldson of Parke, Davis and Co., Detroit, Mich., and to Dr. Adley B. Nichols of the U. S. Pharmacopeia for supplies of the hormone.

TABLE I. Comparison of Thyroidal I^{131} and P^{32} Uptake in Normal Metamorphosis.

Exp. No.	Stage of development* H.L.	B.L.	No. of animals	Mean thyroid I^{131} uptake % of inj. dose (10^2)		Mean thyroid P^{32} uptake % of inj. dose (10^2)	
1	3.3 ± .5	22.5 ± 1.2	19	11.7 ± 1.8	P <.001‡		
	13.9 ± 1.9	29.5 ± 1.0	18	49.1 ± 6.3			
	1.8 ± .3	18.9 ± 1.0	8	11.6 ± 3.3	P <.001		
	25.0 ± 2.4†	24.0 ± 1.2	4	242.0 ±54.0			
	2.3 ± .5	22.0 ± 1.0	16			.49 ± .09	P n.s.
	15.1 ± 2.3	30.4 ± 1.4	14			.74 ± .15	
2	2.8 ± 1.0	21.2 ± 1.9	12	7.0 ± 1.0	P <.001		
	13.5 ± 2.5	25.0 ± 1.5	8	22.8 ± 3.6	P <.001		
	34.0 ± 1.0†	24.5 ± 1.0	2	89.7 ± 5.0			
	1.4 ± .3	19.0 ± 1.0	8			.25 ± .07	P <.001
	25.9 ± 2.0†	25.5 ± 1.2	8			.97 ± .08	

* H.L. and B.L. refer to mean hindlimb and body lengths in mm ± avg dev. In other columns, values represent mean ± stand. error of mean.

† Animals in advanced stages of metamorphosis (imminent or actual forelimb emergence).

‡ Statistical significance calculated from "T" test tables. "P" values >.05 are not considered significant (n.s.).

tegument and subjacent musculature. The "os thyroideum" (urobranchial cartilage) and the dorsolaterally placed thyroid lobes were then visualized under binocular dissecting microscope by removal of the glossal muscles and heart posteriorly. This permitted the vascularized glands to be observed clearly in their normal anatomic position ventral to the basal plate of the hyoid (Fig. 2). The cartilage plate with attached thyroids was then dissected free from the lower jaw, removed to filter paper, and the thyroid lobes gently stripped off with watchmaker's forceps and transferred to a stainless steel planchette. Radioactivity in the gland was measured immediately after dissection by a thin mica end-window beta sensitive Geiger-Müller counter under conditions of constant geometry§ for 200-300 seconds. Utilizing this procedure, it was possible to dissect, and measure the radioactivity in, the thyroids of 10-12 tadpoles in about one hour.

Results. A comparison of thyroidal I^{131} and P^{32} uptakes obtained from parallel experiments on normal and TSH treated tadpoles reveals that the larval thyroid concentrates radioiodine much more effectively than radiophosphorus (Tables I and II). Measured under similar conditions, mean percentages of injected radioiodine accumulating in the thyroids of young tadpoles were approximately 10-20 times greater than with P^{32}. This difference in uptake, moreover, was exaggerated throughout normal metamorphosis. The I^{131} collection of thyroids from larvae in advanced stages of development (Table I) increased markedly. The effect was not paralleled with radiophosphorus; the slight increase in P^{32} uptake by the thyroid which occurred in tadpoles with well developed hindlimbs was not statistically significant. Appreciable and significant increase of P^{32} uptake by the thyroid did occur, however, in the climactic stages of metamorphosis (forelimb emergence) although the rise was far less than that with I^{131}. The marked augmentation in uptake of I^{131} by the thyroid with advancing development suggests that the avidity of the gland (per unit mass) for radioiodine may increase progressively in the metamorphic process, and at a greater rate than do growth changes in the gland.

The results obtained with exogenous thyrotrophin are summarized and statistically evaluated in Tables II and III. It is evident that a single injection of TSH in proper dosage in-

§ Small size of larval thyroid obviates any major difficulty with respect to geometry of radiometric measurement; radioactivity of the dried glands measured 24 hr later in the same planchette is not significantly altered when correction is made for isotopic decay.

TABLE II. Effect of a Single Dose of TSH on Thyroidal P^{32} and I^{131} Accumulation in the Young Tadpole.*

Exp. No.	Dose TSH (μg)	No. of animals†	Mean thyroid P^{32} % of inj. dose (10^2)	Increase of normal (%)	Level of significance‡	Mean thyroid I^{131} % of inj. dose (10^2)	Increase of normal (%)	Level of significance
1	0	30	.26 ± .04			1.9 ± .17		
	1	30	.31 ± .09	19.2	P n.s.	2.9 ± .30	52.6	P <.01
	20	30	.69 ± .05	165.4	P <.001	2.8 ± .25	47.4	P <.01
	100	13				1.9 ± .28	0	P n.s.
2	0	46	.35 ± .05			3.2 ± .18		
	.5	18	.43 ± .06	22.8	P n.s.			
	2	32	.70 ± .10	100.0	P <.005	4.6 ± .48	43.8	P <.02
	10	30	1.05 ± .16	200.0	P <.001	3.7 ± .32	15.7	P n.s.
	50	15	.78 ± .10	122.8	P <.005			

* Animals with hindlimbs ranging from 1-6 mm; 4-8 days from field.
† Divided equally between P^{32} and I^{131} groups.
‡ Calculated from "T" test tables. "P" values in each case are compared against the control ("0" TSH). "P" values >.05 are not considered significant (n.s.).

creases radioiodine or radiophosphorus accumulation in the thyroids of tadpoles in early hindlimb stages. The minimal effective dose of hormone required to induce significant uptakes for both radioactive tracers lay at the 1-2 μg level (Table II). The magnitude and range of thyroidal response to increasing doses of thyrotrophin were greater with P^{32} than with I^{131} but in each instance high dose levels resulted in a diminution of thyroidal radioactivity from peak values which fell to approximately normal levels (radioiodine). The effects obtained from continued stimulation of the thyroid by a series of TSH injections were more marked (Table III). The administration of thyrotrophin (U.S.P.) to the stasis tadpole (starved, non-metamorphosing) once daily, over a 4-day period, resulted in significant increases of thyroidal I^{131} uptake ranging from about 250-750% of normal. The proportionality of response to increasing dose of TSH was much greater than that obtained with a single injection of equivalent amounts of the hormone.‖ The greater increments and wider range of thyroidal radioiodine uptakes produced with chronic TSH treatment were accompanied by somatic changes in the tadpoles indicative of accelerated metamorphosis. In animals receiving the higher doses of thyrotrophin, significant increases in hindlimb extension were noted as well as constriction of the body and head changes characteristic of advanced development. The thyroids of these animals were found to be enlarged and highly vascularized at dissection. It is noteworthy that the minimal effective dose of TSH required to induce a significant increase in thyroidal I^{131} accumulation was of the same order of magnitude with both single and multiple injections. Chronic treatment with high doses of TSH did not reduce radioactivity in the gland, however.

Discussion. The finding of substantial increase in radioiodine accumulation by the thyroid during metamorphosis closely accords with results of previous studies establishing the basic importance of thyroid-pituitary interaction in amphibian development. The growth and histologic manifestations of secretory activity in the thyroid of developing Rana tadpoles have been shown to increase progressively to a maximum, coinciding closely with the appearance of the climax stages of metamorphosis(4-6). These thyroidal changes and their metamorphic sequelae in turn are dependent upon concomitant changes in thyrotrophic hormone secretion from the tadpole's anterior pituitary and can be induced precociously with exogenous TSH(7-10). It is quite likely, therefore, that the enhanced I^{131} uptake by the thyroid in normal metamorphosis is a further expression

‖ 1 μg of Parke, Davis preparation (50P4) is equivalent to approximately 2 milliunits of U.S.P. Reference Standard.

TABLE III. Thyroidal Radioiodine Collection in the Stasis Tadpole* after Chronic Treatment with U.S.P. Thyrotrophin.

No. of animals	Mean increase in hindlimb growth (mm)	Dose TSH‡ (U.S.P. units)	Thyroid I^{131}, mean counts per 100 sec.	Mean % increase of normal	Level of significance‖
14	—	0	440 ± 45§	—	—
14	.9	.0002	447 ± 53	1.6	P n.s.
15	1.1	.0012	471 ± 76	7.0	P n.s.
14	1.4	.006	1500 ± 150	240.9	P <.001
14	1.7†	.03	3251 ± 499	638.9	P <.001
14	2.0†	.15	3793 ± 623	762.0	P <.001

* Starved, non-metamorphosing.
† Body changes indicative of advanced metamorphosis.
‡ Total dose given over experimental period (4 days).
§ Mean number of counts ± stand. error of mean.
‖ "P" values <.001 are highly significant; "P" n.s. (not significant).

of the increased tempo of thyroid function resulting from augmented titers of circulating endogenous thyrotrophin. It cannot be stated with certainty how much of the radioiodine accumulating in the thyroid reflects increased growth as against enhanced avidity of the gland per unit mass. The small size of the larval thyroid precludes direct weight measurements but from dimensions obtained with camera lucida and from histologic sections the estimated weight of the developing gland ranges from 50 to 200 μg. The concentration of radioiodine in the thyroid during advanced metamorphosis is far greater than can be accounted for by increased size of the gland, and uptakes may actually exceed those described by Bradley(11) for the frog (*Rana pipiens*). A detailed study of thyroidal uptake through development and into post-metamorphic stages is indicated.

The significance of phosphorus uptake in thyroid function (unlike that of iodine which is an essential component of thyroid hormone) is poorly understood. Chemical studies by Borell(14) pointed to a direct relationship between the phosphorus content of the thyroid and the amount of follicular epithelium. Borell and Holmgren(15) and Lamberg(16) for guinea pig and chick respectively, found good correlation between acinar cell height increase and P^{32} uptake in TSH stimulated thyroids. The present study indicates that the augmentation of P^{32} uptake with TSH also holds for the amphibian thyroid. The similarity of the response in representatives of at least three vertebrate classes seems highly significant, and it may well be that phosphorus has special importance as a component of the growing thyroid cell(14). It remains to be shown, however, whether or not the uptake of phosphorus by the thyroid in metamorphosis truly parallels growth of the gland, and whether it relates specifically to colloid or to epithelium. The relationship between iodine and phosphorus metabolism in the hypertrophic gland should also be investigated.

The increases in thyroidal I^{131} and P^{32} uptakes induced with relatively small doses of TSH are of particular interest as regards the problem of thyrotrophin assay. The dose response curves obtained with a single injection of the hormone did not display a range of proportionality which could lead to precise estimation of TSH in blood and pituitary, as now routinely accomplished with microhistometric measurement of thyroid cell height in the stasis tadpole(13,12). Large doses of TSH tended to decrease rather than increase radioactivity in the thyroid. It is conceivable that purging of thyroid hormone from the gland with sharp and vigorous stimulation may induce transformation changes (fluid loss?) in the test animal which temporarily diminishes the amount of radioactive tracer available to the gland. Whatever the cause, the effect was overcome by chronic stimulation of the thyroid with TSH. In fact, thyroidal I^{131} uptakes in stasis tadpoles given a series of TSH injections were sufficiently large over a wider range of dose level to merit further investigation of the response as to its usefulness for thyrotrophin assay. A detailed compari-

son of histometric and radiometric methods for TSH detection in the stasis tadpole will be considered elsewhere.

Summary and conclusions. 1) The uptake of I^{131} and P^{32} by the developing thyroid of Rana tadpoles has been determined in normal and accelerated metamorphosis. Radioactive measurements of the isolated gland (estimated weight, 50-200 μg) disclose a progressive increase in radioiodine accumulation throughout normal development. The effect is not paralleled with radiophosphorus although significantly increased P^{32} thyroidal uptakes eventually occur in advanced development. Exogenous thyrotrophin augments I^{131} and P^{32} accumulation in the thyroids of young tadpoles. The applicability of the thyroidal response to the bioassay of thyrotrophin is discussed. 2) The enhancement of iodine and phosphorus uptake by the larval thyroid is considered to reflect increased tempo of thyroid function in amphibian development. The fundamental rôle of pituitary-thyroid interaction in anuran metamorphosis is reemphasized.

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Lack of Lipotropic Action by Thiomethyl Derivatives of Glucose.* (22584)

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The fatty infiltration of the livers of rats on low protein diets is generally ascribed to a deficient synthesis of choline as a result of the inadequate supply of biologically transferable ("labile") methyl groups. In methionine, which is the major dietary source of such groups, the methyl is attached to a sulfur atom; and the same type of attachment is present in methionine sulfoxide and in various thetins (dimethylpropio-, dimethyl-, methylethyl thetin, and methionine ethylsulfonium), which have been shown to be efficient methyl donors in the rat(1). It seemed possible that other compounds possessing terminal thiomethyl groups may also be active in this respect. In the present investigation, the lipotropic action of 2 such compounds, methyl-thioglucoside (MTG) ($CH_3 \cdot S \cdot CH_2 \cdot (CHOH)_4 \cdot CH_2OH$) and glucose dimethylmercaptal (GDM) ($(CH_3 \cdot S)_2 \cdot CH \cdot (CHOH)_4 \cdot CH_2OH$), was tested.†

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