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Requirement for RNA[1] and Protein Synthesis for Induced Regression of the Tadpole Tail in Organ Culture

J. R. TATA

National Institute for Medical Research, Mill Hill, London, England

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INTRODUCTION

Tissue regression is an important and precisely timed event occurring during embryonic development. The resorption of the anuran tadpole tail is one of the most striking features of amphibian metamorphosis, and the increase in the activity of several hydrolases in the regressing tissue has now been well established (see Bennett and Frieden, 1962; Weber, 1963; Gross, 1964). From measurement of specific activities of hydrolytic enzymes alone, it has not been possible to decide whether the increase during tail resorption reflected activation of latent enzymes (see de Duve, 1963), or a selective protection of the enzymes against degradation, or a synthesis of new enzyme molecules *in situ.*

The technique, introduced by Shaffer (1963), of maintaining *Xenopus* tadpole tails in organ cultures and the induction of regression of the isolated tail by the addition of thyroid hormone to the culture medium, offer a valuable tool in resolving some of these problems. It would also help in deciding whether regression was initiated by hydrolases present in invading phagocytic bodies and eliminate some of the complications resulting from the varied developmental changes in other tissues during metamorphosis. Adopting Shaffer's technique, Weber (1963) concluded that the rise in cathepsin during thyroxine-induced regression was unlikely to be due to an activation of the latent enzymes but more likely to be due to additional enzyme formation. Alteration in the properties or distribution during metamorphosis of tail enzymes such as DNase (Coleman,

[1] Abbreviations: T_3, 3,5,3′-triiodo-L-thyronine; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

1962), cathepsin (Weber, 1963), and β-glucuronidase (Kubler and Frieden, 1964) have also provided indirect evidence in support of this idea. It is however essential, as a preliminary step to further work on this problem, to establish that the cultured tail can synthesize protein and that the synthetic machinery can be mobilized for the process of regression.

The experiments described below were initially undertaken to determine the capacity of *Rana temporaria* tails, maintained in organ cultures, to synthesize RNA and protein and to study the response to induced regression. Although the synthesis of specific proteins has not been demonstrated, our results show that a sustained generation of RNA and protein is essential for regression to occur.

MATERIALS AND METHODS

Rana temporaria tadpoles, at least 40 days before spontaneous metamorphosis, were obtained from L. Haig & Co. (Beam Brook, Newdigate, Surrey, England). Tails for organ culture were amputated from tadpoles of uniform size (tail length, 14–16 mm) after the animals had been chilled in ice water for 10 minutes. The method of culture was a simplified version of that described by Shaffer (1963). The simplification consisted of not mounting the tails on special supports, nor was it found necessary to pay particular attention to shaking or gassing the culture medium. Seven tails were placed in the lids of plastic organ culture dishes, 6 cm in diameter and containing 4 ml of the culture medium. The open dish was placed in a larger, closed culture dish (8 cm diameter) and surrounded by water-saturated cotton wool. The volume of the medium in the culture dish was critical. The composition of the culture medium in a final volume of 200 ml was as follows: 150 ml of Gey's solution buffered with 0.0025 *M* Tris-HCl at pH 7.6, 10 ml of 10-day-old chick embryo extract (supernatant from 1 embryo homogenized per milliliter of the Gey's solution), 10 ml of calf serum (clear supernatant obtained after heating to 60° for 10 minutes), 100,000 units penicillin, 125 mg streptomycin, 10,000 units mycostatin, and 5 mg Terramycin. The culture dishes were placed in a constant temperature room at $20 \pm 1°$ and were gently shaken for 20–30 seconds by hand, three times a day. The medium was replaced every 48 hours throughout the duration of the experiment. There was some twitching of the tails for the first 2 hours after amputation and a shortening of less than 10% in length

during the first 20 hours of culture. Experiments were therefore usually begun after this period.

Regression of the isolated tails was induced by adding 1 μg of 3,5,3′-triiodo-L-thyronine to the culture medium. It was quantitated by measuring the length of the tail every 18 or 24 hours and recording its weight at the end of the period of culture. Before weighing, the tails were washed several times at 0° first with 5-ml batches of buffered Gey's solution and then with 0.15 *M* NaCl. After weighing, they were homogenized at 0° in 9 volumes of 0.15 *M* NaCl in a Kontes all-glass homogenizer for chemical and enzyme determinations.

RNA and protein synthesis was estimated from the incorporation of H^3-labeled uridine and a mixture of C^{14}-labeled amino acids, respectively. A 1–10 μc sample of the isotope was included in 4 ml of the culture medium. For radioactive nucleic acids, RNA was extracted from the homogenate and separated from DNA according to the method of Tata and Widnell (1966). C^{14}-labeled protein was precipitated from the homogenate with 5% trichloroacetic acid (TCA) and the precipitate washed as follows: 2 times with 5% TCA acid at 0°, 2 times with TCA at 90°, once with 95% ethanol, 2 times with ether–ethanol (3:1). H^3 and C^{14} were measured in a Packard Tricarb liquid scintillation spectrometer after heating the sample in 0.5 ml of 5% TCA at 90° for 15 minutes and dissolving it in 10 ml of the dioxane scintillation fluid containing 18% naphthalene (Butler, 1961). Chemical determination of RNA and DNA were performed according to Ceriotti (1955) and Burton (1956), and protein was measured by the method of Lowry *et al.* (1951). Acid phosphatase was determined according to Frieden and Mathews (1958) using nitrophenyl phosphate as the substrate, cathepsin as described by Weber (1963), and ribonuclease by recording the acid-soluble material absorbing at 260 mμ released from added yeast RNA.

RNA and protein synthesis was inhibited by the inclusion in the culture medium of actinomycin D, cycloheximide, and puromycin in appropriate amounts. The tails were usually exposed to the inhibitor after initial twitching and shortening during the first 20 hours.

3,5,3′-Triiodo-L-thyronine was obtained from Glaxo Laboratories, Greenford, England; actinomycin D was from Merck, Sharp & Dohme, Rahway, New Jersey; cycloheximide from California Corporation for Biochemical Research, Lucerne, Switzerland; puromycin was from

Lederle Laboratories, Pearl River, New York. All substances were dissolved in a small volume of 0.15 *M* NaCl and diluted with the culture medium just before use. H^3-labeled uridine (specific activity 1220 mc/m*M*) and C^{14}-labeled amino acids (*Chlorella* protein hydrolyzate, specific activity about 600 μc/mg) were obtained from the Radiochemical Centre, Amersham, England.

RESULTS

Induced Regression in Isolated Tails

The rate of regression of the isolated tails in the presence or absence of triiodothyronine in the culture medium is shown in Fig. 1. In the control samples, there was less than a 10% reduction in the

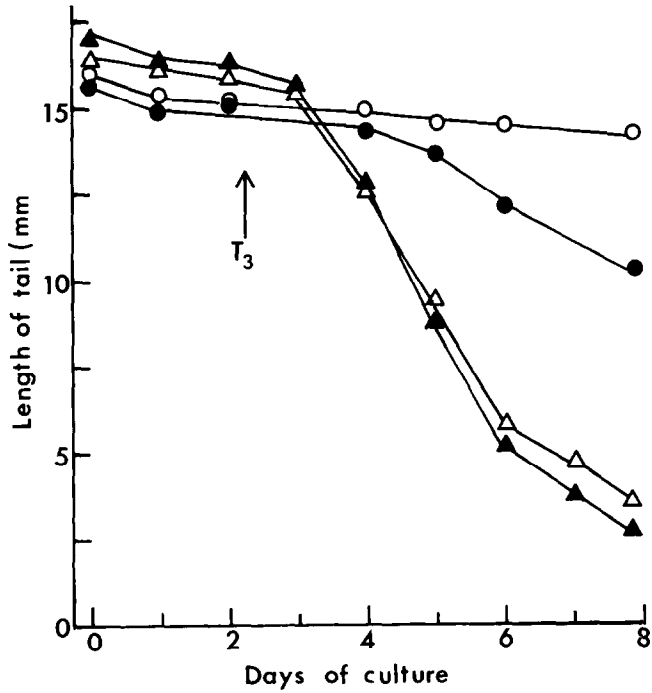

FIG. 1. Regression of *Rana temporaria* tadpole tails induced *in vitro* at different concentrations of triiodothyronine added to the medium 2 days after amputation. ○——○, Control, no T_3 added; ●——●, T_3, 0.25 μg/ml; △——△, T_3, 1.0 μg/ml; ▲——▲, T_3, 2.5 μg/ml.

length during 7 days of culture whereas a 80–90% reduction had occurred in preparations to which the hormone was added. The rate of reduction in length did not parallel the loss of weight in the samples treated with the hormone; this dissociation was also observed when the amounts of different tissue constituents were measured. It can be seen from Table 1 that, at the end of the culture period, the tails had lost protein, RNA, and DNA to different extents. At the onset of regression, the tissue underwent dehydration to a greater extent than degradation of these constituents with the result that an increase of

TABLE 1

CHANGES IN WET WEIGHT, PROTEIN, DNA, AND RNA ACCOMPANYING T_3-INDUCED REGRESSION OF ISOLATED TAILS[a]

Tails	Culture period (days)	Length (mm)	Wet weight (mg)	Protein		DNA		RNA		RNA: DNA
				Mg/ gm wet wt.	Mg/ tail	Mg/ gm	Mg/ tail	Mg/ gm	Mg/ tail	
Control	0	15.9	34.0	44.8	1.52	1.20	0.41	1.71	0.57	1.41
	2	15.1	26.8	46.5	1.25	1.41	0.38	1.82	0.49	1.29
	4	14.6	23.3	50.0	1.16	1.50	0.35	2.22	0.52	1.48
	7	14.4	20.7	40.5	0.84	1.02	0.21	1.41	0.30	1.39
Induced regression	0	16.2	38.0	43.3	1.64	1.06	0.40	1.61	0.61	1.52
	2	15.9	31.4	49.5	1.55	1.32	0.41	1.90	0.60	1.50
	4	10.5	20.0	44.0	0.88	1.20	0.24	2.08	0.33	1.36
	7	4.0	6.1	71.0	0.43	2.31	0.14	2.65	0.15	1.09

[a] The values are for the same samples illustrated in Figs. 1 and 2. Each value is the average of 3 separate determinations on 7 tails pooled from each culture dish, with standard deviation within ± 10%. Other conditions as in text and Figs. 1 and 2.

protein and nucleic acids was noted per milligram wet weight, but a net loss of these substances when recorded per whole tail. A similar increase in the dry matter content of the tails during the initial stages of regression has been observed in metamorphosis induced in the intact tadpole (Lapiere and Gross, 1963; Gross, 1964; Domjan and Tata, unpublished).

In our initial experiments, we determined the activity of a few hydrolytic enzymes in the regressing tails (Domjan and Tata, unpublished). There was a 2-fold increase in the activity of acid phosphatase, cathepsin, and ribonuclease when the results were expressed either on the basis of milligrams protein or milligram wet weight of the tissue (see Table 2). However, the amount of acid (and alkaline)

TABLE 2

THE EFFECT OF T_3-INDUCED REGRESSION ON THE ACTIVITY OF SOME HYDROLYTIC ENZYMES OF THE ISOLATED TAIL MAINTAINED IN ORGAN CULTURE[a]

Enzyme	Days of culture	Enzyme activity in tail			
		Nonregressing		Regressing	
		Per mg protein	Per tail	Per mg protein	Per tail
Acid phosphatase (Δ μg P released/20 minutes)	0	36	54	41	60
	3	42	51	54	51
	6	39	63	107	73
Cathepsin (Δ μg acid-soluble N/hour)	0	6	10	5	11
	3	8	9	19	23
	6	11	18	48	35
Ribonuclease (Δ E_{260}/30 minutes)	0	0.024	0.039	0.020	0.035
	3	0.031	0.042	0.048	0.077
	6	0.038	0.041	0.079	0.049

[a] Regression was induced by the addition of 0.8 μg T_3 to the medium on the second day of culture. Values are average for 3 experiments with 7 tails for each determination.

phosphatase and ribonuclease per tail did not change appreciably throughout the period of culture in regressing and nonregressing tails. There was an increase in cathepsin values even when the results were calculated per tail. Similar findings have been made in isolated *Xenopus* tails by Weber (1963), who showed a 2- 3-fold increase in total cathepsin activity per tail, although with the concomitant loss of total protein the increases expressed per milligram protein were considerably larger.

RNA and Protein Synthesis during the Culture of Isolated Tails

Table 3 shows the incorporation of H^3-uridine into RNA by the isolated tails when the radioactive precursor was added to the medium at different times during the culture of regressing and nonregressing samples. Preliminary experiments showed that the incorporation of H^3-uridine into RNA of the tail was almost linear between 4 and 12 hours after the addition of the isotope to the nonregressing samples. It can be seen that the cultured tails retained the capacity to actively

TABLE 3

THE INCORPORATION OF H^3-URIDINE INTO RNA BY NONREGRESSING AND REGRESSING ISOLATED TAILS AND THE EFFECT OF ACTINOMYCIN D AND CYCLOHEXIMIDE[a]

Treatment	Time H^3-uridine added (days after amputation)	Length of tail when H^3-uridine added (mm)	Total H^3-RNA per tail (cpm)	Specific activity (cpm/mg RNA)
None	1	15.6	2,250	3,840
	2	15.3	4,800	8,230
	3	15.3	6,450	11,100
	4	15.1	5,400	10,500
T_3 (1 μg/ml)	1	15.2	2,600	4,170
	2	14.5	4,000	7,580
	3	12.7	13,680	33,250
	4	10.1	11,350	32,600
Actinomycin D (5 μg/ml)	2	15.4	960	2,060
	3	15.3	380	952
T_3 and Actinomycin D	2	15.6	1,130	2,230
	3	15.4	80	184
Cycloheximide (30 μg/ml)	2	14.7	3,700	7,610
	3	14.7	740	1,735
T_3 + cycloheximide	2	14.9	1,250	1,807
	3	14.7	900	2,060

[a] Regression was induced by T_3 added to the medium at the beginning of culture. Tails were washed with nonradioactive uridine, 12.5 hours after the addition of 10 μc H^3-labeled uridine and then homogenized. Each value is an average of three determinations in duplicate with variations of ± 5–15%.

synthesize RNA for at least 4 days after amputation. Whether regression was induced or not, the tails exhibited a low incorporation activity when the H^3-uridine was added on the first day after amputation. If, however, the radioactive precursor was added on the second or third day of culture, maximum activity was observed and then activity remained constant or decreased. At the time of the highest incorporation activity, the specific activity of RNA in tails with induced regression was 3–5 times higher than that in the noninduced controls. There was little net accumulation of RNA in the tissue (see Table 2 for values); this suggested that regression was accompanied by the acceleration of both the synthesis and turnover of RNA.

The incorporation of C^{14}-labeled amino acids into protein was not

completely linear but continued to rise for 10–12 hours after the addition of the isotope to control samples. A somewhat different pattern was observed when the incorporation of C^{14}-labeled amino acids into protein was studied (Table 4). The main difference between the kinetics of labeling of RNA and that of protein was that the capacity

TABLE 4

THE INCORPORATION OF C^{14}-LABELED AMINO ACIDS INTO PROTEIN BY ISOLATED TAILS, WITH OR WITHOUT T_3-INDUCED REGRESSION AND THE EFFECT OF PUROMYCIN AND CYCLOHEXIMIDE[a]

Treatment	Time of addition of C^{14}-amino acids (days after amputation)	Length of tail when C^{14}-amino acids added (mm)	Total C^{14}-protein per tail (cpm)	Specific activity (cpm/mg protein)
None	1	15.0	2,150	1,349
	2	14.9	6,000	5,080
	3	14.8	5,700	4,860
	4	14.7	2,150	2,040
T_3 (1 μg/ml)	1	15.2	7,800	4,750
	2	12.4	32,100	19,900
	3	10.2	15,270	13,520
	4	8.4	10,350	12,800
Puromycin (100 μg/ml)	2	15.5	1,100	600
	3	15.5	3,020	1,640
T_3 and puromycin	2	15.4	1,290	787
	3	15.2	2,870	2,010
Cycloheximide (30 μg/ml)	2	14.7	3,800	2,390
	3	14.7	2,150	1,410
T_3 and cycloheximide	2	14.8	2,490	1,140
	3	14.6	2,200	1,500

[a] Triiodothyronine and the inhibitors were present, where indicated, from the beginning of the experiment. C^{14}-labeled amino acids (1.6 μc) were added at different times during the culture, and the tails were withdrawn and washed with nonradioactive casein hydrolyzate 8.8 hours after the addition of the isotope. Each value is an average of 3 determinations with variations in the range of ± 10–20%.

to incorporate amino acids into protein by nonregressing tails was at its highest level 48 hours after amputation. In the T_3-induced regressing tails, the specific activity of the radioactive protein was 4–8 times higher than in controls. This stimulation was particularly marked on the 2nd day of the culture, the hormone having been included in the medium from the beginning of the culture period. In view of the

limited scope of these experiments, it is not possible to explain why incorporation of amino acids into protein was in induced accelerated samples before a significant increase in the incorporation of H^3-uridine into RNA could be detected. As also noted earlier (Table 1) there was an increase in the protein content per unit wet weight of the tissue, but there was a loss of total protein per tail which is reflected in the values for total C^{14}-labeled protein per tail.

Abolition of T_3-Induced Regression by Inhibitors of RNA and Protein Synthesis

In order to test whether stimulated RNA and protein synthesis observed during induced regression of the tail had a bearing on the process of regression itself, cultures were performed in the presence of actinomycin D, cycloheximide, and puromycin. Figure 2 shows that actinomycin D, which is well established as an inhibitor of both messenger and ribosomal RNA synthesis (see Reich and Goldberg, 1964) was very effective in preventing the T_3-induced regression of the cultured tails. The inhibition was proportional to the concentration up to 4 μg of actinomycin D per milliliter when regression was induced by less than 1 μg T_3 per milliliter. Figure 2 also shows that the inhibition produced by 5 μg of actinomycin D could be partially relieved by increasing the amount of the hormone to 2.5 μg T_3 per milliliter. It should be noted, as shown earlier (Fig. 1), that increasing the T_3 level from 1 μg/ml to 2.5 μg/ml of the culture medium does not accelerate any further the rate of tail regression in the absence of the inhibitor. The effect of actinomycin D could not be reversed by washing the organs and continuing the culture in an inhibitor-free T_3-containing medium even if the hormone was added initially at the same time as the inhibitor. In Fig. 3 are shown the results of an experiment in which the tails failed to regress even 5 days after the removal of actinomycin D, although amounts of T_3 sufficient to cause rapid regression were maintained. Actinomycin D is known to be extremely firmly bound to DNA, and irreversibility of its inhibitory effects have also been observed in other systems (see Reich and Goldberg, 1964).

Puromycin and cycloheximide are effective inhibitors of protein synthesis (see Darken, 1964; Siegel and Sisler, 1964), and both these substances are also known to suppress ribosomal RNA synthesis (Tamaoki and Mueller, 1963; Fiala and Davis, 1965). Figure 4

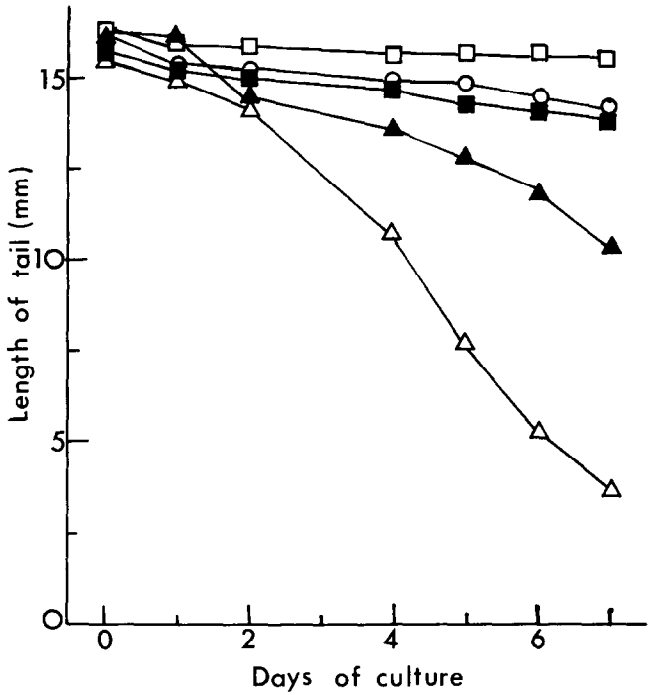

FIG. 2. Inhibition by actinomycin D of the regression of isolated tadpole tails induced by triiodothyronine. Both the hormone and inhibitor were present in the culture medium from the beginning. ○——○, Control; △——△, T_3, 1 μg/ml; □——□, actinomycin D, 5 μg/ml; ■——■, 1 μg T_3 + 5 μg actinomycin D per milliliter. ▲——▲, 2.5 μg T_3 + 5 μg actinomycin D per milliliter. Note in Fig. 2 that there is no difference in the rate of regression induced by 1.0 or 2.5 μg T_3 per milliliter in the medium.

shows that cycloheximide and puromycin abolished the induced regression very effectively. These compounds, as well as actinomycin D (Fig. 3), also prevented the small amount (about 10% reduction in length) of regression that took place in control samples. After 5 days of culture in its presence, puromycin was found to lead to tissue damage, largely seen as a fraying of the fin.

That the inhibition of regression of the tails by the above substances was indeed produced under conditions in which the RNA and protein synthesis by the tissue had been suppressed was verified by

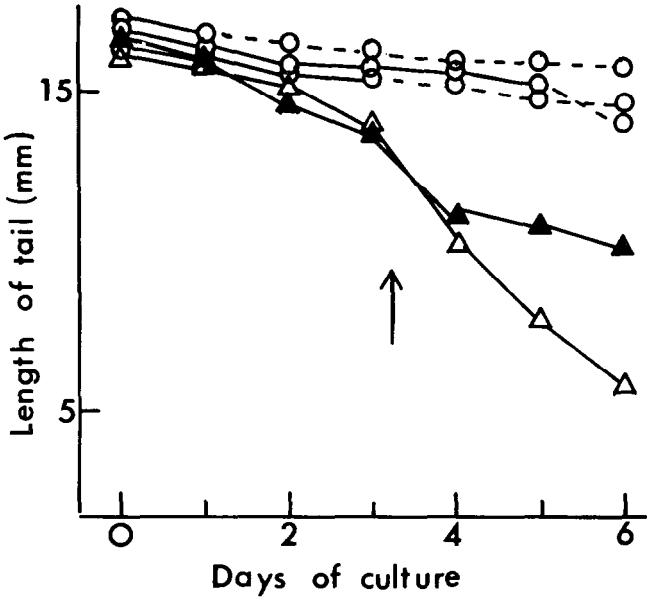

FIG. 3. Irreversibility of the inhibition by actinomycin D of T_3-induced regression of the isolated tadpole tail and the effectiveness of actinomycin D when added during the course of tissue resorption. ○——○, 1 μg T_3 and 5 μg actinomycin were present in the medium on day 0 of the culture; the tails were washed free of the medium after different periods of culture and the incubation was continued only with 1 μg T_3 per milliliter as shown by dashed lines. △——△, ▲——▲, culture begun in control medium and 1 μg T_3 added per milliliter after 1 day; △——△, no actinomycin D; ▲——▲, 5 μg actinomycin D added after 3 days (vertical arrow) and culture continued in the presence of 1 μg T_3 per milliliter.

measuring the effect of the inhibitors on the incorporation of tritiated uridine and C^{14}-labeled amino acids. As shown in Table 3, both actinomycin D and cycloheximide substantially inhibited the incorporation of H^3-uridine into RNA in the T_3-induced as well as uninduced tails at concentrations of the inhibitors which suppressed tissue regression. Similarly, puromycin and cycloheximide inhibited the incorporation of C^{14}-labeled amino acids into protein (Table 4). There was good correlation also between the degree of partial inhibition of regression and that of RNA and protein synthesis at lower levels of the inhibitors.

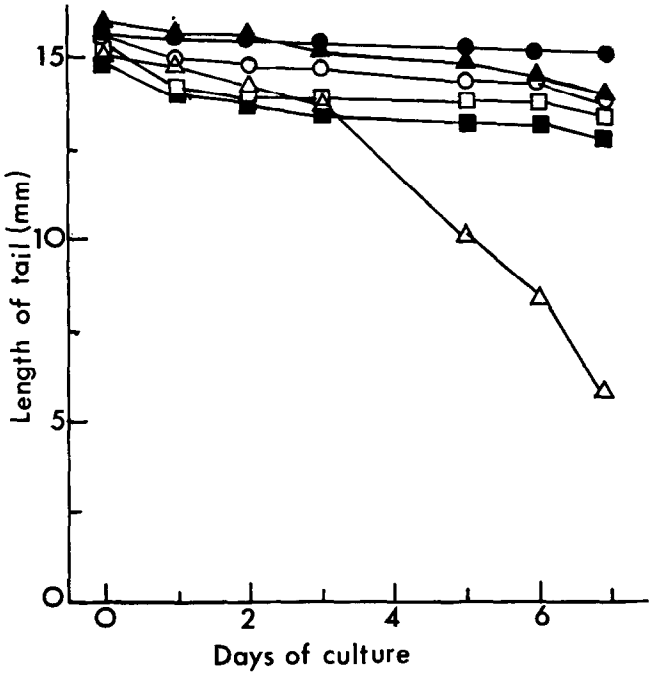

FIG. 4. Inhibition by puromycin and cycloheximide of T_3-induced regression of the isolated tadpole tail. The hormone and inhibitors were added 18 hours after culture was begun. ○——○, control; △——△, T_3, 0.8 μg/ml, puromycin, 80 μg/ml; ▲——▲, 0.8 μg T_3 and 80 μg puromycin per milliliter; □——□, cycloheximide, 40 μg/ml; ■——■, 0.8 μg T_3 and 40 μg cycloheximide per milliliter.

DISCUSSION

Isolated tails of the *Rana temporaria* tadpoles can be maintained in organ cultures for several days under conditions simpler than those used by Shaffer (1963) and Weber (1963) for *Xenopus* tadpole tails. The cultures are viable in the sense that regression can be induced *in vitro* by the addition of thyroid hormone to the culture medium and, furthermore, the rate of induced regression is nearly the same in isolated tails as in the intact tadpole (see Bennett and Frieden, 1962; Weber, 1963). Weber (1963), had shown that there was no qualitative differences in the properties of cathepsin in the freshly amputated *Xenopus* tail or in samples that had been cultured for 9 days. Our work has also shown that the isolated tail maintained in

organ cultures is amenable to experiments designed to study RNA and protein synthesis.

The activity of a number of hydrolytic enzymes in the regressing tadpole tail increases substantially both in spontaneous and in thyroid hormone-induced metamorphosis (Weber and Niehus, 1961; Bennett and Frieden, 1962; Frieden and Mathews, 1958; Weber, 1963; Coleman, 1962; Lapiere and Gross, 1963; Gross, 1964; Kubler and Frieden, 1964; Eeckhout, 1964; Domjan and Tata, unpublished). In the case of acid and alkaline phosphatases and ribonuclease (Weber, 1963; Eeckhout, 1964; Domjan and Tata, unpublished) there is no increase in total enzyme per tail whereas a net increase in total enzyme activity has been found for DNase, cathepsin, β-glucuronidase and collagenase (Weber, 1963; Kubler and Frieden, 1964; Lapiere and Gross, 1963; Gross, 1964). Although acid phosphatase has been localized in the *Xenopus* tail macrophages during metamorphosis (Salzmann and Weber, 1963), it is not certain whether the increase in various hydrolase activities is due to a selective activation, synthesis, or a protection against degradation of these enzymes. The question is further complicated by the finding that some enzymes, such as β-glucuronidase and acid phosphatase, may be distributed in both the particulate and nonparticulate fractions of the regressing tissue (Weber, 1963; Kubler and Frieden, 1964). From his examination of the enhanced cathepsin activity, accompanying induced regression of cultured *Xenopus* tails, Weber (1963) concluded that the increase in enzyme activity was due not to an activation of latent enzyme by the hormone but to a synthesis of additional enzyme or new macrophages in the tail during regression.

Thus, whereas the increase in activity of enzymes involved in tissue regression (whether caused by activation, synthesis, or selective protection), is well established, there has been no direct evidence about the capacity of the resting and regressing tadpole tail to synthesize protein. In the intact tadpole, induction of metamorphosis with thyroxine or triiodothyronine has been shown to lead to a marked stimulation of RNA and protein synthesis in the liver (Finamore and Frieden, 1961; Tatibana and Cohen, 1964; Tata, 1965). Marked alterations in the synthetic activity of very active tissues like the liver, and the variations in the pool sizes of metabolites due to regression of other tissues, e.g., the intestine and gills, would certainly complicate the study of protein synthesis in the whole animal of a metabolically

less active tissue like the tail. Our isotopic experiments in the organ cultures therefore conclusively demonstrate that the tail is capable of synthesizing RNA and protein. They also show that the synthesis of both constituents is elevated after the induction of regression. Lapiere and Gross (1963), when studying the thyroxine-induced breakdown of collagen in the tail of the intact tadpole, found that collagen continued to be synthesized at an undiminished rate in the face of increasing collagenolytic activity during induced resorption. He also found that the "old" collagen fibrils were attacked by the tail collagenase in preference to newly deposited protein.

It was not within the scope of our experiments to demonstrate the synthesis of specific protein molecules or reveal the site of their synthesis. Furthermore, the results of experiments involving the inhibitors of RNA and protein synthesis do not indicate the site of action of the hormone and ought to be interpreted with caution, especially in view of the multiple effects of the inhibitors. For example, actinomycin D which was initially considered to be a specific inhibitor of messenger RNA synthesis now appears to be an even more potent inhibitor of ribosomal RNA synthesis (see Reich and Goldberg, 1964; Perry, 1963). Similarly, puromycin and cycloheximide which are commonly used to suppress protein synthesis (Darken, 1964; Siegel and Sisler, 1964) are also potent inhibitors of nuclear RNA synthesis (Tamaoki and Mueller, 1963; Fiala and Davis, 1965). What can be deduced from our studies with these inhibitors is that an undisturbed and even accelerated generation of RNA and/or protein seems to be necessary for regression of the tail to occur. The results therefore support the idea of a synthesis of new hydrolytic enzyme molecules, perhaps with altered characteristics (Weber, 1963) in preference to an activation of latent lysosomal enzymes (see de Duve, 1963) during metamorphosis. It would be interesting to decide whether the formation of enzymes *de novo* necessitates the transcription of new genetic information or whether the tissue regression is triggered off by a mechanism at the level of translation of preformed messenger RNA. The latter phenomenon seems to initiate development in other systems, such as in the primary differentiation of the fertilized sea urchin egg (Hultin, 1964; Gross and Cousineau, 1964), the initiation of hemoglobin synthesis (Wilt, 1965), and the initiation of down feather growth (Bell *et al.*, 1965). In one of these systems (Bell *et al.*, 1965) and in the tadpole liver during metamorphosis (Tata, 1965),

structural elements of the protein synthesizing apparatus are known to undergo a reorganization, and it would be interesting to know whether similar intracellular structural changes accompany regression of the tail. Marked alterations in the mutual orientation of cells and extracellular components as regression proceeds have already been described for the tadpole tail, intestine, and neurons (Weber, 1964; Bonneville, 1963; Weiss and Rossetti, 1951).

The induction *in vitro* of regression complements the demonstration by Kaltenbach (1959) of a direct, and not a systemic, effect of thyroid hormone on the tail in the intact tadpole. Direct action of thyroid hormone on the development of limb buds, transformation of the epithelium and the conversion of visual pigments have also been described (see Bennett and Frieden, 1962; Wilt, 1959; Ohtsu *et al.*, 1964). The mechanism by which the same hormone initiates such varied developmental changes is not yet understood, but it is important to note that the RNA and protein synthesis are also enhanced in the tadpole liver in which thyroid hormone does not induce extensive synthesis of hydrolases but induces that of other proteins like the urea cycle enzymes and serum albumin (Finamore and Frieden, 1960; Tatibana and Cohen, 1964; Tata, 1965). The nature of proteins whose synthesis is induced or selectively accelerated and therefore the type of developmental change initiated, will be determined by the differences in genetically predetermined responses of different cells to the hormone. Organ cultures have already been shown to be useful in extending studies on the action of biologically active substances on whole animals (see Fell, 1964). The ease of inducing programmed cell death at a precise time and the analysis of biosynthetic functions of the system renders tadpole tail regression in organ culture a valuable tool in studying one facet of embryonic development.

SUMMARY

A simple method for maintaining the tail of *Rana temporaria* tadpoles for up to 8 days in organ culture has been described. Regression of the isolated tail was induced by the addition of triiodothyronine to the culture medium. The rate of regression and the increase in activity of some hydrolytic enzymes were comparable to those observed in the tails of intact tadpoles undergoing metamorphosis.

Cultured tails are capable of synthesizing RNA and protein as seen from the incorporation of H^3-labeled uridine and C^{14}-labeled amino

acids. Hormone-induced regression was accompanied by an accelerated synthesis of both RNA and protein. Actinomycin D, puromycin, and cycloheximide abolished regression and increase in hydrolase activity in the isolated tails induced by triiodothyronine. Under conditions in which regression was inhibited, actinomycin D and cycloheximide blocked the incorporation of H^3-uridine into RNA, and puromycin and cycloheximide that of C^{14}-amino acids into protein. A continuous generation of RNA and protein is associated with the hormone-induced regression of the isolated tadpole tail.

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