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A Study on Effect of T3, T4 In the Development andMetamorphosis of Rana Curtipes

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Abstract:

The basic hormonal regulation of amphibian metamorphosis was established by Gudernatch (1914). It has been well established that development and metamorphosis of amphibians are under the control of several hormones. Of these, thyroid hormone has long been known as thekey hormone inducing metamorphic changes in amphibians [Frieden and Just, 1970). AlthoughT4 is normally more abundant than T3 , it is generally accepted that the latter is responsible for most of the physiological actions of the thyroid hormones in mammals. According to Galton (1983) conversion of T4 to T3 is accelerated as metamorphosis proceeds, the latter being biologically more active than T4 . T3 binding sites in the target tissue are increased during metamorphosis. Leloup and Buscaglia (1977) recorded the importance of T3 during metamorphic climax.

Keywords: Rana Curtipes, hormone, T3, T4.

I. Introduction

Frieden and Just (1970) demonstrated that although in premetamorphic tadpoles T) and T4 are below the detectable level of RIA, they respond physiologically to exogenous T3 or Ttl' Adrenal steroids are known to potentiate the action of thyroid hormone by agumenting the nuclear binding capacity for thyroid hormone in the target tissue. When corticoids are given to tadpoles in early sta~es of development (premetamorphosis), metamorphosis is arrested. However when they are administered in advanced stages of development, metamorphosis is accelerated. Injections of glucocorticoids or ACTH, into prometamorphic or thyroxine treated premetamorphic tadpoles, accelerated the development and metamorphosis. Kaltenbach (1968) and, Dodd and Dodd (1976) have described adrenal steroids as regulatory factors in T) or T4 induced metamorphosis.

Experiments with Bufo bufo tadpoles showed that hydrocortisone, cortisone and glucocorticoids accelerate T4 induced metamorphosis. But deoxycorticosterone had no effect on the tail shortening of either Bufo or Bufo vulgaris tadpoles. Moreover high concentrations of deoxycorticosterone produced toxic effects too. Kobayashi (1958) observed that a combination of immersion and implantation techniques, with deoxycorticosterone promote T4 induced metamorphosis of Rana japonicus tadpoles. Hydrocortisone accetate, cortisone and deoxycorticosterone enhance T4 induced fin resorption in Rana pipiens tadpoles.

RIA for these corticoids have been performed in tadpoles of many amphibian species such as Rana catesbeiana, Xenopus laevis, Bufo japonicus and Amblystorna tigrinum. The results of these studies indicate that elevation of corticoid levels occurs in synchronization with the elevation of thyroid hormone levels. This condition of relatively high levels of circulating corticoids in metamorphosing tadpoles suggests that adrenal steroids physiologically participate in metamorphic events.

Although there are several reports as mentioned above, the evidence that exogenous corticosterone acts together with thyroid hormone to accelerate metamorphosis is scanty. Thisstudy was therefore designed to find out whether corticosterone affects metamorphosis in Rana curtipes tadpoles. Further, this study was also undertaken to investigate the difference in effectof T3 and T4 on the development and metamorphosis of Rana curtipes tadpoles, tropical species.

II. Materials and Methods

In this experiment 36 laboratory acclimated {29 + 2°c, 12:12 h L:D) tadpoles ranging from Stage IX to XI, with an average, tail length 42.3 mm and body weight 5 g were used. They were fed with boiled spinach.

Chemicals

Triiodo-L-thyronine (T3), L-thyroxine and corticosterone were procured from Sigma Chemical C0., USA and all other chemicals used were of analytical grade. T3 and T4 were dissolved in minimum quantity of 0.01 N NaOH, diluted with distilled water, neutralised with an equal volume of 0.01 N HCl. These were added to the aquaria to get a final concentration of 50 ng T3 or T4 per ml. Corticosterone was dissolved in ethanol and added to aquaria to produce a final concentration of l.04p g/ml. The final concentration of ethanol added to the aquaria as solvent for corticosterone was 0.01%.

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Experimental design

The thirty six tadpoles were divided into six groups of six each and received the following treatment.

Group I Tadpoles were kept in pond water containing hormone vehicles and served as control.Group II Treated with corticosterone

Group III Treated with T4

Group IV Treated with T4 + corticosteroneGroup V
Treated with T3

Group VI T3 and corticosterone treatment

Group III and IV were exposed to T4 and Groups V and VI were exposed to T3 for 24 hourson day O (zero) and 8th day. They were again exposed for 48 hours from day 20 through 22. The corticosterone of Group II, IV and VI were replenished on every second day. The tadpolesin all groups were fed boiled spinach until the T3 or T4 alone treated animals stopped feeding.Changes in tail length and body weight were recorded in all tadpoles during the period of theexperiment at regular intervals.

The two 24 hours exposures with thyroid hormones on zero day and 8th day were done to produce a low level of thyroid hormones, similar to prometamorphic stages, so that any acceleration by corticosterone is apparent. Similarly the 48 hours of treatment with thyroid hormones was done to produce high T3 and T4 levels in tadpoles similar to that in normal metamorphic climax stages.Statistical analysis of data was done by one way classification of ANOVA [Snedcor and Cohran, 1967].

III. Results and Discussion

In the absence of thyroid hormone, corticosterone had no effect on tail regression during metamorphosis of Rana curtipes. Corticosterone did not show any significant effect, either in the individual or combined status on body weight change.

In Rana curtipes too, T3 is more potential than T during metamorphosis. The tadpoles which did not receive T3 or T4 (control or corticosterone alone treatment) showed no effect on tail regression. The group treated with corticosterone alone had no difference in its action with control upto 20th day, after which it showed slight difference. The T4 treated tadpoles (GroupIII) had slight tail resorption which became significant (1.2%) by 14th day. By 24th day T4 caused about 24.5% resorption of the tail. The Group IV rT4 and corticosterone treatment) tadpoles showed significant tail resorption three days before the T4 alone treated groups (Group III). A 30.8% resorption of tail length was observed by day 24. Group V stopped feeding on 14th day and tadpoles exposed to T3 alone displayed a marked tail · h t d This group had 1% tail reduction then T4 treae groups. reduction on 10th day of experiment and 7.9% reduction on 14th day compared to 1.2% of T4 treated group (IV). It showed a 5% significant differenceover control on 8th day itself. The data indicate that T3 tiggered tail resorption 2 to 3 days earlier than T4 in Rana curtipes tadpoles. A 30.1% reduction in tail length was noted by day 24.

Table 1: Percentage change in tail length following treatment with 13,14 andcorticosterone

Days of measure-ment	Control (M_1)	Corticosterone (M_2)	T_4 (M_3)	T_4 and corticosterone (M_4)	T_3 (M_5)	T_3 and corticosterone (M_6)
0	0	0	0	0	0	0
2	Nil	Nil	Nil	Nil	Nil	Nil
4	0.40 ± 0.06	0.30 ± 0.08	0.30 ± 0.07	0.40 ± 0.09	0.4 ± 0.06	0.30 ± 0.07
6	0.70 ± 0.13	0.61 ± 0.06	0.70 ± 0.10	0.60 ± 0.14	0.53 ± 0.13	Decrease 1.20 ± 0.46
8	0.71 ± 0.12	0.80 ± 0.12	0.80 ± 0.14	0.49 ± 0.14	0.30 ± 0.10	3.20 ± 0.75
10	0.72 ± 0.14	0.92 ± 0.16	0.60 ± 0.14	0.34 ± 0.09	Decrease 1.00 ± 0.20	5.60 ± 1.45
12	0.78 ± 0.22	1.10 ± 0.21	0.50 ± 0.12	Decrease 3.10 ± 0.47	3.00 ± 0.46	9.20 ± 1.30
14	1.06 ± 0.25	0.94 ± 0.14	Decrease 1.20 ± 0.23	8.20 ± 1.50	7.90 ± 1.30	15.60 ± 1.80
16	1.30 ± 0.22	Decrease 0.10 ± 0.03	3.20 ± 0.38	13.00 ± 1.81	12.50 ± 1.62	20.30 ± 2.4
18	0.70 ± 0.17	0.18 ± 0.09	7.50 ± 1.35	18.90 ± 1.67	16.10 ± 1.33	25.50 ± 2.56
20	Decrease 1.20 ± 0.29	3.20 ± 0.28	10.60 ± 1.69	23.90 ± 2.30	22.30 ± 1.54	30.20 ± 2.15
22	2.00 ± 0.57	3.80 ± 0.74	17.30 ± 1.32	27.90 ± 1.83	25.40 ± 1.52	33.60 ± 1.87
24	2.40 ± 0.44	5.40 ± 0.47	22.48 ± 1.60	30.80 ± 1.93	30.10 ± 2.08	38.40 ± 1.40

The extent of tail resorption is expressed as the percentage change from the initial length

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T3 and corticosterone in the combined status regressed the tail length rapidly and drastically than any other group. Significant change over control was shown 6 days after the experiment began. By day 24, this category had 38.4% decrease in tail length which is 21.6% more than the effect of T4 corticosterone treatment.

Table 2: ANOVA table for comparing different groups of tadpoles at different stages

Stages: Days of measurements	Source of variation	df	SS	MSS	F	'p' value 0.05	'p' value 0.01
4	Between treatments	5	0.08	0.02			
	Error	30	0.18	0.06	2.82	>F0.05	< F0.01
	Total	35	0.26				
6	Between treatments	5	16.86	3.37			
	Error	30	1.36	0.05	74.12	>F0.05	> F0.01
	Total	35	18.22				
8	Between treatments	5	74.11	14.82			
	Error	30	3.17	0.10	140.06	>F0.05	> F0.01
	Total	35	77.28				
10	Between treatments	5	189.04	37.8			
	Error	30	11.15	0.37	101.69	>F0.05	> F0.01
	Total	35	200.19				
12	Between treatments	5	462.16	92.43			
	Error	30	10.69	0.36	259.36	>F0.05	> F0.01
	Total	35	472.85				
14	Between treatments	5	1303.93	260.78			
	Error	30	37.22	1.24	210.15	>F0.05	> F0.01
	Total	35	1341.15				
16	Between treatments	5	2210.84	442.17			
	Error	30	59.96	1.99	221.24	>F0.05	> F0.01
	Total	35	2270.80				
18	Between treatments	5	3272.19	654.44			
	Error	30	84.38	2.81	232.67	>F0.05	> F0.01
	Total	35	3356.57				
20	Between treatments	5	4339.81	867.96			
	Error	30	81.03	2.70	321.32	>F0.05	> F0.01
	Total	35	4420.84				
22	Between treatments	5	5121.56	1024.31			
	Error	30	59.06	1.96	520.30	>F0.05	> F0.01
	Total	35	5180.62				
24	Between treatments	5	6425.95	1285.19			
	Error	30	66.17	2.21	582.71	>F0.05	> F0.01
	Total	35	6492.12				

F5, 35 at 5% = 2.53
F5, 35 at 1% = 3.70

In T4 treated tadpoles, tails shortened after a latent period of 12 days. This lag period was reduced to 10 days in T4 + corticosterone group. Similarly the T3 corticosterone combination treatment had reduced the latent period to six days from eight days of T3 alone exposed group. The change in body weight of Rana curtipes during the present study fell into three catesories. The first category, in which the control and corticosterone alone treated tadpoles belonged, showed no significant difference between them. This indicated that corticosterone alone had no effect on body weight loss.

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Table 3: Comparison between different groups of tadpoles at different stages by using(CD Method)

Pair of treatment compared	Absolute difference between groups of tadpoles on day										
	4	6	8	10	12	14	16	18	20	22	24
M_1 Vs M_2	0.08^{ns}	0.09^{ns}	0.09^{ns}	0.19^{ns}	0.32^{ns}	0.12^{ns}	1.40^{ns}	0.88^{ns}	2.27*	1.80*	1.80*
M_1 Vs M_3	0.09^{ns}	0.003^{ns}	0.09^{ns}	0.12^{ns}	0.37^{ns}	2.26**	4.50**	8.20**	9.73**	15.30**	20.08**
M_1 Vs M_4	0^{ns}	0.09^{ns}	0.22^{ns}	0.38^{ns}	3.88**	9.26**	14.30**	19.57**	23.03**	25.90**	28.40**
M_1 Vs M_5	0^{ns}	0.17^{ns}	0.41*	1.72**	3.78**	8.96**	13.80**	16.80**	21.43**	23.40**	27.70**
M_1 Vs M_6	0.08^{ns}	1.90**	3.91**	6.32**	9.98**	16.66**	21.60**	25.53**	29.33**	31.60**	36.00**
M_3 Vs M_4	0.07^{ns}	0.10^{ns}	0.31^{ns}	0.26^{ns}	3.51**	7.00**	9.80**	11.37**	13.3**	10.60**	8.32**
M_3 Vs M_5	0.09^{ns}	0.17^{ns}	0.50*	1.60**	3.41**	6.70**	9.30**	8.60**	11.7**	8.10**	7.62**
M_5 Vs M_6	0.08^{ns}	1.73**	3.50**	4.60**	6.20**	7.70**	7.79**	8.73**	7.89**	8.19**	8.29**
CD at 5%	0.10	0.25	0.38	0.72	0.70	1.31	1.66	1.98	1.94	1.65	1.75
CD at 1%	0.18	0.34	0.52	0.97	0.95	1.77	2.24	2.66	2.61	2.22	2.36

* Significance at 5% level
** Significance at 1% level
ns Not significant

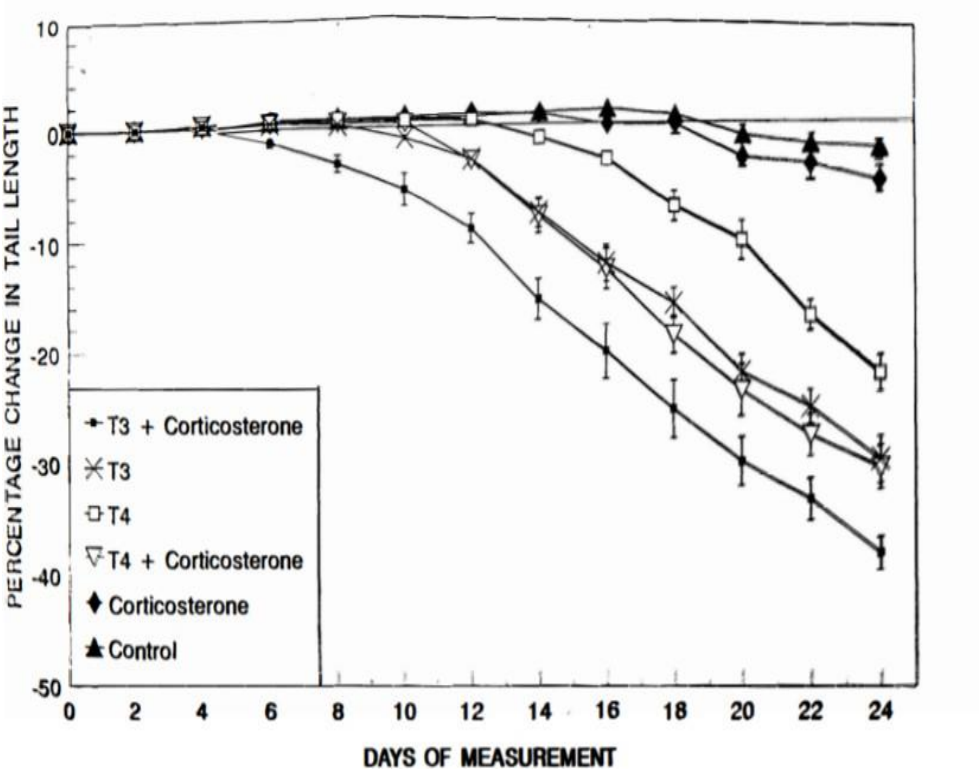

Figure 1: Percentage change in tail length of Rana curtipes tadpoles following treatmentwith T3" T4 and corticosterone. The bars indicate standard deviation

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Stages: Days of measurements	Source of variation	df	SS	MSS	F	'p' value 0.05	'p' value 0.01
4	Between treatments	5	63.59	12.72	78.23	> F0.05	> F0.01
	Error	30	4.88	0.16			
	Total	35	68.47				
6	Between treatments	5	327.06	65.41	146.39	> F0.05	> F0.01
	Error	30	13.40	0.45			
	Total	35	340.46				
8	Between treatments	5	681.45	136.29	120.46	> F0.05	> F0.01
	Error	30	33.94	1.13			
	Total	35	715.39				
10	Between treatments	5	1141.41	288.28	115.99	> F0.05	> F0.01
	Error	30	59.05	1.97			
	Total	35	1200.46				
12	Between treatments	5	1541.60	308.32	170.65	> F0.05	> F0.01
	Error	30	54.20	1.81			
	Total	35	1595.80				
14	Between treatments	5	1769.68	353.94	166.73	> F0.05	> F0.01
	Error	30	63.68	2.12			
	Total	35	1833.36				
16	Between treatments	5	1891.56	378.31	205.15	> F0.05	> F0.01
	Error	30	55.32	1.88			
	Total	35	1446.88				

Table 4: Percentage change in body weight following treatment with T3, T4 andcorticosterone

Days of measure-ment	Control (M_1)	Corticosterone (M_2)	T_4 (M_3)	T_4 and corticosterone (M_4)	T_3 (M_5)	T_3 and corticosterone (M_6)
0	0	0	0	0	0	0
2	Nil	Nil	Nil	Nil	Nil	Nil
4	1.00 ± 0.20	0.60 ± 0.14	Decrease 0.60 ± 0.15	Decrease 0.60 ± 0.14	Decrease 2.40 ± 0.64	Decrease 2.50 ± 0.68
6	1.50 ± 0.23	1.30 ± 0.22	1.30 ± 0.29	1.50 ± 0.22	5.80 ± 0.92	6.00 ± 1.18
8	1.30 ± 0.26	1.20 ± 0.20	4.90 ± 0.95	6.00 ± 0.87	8.20 ± 1.70	10.10 ± 1.51
10	0.50 ± 0.12	0.68 ± 0.13	7.50 ± 1.93	9.80 ± 1.72	12.10 ± 1.55	13.50 ± 1.65
12	*Decrease 0.92 ± 0.16*	*Decrease 0.71 ± 0.13*	*10.10 ± 1.46*	*11.60 ± 1.67*	15.50 ± 1.28	17.60 ± 2.10
14	*2.00 ± 0.6*	*1.80 ± 0.17*	*12.20 ± 1.08*	12.30 ± 1.26	17.30 ± 1.58	20.30 ± 2.66
16	*3.20 ± 0.4*	*2.40 ± 0.44*	*13.50 ± 0.96*	14.50 ± 1.74	19.30 ± 1.81	21.90 ± 1.86
18	3.60 ± 0.64	2.60 ± 0.77	14.80 ± 1.97	17.60 ± 1.54	21.00 ± 1.79	24.30 ± 1.64
20	4.90 ± 0.97	3.40 ± 0.96	18.40 ± 1.45	19.70 ± 1.46	23.00 ± 1.99	26.40 ± 2.01
22	5.20 ± 0.75	4.60 ± 0.51	20.20 ± 1.43	21.00 ± 1.58	25.20 ± 1.78	27.00 ± 2.04
24	5.80 ± 0.92	4.80 ± 0.63	20.80 ± 2.20	23.20 ± 2.28	28.10 ± 1.2	27.30 ± 1.33

The extent of body weight change is expressed as the percentage change from the initial weight

Table 5: ANOVA table for comparing different groups of tadpoles at different stages

18	Between treatments	5	2446.85	489.37	220.71	> F0.05	> F0.01
	Error	30	66.51	2.21			
	Total	35	2513.36				
20	Between treatments	5	2751.44	550.29	262.79	> F0.05	> F0.01
	Error	30	62.94	2.09			
	Total	35	2814.38				
22	Between treatments	5	2921.87	584.38	292.88	> F0.05	> F0.01
	Error	30	59.86	1.99			
	Total	35	2981.74				
24	Between treatments	5	3073.56	614.71	340.20	> F0.05	> F0.01
	Error	30	54.20	1.81			
	Total	35	3127.76				

F5, 35 at 5% = 2.53
F5, 35 at 1% = 3.70

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Table 6: Comparison between different groups of tadpoles at different stages by using(CD Method) g analysis or variance

Pair of treatment compared	Absolute difference between groups of tadpoles on day										
	4	6	8	10	12	14	16	18	20	22	24
M_1 Vs M_2	0.40^{ns}	0.19^{ns}	0.10^{ns}	0.27^{ns}	0.21^{ns}	0.20^{ns}	0.50^{ns}	1.00^{ns}	1.50^{ns}	0.60^{ns}	1.50^{ns}
M_1 Vs M_3	1.60**	2.60**	6.20**	7.91**	9.17**	10.20**	10.30**	11.20**	13.50**	15.02**	13.58**
M_1 Vs M_4	1.60**	3.00**	7.30**	10.21**	10.68**	10.30**	11.30**	14.00**	14.80**	15.88**	16.00**
M_1 Vs M_5	3.40**	7.30**	9.43**	12.51**	14.58**	15.30**	15.10**	17.48**	18.10**	20.00**	20.90**
M_1 Vs M_6	3.47**	7.50**	11.40**	13.91**	60.66**	18.30**	18.70**	20.57**	21.50**	21.80**	20.10**
M_3 Vs M_4	0^{ns}	0.34^{ns}	1.10^{ns}	1.10^{ns}	1.20^{ns}	0.10^{ns}	1.00^{ns}	1.48^{ns}	1.30^{ns}	0.87^{ns}	1.50^{ns}
M_3 Vs M_5	1.80**	4.64**	3.23**	4.60**	5.40**	5.10**	4.80**	6.28**	4.60**	4.98**	7.32**
M_5 Vs M_6	0.07^{ns}	0.20^{ns}	1.07^{ns}	1.04^{ns}	1.18^{ns}	1.30^{ns}	1.36^{ns}	1.41^{ns}	1.34^{ns}	0.94^{ns}	0.79^{ns}
CD at 5%	0.48	0.79	1.12	1.14	1.33	1.62	1.43	1.54	1.71	1.66	1.52
CD at 1%	0.64	1.06	1.51	1.55	1.79	2.18	1.92	2.07	2.30	2.24	2.13

* Significance at 5% level
** Significance at 1% level
ns Not significant

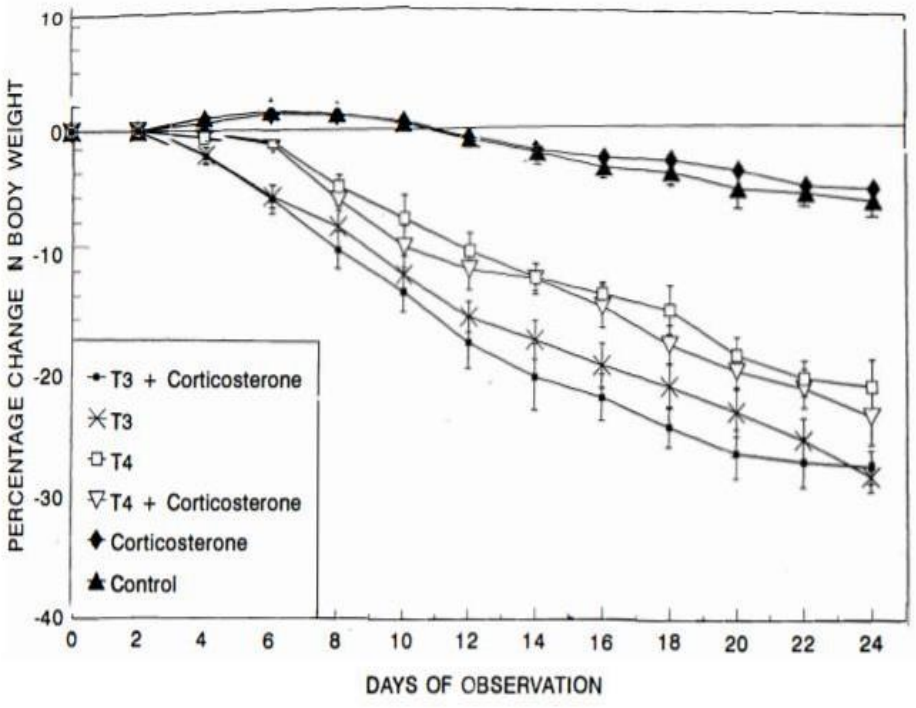

Figure 2: Percentage change in body weight of Rana curtipes tadpoles following treatment with T3, T4 and corticosterone. The bars indicate standared deviation

However, it is surprising to note that, corticosterone alone treated tadpoles experienced a slight gain in body weight by four to six days after the initiation of treatment. The T4 treated group (III & IV) included in the second category showed no statistically significant difference between them. While T4 alone exposed group

showed 20.8 + 2.2% decrease, T4 and corticosterone combined treatment expressed only 23.2 + 2.28% decrease by 24th day. Similarly the T3 exposed groups (V & VI) were found to be identical category. Since there too, no significant difference occurred between them.

Corticosterone even in combined status with either T3 or T4 did not accelerate the body weight loss in Rana curtipes. But statistically significant difference was noted between T3 and T4 , both in the regression of tail length and body weight loss. T3 was found more active than T4 , which is consistant with several earlier observations.

IV. Conclusion

In the present study corticosterone exhibits a prominent effect on the tail resorption of Rana curtipes tadpoles maintained in a sub-threshold concentration of T4 and T3. However, corticosterone does not effect shrinkage of tail in the absence of T3 or T4. This confirms the corticosterone induced augmentation of thyroid hormone effect in amphibian metamorphosis, for the first time in a tropical species, Rana curtipes. Present results indicate that neither the control tails nor those exposed to corticosterone undergoes appreciable shortening.

In tadpoles, treated with T4 , the tail shorten after a latent period of 12 days. This latent period is shorter in groups that is treated with T4 and corticosterone. Likewise T3 corticosterone combination also reduces the lag period from 8 to 6 days in this study.

These observations agree with the earlier reports and confirm the role of these hormones in a tropical species for the first time. Frieden and Naile (1955) observed that cortisol, cortisone and cortisone acetate caused augmentation of thyroid hormone induced tail reduction in Bufo bufo tadpoles. Deoxycorticosterone acetate synergise with thyroxine in Xenopus laevis. Aldosterone is more effective than corticosterone and cortisol is less potent than both corticosterone and aldosterone in augmenting thyroid hormones during metamorphosis. Hartman and Platt (1984) reported that corticosterone accelerates thyroid hormone induced tissue regression in the tail fin of Tiger salamanders.

Carr and Norris (1988) noted an increase in plasma corticosterone level during metamorphosis in Ambystoma tigrinum and an augmention in nuclear binding capacity for T3 with corticoids in Rana catesbeiana was reported by Suzuki and Kikuyama (1983). The increase in urea excretion was found to be strongly correlated with tail regres. Medda and Frieden (1970) noted that ACTH increases urea excretion with thyroid hormones. Other investigators have also· reported similar effects.

The above reports and the present result, that corticosterone augments T3, T4 induced tail regression are consistant with the observations of Kobayashi (1958) and Carr and Norris (1988) that corticoids synergize with the destructive actions of thyroid hormones, including shortening of the trunk, resorption of the opercular integumental resorption. Total body dehydration has long been known to occur in anuran tadpoles as they undergo metamorphosis [Etkin, 1932]. Just et al. (1977) examined the plasma osmotic pressure of Rana catesbeiana tadpoles and found that there is an 8% increase in osmotic pressure during metamorphosis. This increase is largely accounted for by increase in sodium, potassium and bicarbonates. Corticosterone, cortisol and deoxycorticosterone have sodium preserving and consequently water conserving effect in the tadpoles kidney [Kobayashi, 1958].

The augmenting effect of corticosterone on T3 or T4 induced tail resorption and the lack of corticosterone influence on weight loss in Rana curtipes tadpoles are consistant with the observation by Kobayashi (1958) that corticosterone synergizes with only the catabolic actions of thyroid hormones.

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