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THE EFFECT OF AUREOMYCIN ON THE ORGAN WEIGHTS OF THE GROWING TADPOLES[1]

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ABSTRACT

Two groups of thyroidectomized tadpoles of *Rana catesbeiana* were reared in chlortetracycline HCl medium and tap water respectively. After 34 weeks of treatment, the wet and dry weights of the liver, fat bodies, kidney, ovary, GI tract and tail as well as the whole tadpole were compared between the 2 groups. Critical appraisal of absolute weights showed that growth of somatic organs was depressed, some remarkably and others less so; while the ovaries were not affected by aureomycin. Study on percentage weight also indicated a differential effect of the antibiotic on organs. The effect was evident again, when studied by the regression of organ weights on body weights, showing regression coefficients in most organs significantly altered after aureomycin treatment. The implications of these results are discussed.

It was reported previously that aureomycin-treated tadpoles suffered a decreased growth rate both in body weight and body size and that they took less food than the controls (1, 2). It was suggested then that the digestive system and other visceral organs might be hampered by the treatment. Therefore a critical investigation of organ weights of control and auremycin-treated tadpoles was made in the present study to ascertain whether any organs were affected specifically.

MATERIALS AND METHODS

The materials were thyroidectomized tadpoles of previous experiment from a single pair of *Rana catesbeiana* (2). After 34 weeks of experimentation, 20 tadpoles taken randomly from each of the control and aureomycin-treated groups were killed with ether. The tadpoles were weighed individually with a torsion balance to the hundredth of a gram. They were then dissected. The liver, kidneys, ovaries, fat bodies and GI tract (from the oesophagus to the end of the rectum) of each tadpole were weighed with an analytical balance to a sensitivity of 0.2 mg while the tail weight was taken by the torsion balance. The organs after weighing and the rest of the carcases were dried to constant weight in an oven at 110 C for 2 to 3 days. They were then weighed by a microtorsion balance (Vereenigde Draadfabrieken Nijmegen, Holland) with a sensitivity of 0.05 mg except the tails and the rest of the carcases, these latter by the analytical balance. Ten other tadpoles in each group were sacrificed for microscopic study to be reported elsewhere.

RESULTS

It is of interest to note that all 60 tadpoles, whether control or aureomycin-

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treated, were females with ovaries showing auxocytes at the time of sacrifice when they were 12.5 months old. Critical appraisal of the organ weights of these female tadpoles are reported under the following sections.

I. The weights of the organs:

The average weights of the 20 control and 20 aureomycin-treated tadpoles and their organs are given in TABLE I.

TABLE I shows that the absolute weights, both wet and dry, of the tadpoles and their organs except the ovaries were decreased by aureomycin. The decrease of organ weight was greatest in the fat bodies amounting to 76.41% and 77.04% of the wet and dry control weights respectively, next in the liver with 65.63% and 67.11% reduction and then in the kidney with 55.73% and 44.00% reduction. The GI tract and tail showed the least reduction, amounting to approximately 40% in each case which was similar to that of the treated whole tadpole in terms of the wet weight.

Of the 6 organs studied the ovary was most outstanding in that it was the only organ which responded indifferently to aureomycin. The differences both of wet and dry weights between control and experimental groups were 3.31 ± 3.51 mg and 0.18 ± 0.53 mg respectively which were insignificant statistically. Hence aureomycin did not exert any significant effect on the reproductive organ.

In order to find out whether or not the ratio of the organ weight to the body weight of the treated tadpoles was similar to that of the controls, wet and dry percentage weights of the 6 organs of the 2 groups are also given in TABLE I. It is clear that in both groups the tail was the largest organ among the 6, amounting to more than 40% of the whole tadpole, while the percentage weights of the other 5 organs were small, ranging from less than 1% for the ovary to about 10% for the GI tract.

The effect of aureomycin on the percentage wet weight of the organs was not the same but manifested in 3 different ways: a decrease was noted in the fat bodies, liver and kidney; an augmented effect in the ovary; and the GI tract and tail remained practically unchanged. However, in regard to percentage dry weight the fat bodies and liver remained supressed and the ovary augmented but the kidney, GI tract and tail showed an increase in percentage weight by aureomycin treatment.

Therefore the data in TABLE I show a differential effect of aureomycin on tadpole organs in that by absolute weight, some were markedly reduced in weight and others less so while the reproductive organ, the ovary, was not affected; and by percentage weight, the aureomycin effect was also not even on the 6 organs.

II. The water content of the organs:

The water content of the 6 organs as indicated in TABLE I ranged from 70% to 90% approximately. However, that of the fat bodies was only about 20%. The differences of water content between control and aureomycin-treated organs were not significant at the level of 1% except the kidney and tail. In the former organ after aureomycin treatment the tissues contained 5% less water than the control. Although the treated tail showed a significantly increased water content by 1%, the difference was probably too small for practical consideration when normal water content of the organ was 87.74%.

The 3% difference of water content of the treated over control tadpoles as a whole was also significant. However, the value might not be entirely free from experimental error because the dry weight of the tadpoles was not measured directly but derived from the sum of all the dry weights of the 6 organs and the rest of the carcasses. This might have resulted in loss of blood during dissection and manipulation. It would thus seem that the only organ which was affected by aureomycin in its water content was the kidney.

III. The regression of organ weight on body weight:

The 2 groups of tadpoles were again studied by plotting the organ weights

TABLE I

Absolute and percentage weights of the 6 organs of control and aureomycin-treated tadpoles and their water contents

		Whole tadpole	Fat bodies	Liver	Kidney	Tail	GI tract	Ovaries	Rest of carcase
WET WEIGHT									
Absolute									
gm±SE	C	19.84±0.75	0.7199±0.0489	0.9416±0.0641	0.1541±0.0077	7.42±0.26	1.6667±0.0963	0.0316±0.0016	—
	A	10.99±0.82	0.1698±0.0310	0.3236±0.0355	0.0682±0.0066	4.33±0.34	0.9791±0.0532	0.0283±0.0031	—
Difference	gm±SE	8.85±1.11	0.5501±0.0579	0.6180±0.0733	0.0859±0.0101	3.19±0.43	0.6876±0.1100	0.0033±0.0035	
	%	44.60	76.42	65.63	55.73	42.98	41.25	10.48	
P<0.01		Yes	Yes	Yes	Yes	Yes	Yes	No	
Relative									
%±SE	C	100	3.56±0.13	4.65±0.60	0.77±0.02	37.52±0.46	8.33±0.25	0.16±0.01	—
	A	100	1.29±0.19	2.77±0.14	0.60±0.02	39.08±0.85	9.32±0.39	0.26±0.02	—
Difference±SE			2.27±0.23	1.88±0.62	0.17±0.03	−1.56±0.97	−0.99±0.46	−0.10±0.02	
P<0.01			Yes	Yes	Yes	No	No	Yes	
DRY WEIGHT									
Absolute									
mg±SE	C	2854.38±132.89*	590.48±42.52	184.78±14.69	30.45±1.45	910.40±34.87	164.20±10.79	6.90±0.30	967.18
	A	1319.65±139.23*	135.57±24.77	60.65± 7.10	17.05±1.59	503.63±51.47	105.45± 9.49	7.08±0.44	482.85
Difference	mg±SE	1539.72±192.47	454.90±49.21	123.13±16.32	13.40±2.15	406.77±62.17	58.75±14.37	−0.18±0.53	484.33
	%	53.84	77.04	67.18	44.00	44.64	35.78	2.61	50.08
P<0.01		Yes	Yes	Yes	Yes	Yes	Yes	No	
Relative									
%±SE	C	100	20.55±0.51	6.30±0.25	1.08±0.05	32.13±0.50	5.72±0.23	0.26±0.02	
	A	100	8.18±1.32	4.53±0.13	1.39±0.07	38.29±0.76	9.00±0.75	0.66±0.06	
Difference±SE			12.37±1.42	1.77±0.28	−0.31±0.09	−6.16±0.91	−3.28±0.78	−0.40±0.06	
P<0.01			Yes	Yes	Yes	Yes	Yes	Yes	
WATER CONTENT									
%±SE	C	85.66±0.22	18.54±1.10	80.58±0.43	79.95±0.62	87.74±0.16	90.21±0.06	77.47±1.10	
	A	88.61±0.50	20.32±1.73	81.57±0.36	74.96±0.67	88.92±0.37	89.47±0.64	72.10±0.96	
Difference±SE		−2.95±0.55	−1.78±2.05	−0.99±0.56	4.99±0.91	−1.18±0.40	0.74±0.64	5.37±2.25	
P<0.01		Yes	No	No	Yes	Yes	No	No	

C=Control tadpoles. A=Aureomycin-treated tadpoles. SE=Standard error. *=Sum of all the organ weights and the carcase weight.

against the body weights and thus the regression equations were calculated and lines drawn as shown in TABLE II and *Figs. 1–3* (only some of the typical ones are given).

TABLE II

The regression of organ weights on body weights of control and aureomycin-treated tadpoles

Organ			r	Regression equation	b	Difference of b between C & A	P (t-test)
Fat bodies	WET	C	0.83	Y=0.05316×−335.1	0.05316	0.01742	=0.05
		A	0.67	Y=0.03574×−223.0	0.03574		
	DRY	C	0.96	Y=0.30781×−288.11	0.30781	0.13012	<0.01
		A	0.96	Y=0.17769×−95.66	0.17769		
Liver	WET	C	0.87	Y=0.07437×−534.1	0.07437	0.03331	<0.01
		A	0.95	Y=0.04106×−127.1	0.04106		
	DRY	C	0.94	Y=0.10429×−112.91	0.10429	0.05225	<0.01
		A	0.97	Y=0.05204×−7.30	0.05204		
Kidney	WET	C	0.84	Y=0.00870×−18.6	0.00870	0.00163	>0.3
		A	0.89	Y=0.00707×−9.5	0.00707		
	DRY	C	0.63	Y=0.00676×+11.12	0.00676	−0.00382	>0.1
		A	0.93	Y=0.01058×+3.09	0.01058		
Tail	WET	C	0.93	Y=0.33 ×+910	0.33	−0.09	=0.02
		A	0.96	Y=0.42 ×−330	0.42		
	DRY	C	0.96	Y=0.253×+186.8	0.253	−0.110	<0.01
		A	0.98	Y=0.363×+25.2	0.363		
GI tract	WET	C	0.85	Y=0.11 ×−560	0.11	0.05	<0.01
		A	0.85	Y=0.06 ×−350	0.06		
	DRY	C	0.77	Y=0.063×−16.8	0.063	0.007	>0.5
		A	0.79	Y=0.056×−32.0	0.056		
Ovaries	WET	C	0.60	Y=0.00112×+9.7	0.00112	−0.00113	>0.1
		A	0.68	Y=0.00225×+3.5	0.00225		
	DRY	C	−0.09	Y=−0.00023×+7.46	−0.00023	−0.00199	<0.01
		A	0.60	Y= 0.00176×+4.76	0.00176		

C=Control tadpoles. A=Aureomycin-treated tadpoles. r=Correlation coefficient. b=Regression coefficient.

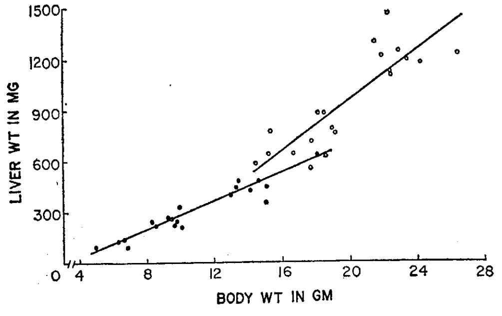

Fig. 1. Regression of wet liver weight on body weight. Circles and dots stand for control and aureomycin-treated tadpoles respectively.

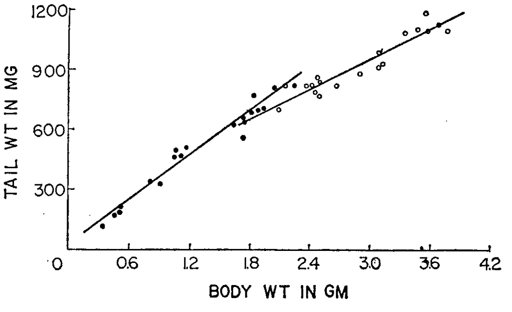

Fig. 2. Regression of dry tail weight on body weight. Circles and dots stand for control and aureomycin-treated tadpoles respectively.

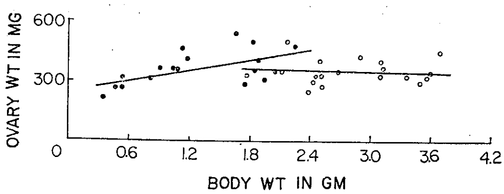

Fig. 3. Regression of dry ovary weight on body weight. Circles and dots stand for control and aureomycin-treated tadpoles respectively.

TABLE II and the scatter diagrams show that the correlation between organ and body weights was positive in all cases except the dry weight of the ovaries of the control group and that the degree of intensity of association was high with correlation coefficients well over 0.8 in most organs except the ovaries where it was about 0.6. The results indicate generally a very close relation between the organ weight and body weight of both control and experimental tadpoles.

The diagrams also depict a clear segregation between the control and experimental dots in that the former group tends to lie in the upper right quadrant indicating larger tadpoles with heavier organ weights; while the latter in the lower left quadrant, representing smaller tadpoles with lighter organ weights. This was the result of aureomycin treatment.

TABLE II shows that the regression coefficients, the increase or decrease of the organ weight for each unit change of of the tadpole body weight, of the 6 control organs were different with each other and may be specific too. The TABLE also indicates a significant alteration of regression coefficients in most organs after aureomycin treatment.

The treated liver had a significantly smaller regression coefficient at 1% level than the control organ in terms of wet and dry weights and so were the fat bodies. In case of the ovary, the regression coefficient was increased by aureomycin treatment although not significantly in wet state but very significantly in dry state from a negative to a positive value. The effect of aurecmycin on regression of the kidney weight was dubious for t-test showed an insignificant difference between the 2 groups. However it appeared that the coefficient of control kidney in wet weight surpassed that of the experimentals and *vice versa* in dry state. The comparison of regression coefficient between control and aureomycin-treated tails both in wet and dry weights showed an appreciable difference in that the experimental tails were augmented over the controls significantly.

The results judged from the regression study (TABLE II) agree closely with those from the analysis of the percentage weight (TABLE I) in the liver, fat bodies, ovaries, kidneys and tail. However, they give opposite results in case of the GI tract. Since the regression analysis is more reliable than the percentage study, the growth of the GI tract is considered significantly suppressed in wet weight but indifferent in dry weight by aureomycin treatment.

DISCUSSION

Sanfilippo, Mustakallio *et al.* and Hsü *et al.* showed that aureomycin treatment in frog tadpoles inhibited metamorphosis (3–5). Thus visceral organs such as the intestine could not be critically compared between control and treated tadpoles either in length or in microscopic structure at the end of the experiment. However, since metamorphosis was prevented by thyroidectomy in this experiment, all tadpoles in the 2 groups remained at stage IV (6) during the experimental period of 34 weeks. This made it possible to compare internal organs between the 2 groups under identical developmental stage.

The results as shown in TABLES I and II that reduction of absolute weights and degree of alteration of percentage weights and regression coefficients were different in various organs clearly indicated a differential and possibly specific effects of aureomycin on the organs.

The reduced body and organ weights of the treated tadpoles could be due to inanition because they took very little food, only one ninth of the control food intake during the latter half of the experimental period (2). However, as it was discussed in the previous report, factors other than malnutrition seemed more responsible on account of the facts that decrease of body width appeared prior to the onset of reduced food intake; and that not a single edematous tadpole was found among the 30 treated ones (2). In addition, the results of the present experiment that no treated organs contained more water than the controls and that no accumulated fluid was observed in body cavities of the treated tadpoles during dissection further supported the view. Therefore it would seem that inanition was not the primary factor but one of the aureomycin effects resulting from poor appetite which secondarily caused the decrease of the weights of some organs especially the fat bodies and possibly the liver and tail.

When tetracycline antibiotics were introduced in the medium of mold culture, Freeman and Circo reported a general reduction in the concentration of the intracellular amino acids except for glutamine and alanine and they also observed a complete disappearence of arginine, lysine, proline, phenylalanine and tyrosine from the intracellular amino acid pool (7). Franklin showed the inhibition of incorporation of C^{14}-leucine into protein of cell-free systems from rat liver and *Escherichia coli* by aureomycin treatment (8). It seems aureomycin owes its antibiotic nature to inhibit protein synthesis through the prevention or reduction of incorporation of certain amino acids but not all. Tissues and organs have their specific proteins. Thus aureomycin might exert different actions on various tissues and organs: some hampered, others augmented and still others unaffected.

Besides the reduction of protein synthesis, aureomycin is also well known for its interference with phosphorelation (9, 10) and oxidation (11). With all these adverse effects imposed upon the tadpoles, they were apt to show a stunted growth. Under such condition various organs reacted according to their own characteristic structures and functions.

1. The fat bodies: During hibernation and prebreeding period when food is scarce, the fat bodies of frogs are consumed as the energy source because they contain stored nutrition (12). In the present experiment, the greatest reduction of absolute weight and percentage weight of this organ among the 6 by aureomycin treatment had the same cause for the treated tadpoles had taken little amount of food (2).

The characteristically low water contents of fat bodies explains why the percentage dry weight of the organ was about 6 times that of the percentage wet weight while the same ratio of other organs was far less.

2. The liver: In frog liver, the amount of glycogen was found at its minimum during the breeding season and reached a maximum in the fall when the frog was less active and had taken much food in summer; and the liver itself was, thus, heavier in the fall than in the spring (13). These facts indicate there is usually a seasonal variation in the weight and size of frog liver which correspond in general to the cyclic changes in glycogen content.

Thyroidectomized tadpoles raised under favorable condition will keep on growing to a huge size without metamorphosis (14). It is conceivable that they will not experience periodic changes in glycogen content and thus suffer no loss in liver weight.

The decrease of liver weight in treated tadpoles might be attributed to the decreased glycogen content in the hepatic cells and/or the degenerative change of the liver tissues. The possibility of glycogen decrease is based on the fact that aureomycin-treated tadpoles took very little food (2) and thus the reserved nutrition was mobilized into energy. Fatty degeneration of the liver as a result of the detrimental effect of aureomycin was reported by Lepper *et al.* and Lin in men, mice, dogs and rats (15–17). The latter author claimed this pathological change of the liver was due to cytochrome C depression caused by the antibiotic.

When fat accumulation took place, the organ was apt to lose weight.

3. The ovary: Berke *et al.* reported that somatic growth was inhibited but the gonad was not affected by aureomycin treatment in the fish, *Lebistes reticulatus* (18). The result agreed with the finding of this study in the ovaries. The experiments on the 2 different species of animals gave the same result that the ovary was unique in resisting the adverse action of auremycin. Perhaps this is one of the characteristics of the germ plasm to maintain itself regardless of the environmental pressure; even hormonal disturbances such as thyroidectomy, thyroid feeding or hypophysectomy did not influence sex differentiation in tadpoles (19–22). The comparatively weak correlation coefficient between the ovary weight and tadpole body weight (0.6, the lowest one among all in this study) and the extremely low correlation coefficient of –0.09 in dry state also help to understand the independence of this reproductive organ on its environment.

However, there was the augmented effect of aureomycin on the ovary as shown by the study with percentage weight and regression coefficient. This can be explained by the fact that the treated tadpoles as a whole were reduced in weight, while their ovaries as an organ were not.

4. The kidney: The reversed effect of aureomycin on percentage weight and regression coefficient of this organ after drying was due to the fact that aureomycin-treated kidney held water 5% less than the control. This might be interpreted to mean renal mechanism of aureomycin-treated tadpoles was deviated from the norm.

Bruggemann *et al.* reported the disturbance of pituitary-adrenal axis in rats by aureomycin treatment which caused the fall of blood eosinophils, increase of adrenal weight and increase of ascorbic acid content (23). Similar result was obtained by Trans *et al.* in rats after a single injection of aureomycin (24). Perek and Bedrak also claimed that aureomycin supplements up to 500 ppm in the diet of chickens increased activity of the adrenal cortex as shown by ascorbic acid level (25). It appeared that aureomycin stimulated ACTH activity and this might as well occur in aureomycin-treated tadpoles.

On account of restriction of sodium due to extremely low food intake of the aureomycin-treated tadpoles in an environment of hypotonic tap water, sodium chloride load on the experimental kidney would be low. If ACTH-stimulating effect of aureomycin happened in the tadpoles, the diuretic action of the mineralocorticoids would dominate over its action of sodium retention and water retention. This accounted for the less water content in the treated kidney than in the control. The fact that no edematous tadpoles were observed in this experiment also favored the view.

Aureomycin might play another role on the pituitary to such an effect that the secretion of antidiuretic hormone was disturbed. The low sodium level in the tadpole due to decreased food intake might result in an decreased osmotic pressure which caused the osmoreceptor-hypothalamic axis to secret less ADH. However diuresis in the present case would be a mild one since there was only a 5% difference of water content between the 2 groups of kidneys and therefore the treated tadpoles survived.

5. The tail and GI tract: While the water content of these organs was not altered by the treatment, the raised percentage weight and regression coefficient in the treated tail and GI tract after drying were very perplexing.

Liver and kidney are simple organs in the sense that they are made up of one dominant tissue, the epithelium; and fat bodies, the adipose cells. On the other hand, the tail and GI tract are composite organs consisting of different tissues such as muscles, connective tissue and epithelium in certain balanced proportion. Should differential action of aureomycin take place in tissues, the result on simple and composite organs would not be the same. In the former case, the antibiotic action would be simple, either depression or augmentation; while with the latter case, the action might be a mixed one. Therefore the percentage weight and regression coefficient of the liver and fat bodies were reduced by aureomycin treatment in both wet and dry state while the same parameters of the tail and GI tract were raised after drying by the treatment.

A reduction in the absolute weight of the small intestine was also observed by Pepper *et al.* in aureomycin fed chicks (26). The same result was obtained in antibiotic-fed pigs too (27). In those cases aureomycin was used to promote growth whereas in the present experiment the effect of the drug was to depress growth. Even so, the result of the reduction of the intestinal weight was just the same. Probably this was due to the fundamental property of aureomycin to hamper against the living system.

The data obtained from this study lend weight to the suggestion that in tadpoles aureomycin also suppressed protein synthesis in that organ weight and body weight were reduced and that the antibiotic might act differentially on tissues and thus on organs too. Further study on microscopic comparison between control and treated organs is necessary to evaluate the view.

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