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HISTOLOGICAL CHANGES IN THE DIGESTIVE SYSTEM OF THYROIDECTOMIZED TADPOLES AFTER AUREOMYCIN TREATMENT

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ABSTRACT

Thyroidectomized tadpoles of *Rana catesbeiana* were reared in 18 ppm aureomycin medium for 34 weeks. Histological sections of the esophagus, stomach, intestine, liver and pancreas were compared with those of controls reared in tap water. The following changes were found in organs of the treated tadpoles. Hepatic plates appeared thin and atrophic; the cytoplasm of parenchymal cells was cloudy and vacuolar; glycogen content was reduced. The gastric glandular epithelium was thin with bulging nuclei and the gland lumen was enlarged. The surface epithelium of the stomach was less adversely affected. The height of the intestinal epithelium was reduced to about half of that of the control; the chromatin substance of epithelial nuclei became pyknotic; the basement membrane appeared indistinct.

INTRODUCTION

In our previous experiment, it was found that thyroidectomized tadpoles after aureomycin treatment suffered from a decreased food intake (1), a retarded growth rate (1, 2), and a differential reduction of organ weights in which fat bodies were reduced by 76%; the liver, kidney, tail and GI tract followed in that order; the ovary had the least reduction of 10% (3). It was thought that the results might be attributed to supression of protein synthesis by aureomycin, the effect of which could be imposed differentially upon tissues and organs. Therefore it was considered to be hightly desirable to make a microscopic study of organ systems, comparing control and aureomycin-treated tadpoles. The present study is restricted to the digestive system.

MATERIALS AND METHODS

Aureomycin is known to inhibit frog metamorphosis (4-8). It is therefore impossible to compare the digestive systems of intact tadpoles under both control and aureomycin-treated conditions. On the other hand, thyroidectomized tadpoles do not undergo metamorphosis. Hence, operated tadpoles of *Rana catesbeiana* were reared in tap water or in 18 ppm aureomycin medium at 19.4 ± 0.2 C in

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order to prevent degradation of aureomycin (9). After 34 weeks under these conditions, 10 control and 10 aureomycin-treated tadpoles were decapitated (2).

Parts of the liver, pancreas, esophagus, stomach and a segment of the center of the spirally coiled intestinal loop were fixed in the ice cold fixative of Lison and Vokaer (85 parts of 95% alcohol saturated with picric acid, 10 parts of 40% of formalin, and 5 parts of glacial acetic acid, 10). The organs were cut in paraffin sections of 5 u in thickness.

Cross sections of tips of the largest lobes of the liver were stained for glycogen using PAS and Hx. Check slides containing consecutive sections of the liver were treated with saliva for 30 minutes before staining. The control and aureomycin-treated sections were placed on the same slides to ensure identical treatment of staining and digestion. Cross sections of the esophagus, stomach, intestine, liver and pancreas were stained with Hx and eosin.

RESULTS

Examination of stained sections revealed that digestive systems of control and aureomycin-treated tadpoles had remained in the early larval stage up to the age of 51 weeks, whereas those of their unoperated pals had metamorphosed into adult structures 34 weeks previously. Comparing the 2 groups of thyroidectomized tadpoles, control and aureomycin-treated, the following microscopic alterations were found:

I. The liver

Focal degenerative changes were found in the livers of treated animals. *Figs. 1* and *2* show hepatic structures and the altered appearance of the treated liver respectively. In altered areas, hepatic cells appeared smaller with cloudy and/or vacuolar cytoplasm, bile canaliculi were smaller and sinusoids were more spacious (*Figs. 3* and *4*).

Sections stained with PAS and Hx demonstrated the presence of PAS-positive granules in hepatic cells of both control and treated livers. The granules were more abundant in the former than in the later (*Figs. 5* and *6*). However, check slides after saliva digestion showed a more pronounced staining reaction in the treated than in control livers (*Figs. 7* and *8*), indicating that PAS-positive granules in control liver were more saliva labile than those in the treated liver. Consequently, the treated liver contained less glycogen.

Figs. 7 and *8* also show the presence of more melanocytes along the walls of sinusoids in treated than in control liver.

II. The stomach

The effect of aureomycin on the stomach was seen mainly in gastric glands. The control stomach (*Figs. 9* and *11*) showed a narrow gland lumen with a thick gland epithelium, whereas the gland lumen of the treated stomach (*Figs. 10* and *12*) was wide and open, with thinning of the glandular epithelium and bulging of nuclei.

The surface epithelium of the stomach was, however, less detrimentally affected; after treatment the epithelium became thinner (*Figs. 13* and *14*).

III. The intestine

The conspicuous changes in the intestine after aureomycin treatment were the decrease of epithelial height to about half of that of the control, and the clumping of chromatin substances in epithelial nuclei (*Figs. 15* and *16*). In the control specimen (*Fig. 15*), some oval shaped nuclei of basal cells wedged below the elongated nuclei of columnar cells and a well defined basement membrane was present; while in the epithelium of treated animals (*Fig. 16*), basal cell nuclei were rarely present and the basement membrane was not clearly seen.

The stromal connective tissue of the control intestine was compact but that of the treated became loose and spacious, with an edematous appearance.

IV. Esophagus and pancreas

No significant changes were observed in the microscopic appearance of these organs after aureomycin treatment with the present technique.

DISCUSSION

This study revealed that under the present experimental conditions the digestive system of the aureomycin-treated tadpoles was detrimentally affected as shown by the degenerative changes in hepatic plates, gastric glands and intestinal epithelium, and the edematous appearance of the stromal connective tissue.

Aureomycin is well known to be capable of interfering with the synthesis of protein (11-13) and nucleic acid (14) and oxidative reactions (15-17). These toxic properties, especially the inhibition of protein synthesis, might exert a detrimental effect on tadpole digestive system resulting in protein depletion as observed histologically by the present study. However, aureomycin-treated tadpoles took less food than the control (1). Nutritional deficency would also induce such similar results.

Another possibility might exist that aureomycin toxification and inanition worked together to hamper the tadpoles. Our previous study found that the earliest signs of aureomycin effect on tadpoles were a decrease of body width beginning in the 4th week and a decrease of food intake starting in the 6th week. The results suggest that the liver and intestine which constitute most of the body width of a tadpole were attacked by the treatment first and then followed the decrease of food intake, resulting eventually in a stunted growth.

Nevertheless, the mechnism of the adverse changes in the tadpole degistive system after aureomycin treatment still remains moot. To solve the problem, paired feeding experiment should be followed.

LITERATURE CITED

1. HSÜ, C. Y., N. W. YÜ and M. M. YOK. 1965. The food intake of aureomycin-treated tadpoles. *Chinese J. Physiol.*, **19:** 313-324.
2. HSÜ, C. Y., N. W. YÜ and H. M. LIANG. 1964. The action of aureomycin on the growth of thyroidectomized tadpoles. *Bull. Inst. Zool., Academia Sinica* **3:** 19-23.
3. HSÜ C. Y., W. C. CHOU and H. M. LIANG. 1965. The effect of aureomycin on the organ weights of the growing tadpoles. *Bull. Inst. Zool., Academia Sinica* **4:** 49-57.
4. SANFILIPPO, G. 1953. Boll. Soc. Ital. Biol. Sper. **29:** 1339. Cited in *Handbook of Toxicology*, vol. II, Antibiotics, 1957, ed. W. S. Spector, W. B. Saunders Co., Philadelphia, U.S.A., p 53.
5. MUSTAKALLIO, K. K. and A. Telkka. 1954. Effect of aureomycin, vitamin B_{12}, folic acid and aminopterin on the metamorphosis of tadpoles. *Ann. Med. Exper. et Biol. Fenniae* **32:** 9-14.
6. HSÜ, C. Y., N. W. YÜ and C. M. PI. 1962. Effect of aureomycin on thyroxin-treated tadpoles. *Chinese J. Physiol.*, **18 (annex):** 87-94.
7. PI, C. M. and C. Y. HSÜ. 1963. The effect of dosage variation on the development of toad tadpoles. *Bull. Inst. Zool., Academia Sinica* **2:** 31-36.
8. YÜ, N. W. and C. Y. HSÜ. 1966. Effect of aureomycin on development of frog tadpoles as related to time of onset of treatment. *Bull. Inst. Zool., Academia Sinica* **5:** 29-33.
9. HSÜ, C. Y. and N. W. YÜ. 1965. The effect of aureomycin on tadpole development as influenced by temperature. *Bull. Inst. Zool., Academia Sinica* **4:** 35-38.
10. PEARSE, A. G. E. 1960. *Histochemistry*, second edition, Little, Brown and Company, Boston, U.S.A., p 63.
11. GALE, E. F. and J. P. FOLKES. 1953. The assimilation of amino acids by bacteria. 19. The inhibition of phenylalanine incorporation in *Staphylococcus aureus* by chloramphenicol and p-chlorphenylalanine. *Biochem. J.* **55:** 730-735.
12. FRANKLIN, T. J. 1963. The inhibition of

incorporation of leucine into protein of cell-free systems from rat liver and *Escherichia coli* by chlortetracycline. *Biochem. J.* **87:** 449-453.
13. FREEMAN, B. A. and R. CIRCO. 1963. Effect of tetracyclines on the intracellular amino acids of molds. *J. Bacteriol.* **86:** 38-44.
14. REGE, D. V. and A. SREENIVASAN. 1954. Influence of folic acid and vitamin B_{12} on the impairment of nucleic acid synthesis in *Lactobacillus casei* by aureomycin. *Nature* **173:** 727-729.
15. Loomis, W. F. 1950. On the mechanism of action of aureomycin. *Science* **111:** 474.
16. VAN METER, J. C. and J. J. OLESON. 1951. Effect of aureomycin on the respiration of normal rat liver homogenates. *Science* **113:** 273.
17. VAN METER, J. C., A. SPECTOR. J. J. OLESON and J. H. WILLIAMS. 1952. In vitro action of aureomycin on oxidative phosphorylation in animal tissues. *Proc. Soc.. Exp. Biol.* **81:** 215-217.

LEGEND OF FIGURES

(Unless otherwise noted, all sections are 5 u, stained with hematoxylin and eosin, × 830)

Fig. 1. Control liver, showing normal hepatic plates. PAS and hematoxylin. × 200.

Fig. 2. Aureomycin-treated liver, showing degenerative hepatic plates. PAS and hematoxylin. × 200.

Fig. 3. Control liver, showing normal parenchymal cells and bile canaliculi. × 1800.

Fig. 4. Aureomycin-treated liver, showing parenchymal cells with cloudy or vacuolar cytoplasm. × 1800.

Fig. 5. Control liver, showing numerous PAS-positive granules in hepatic cells. PAS and hematoxylin.

Fig. 6. Aureomycin-treated liver, showing less PAS-positive granules. PAS and hematoxylin.

Fig. 7. Control liver after saliva digestion, showing labile character of PAS-positive granules. PAS and hematoxylin.

Fig. 8. Aureomycin-treated liver after saliva digestion, showing saliva-resistant and PAS-positive granules. Note the increase of the number of melanocytes. PAS and hematoxylin.

Fig. 9. Control stomach, showing the normal surface epithelium and gastric glands. × 200.

Fig. 10. Aureomycin-treated stomach, showing the degenerative surface epithelium and gastric glands. × 200.

Fig. 11. Gastric glands of the control stomach.

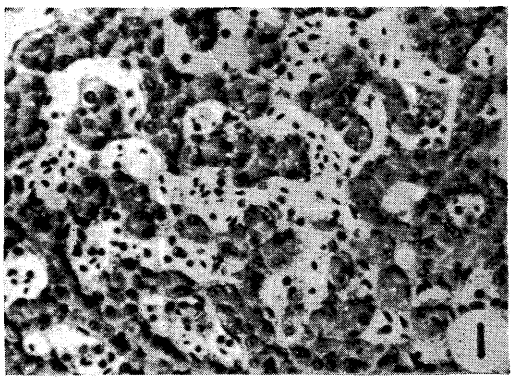
Fig. 12. Gastric glands of the aureomycin-treated stomach.

Fig. 13. Surface epithelium of the control stomach.

Fig. 14. Surface epithelium of the aureomycin-treated stomach.

Fig. 15. Control intestine.

Fig. 16. Aureomycin-treated intestine, showing degenerative epithelium with pyknotic nuclei, indistinct basement membrane and the edematous connective tissue.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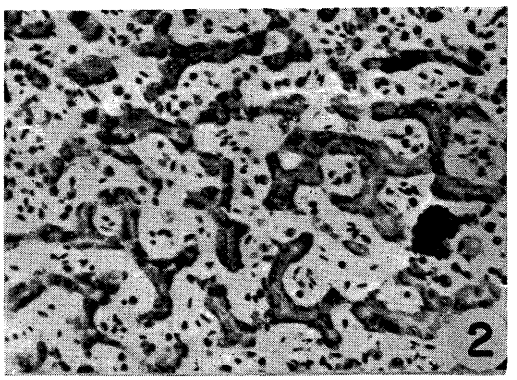
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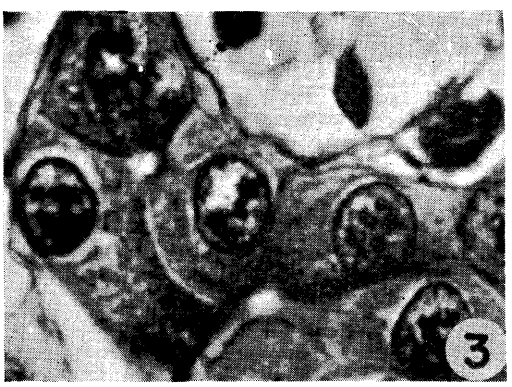
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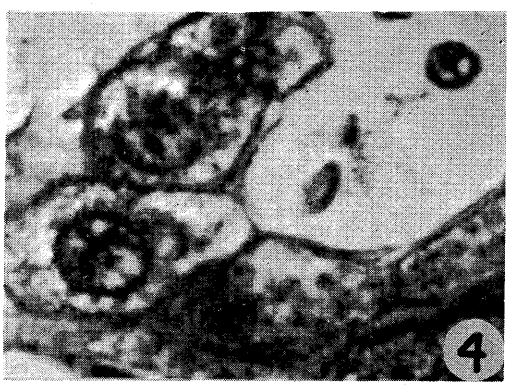
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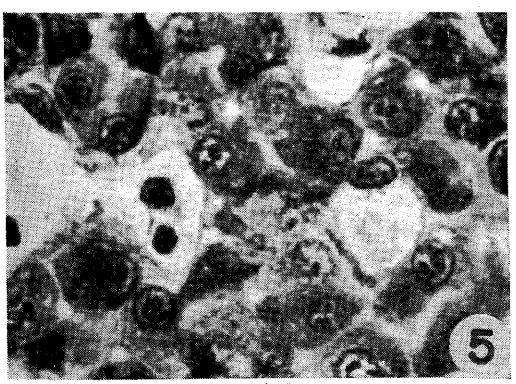
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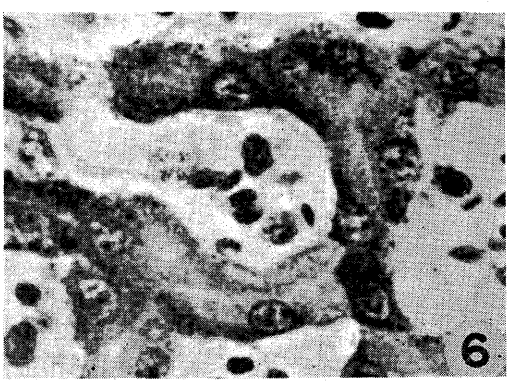
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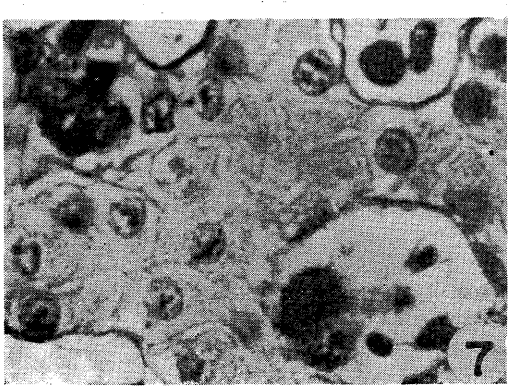
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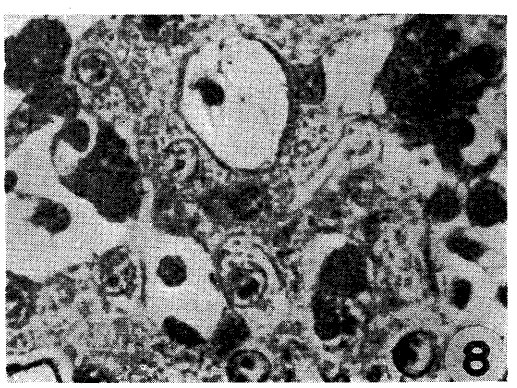
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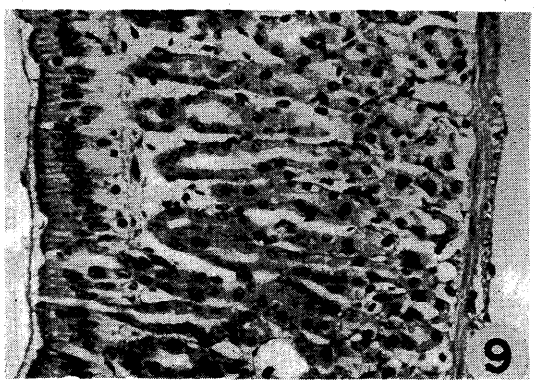
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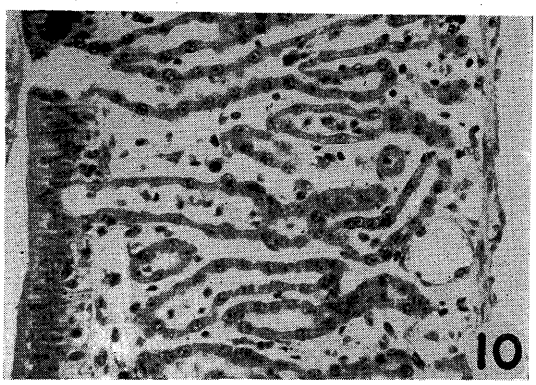
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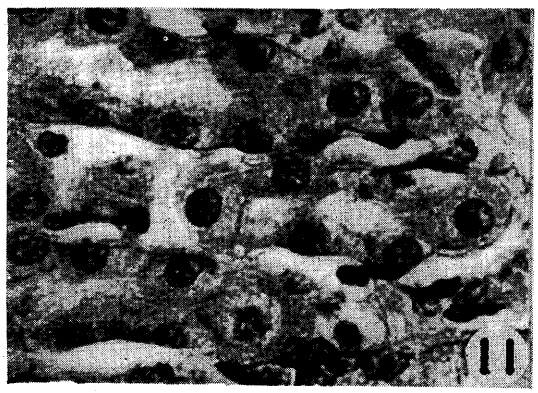
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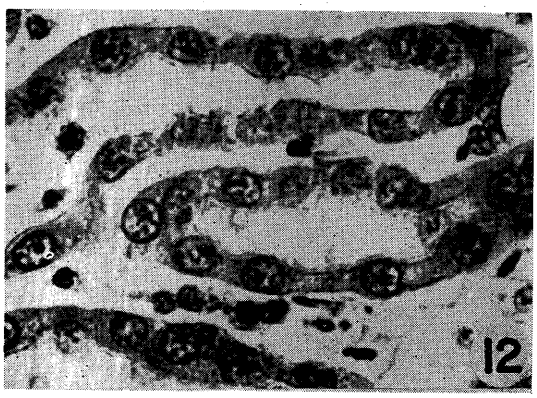
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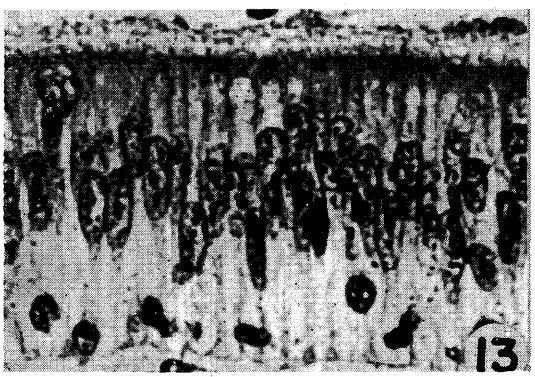
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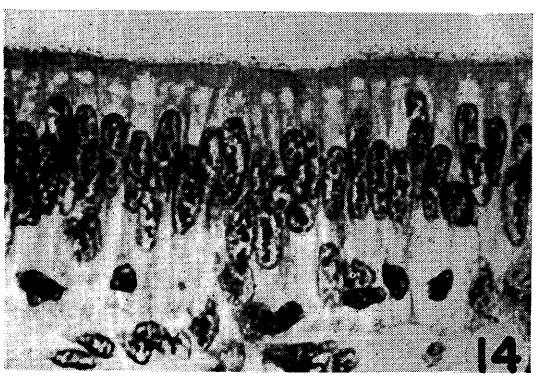
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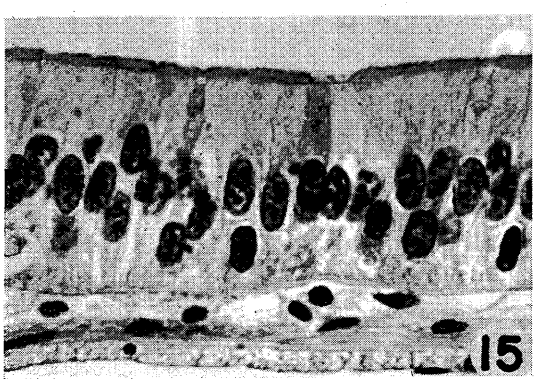
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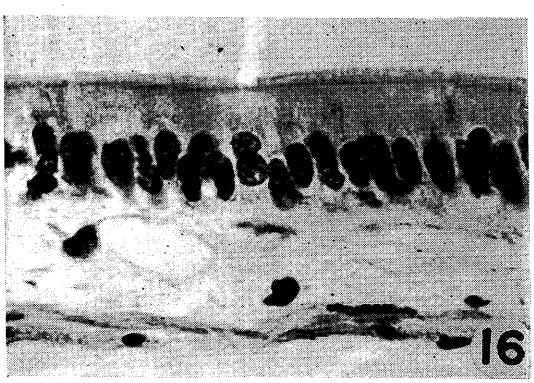
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