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Effect of Thyroxine on Early Development of Chick Limb Buds

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SYNOPSIS: Transplanted hind limb buds of chick embryos at stage 18 were treated with thyroxine, which caused complete growth inhibition at concentrations of 200, 150, and 100 μg/ml. Lower concentrations caused reduction in length and weight as well as a decreased number of feather germs. An increase in the enzyme acid phosphatase and number of lysosomes, in the cells of limb buds, were also observed. Increased acid phosphatase reaction was accompanied by decreased amounts of RNA (in treatment with 50 and 70 μg/ml. concentrations), suggesting a correlation between the two thus showing a thyroxine effect on lysosome activity.

INDEX DESCRIPTORS: Thyroxine, chick limb bud, ectoderm-mesoderm interaction, RNA, acid phosphatase.

Most of the work on chick-limb development has concerned the role of the mesoderm, ectoderm or ectoderm-mesoderm interaction (DeHaan and Ebert, 1964; Zwilling, 1961). Minimal attention has been given to the effect of drugs or hormones on the early development of chick-limbs. According to Miliare (1963) some cytochemical characteristics, such as accumulation of ribonucleic acids (RNA) and alkaline phosphatases, in the cytoplasm, can be detected at early stages of chick-limb development. Thyroxine starts to be released by the thyroid gland of the chick embryo after the twelfth day (Martindale, 1941), while the limb buds become morphologically recognizable after 56 hours of incubation (Hamburger and Hamilton, 1951). Thus, developing chick-limb buds offer a proper embryonic system to test for the effect of thyroxine on early embryonic development.

The physiological action of thyroxine has been frequently investigated. It acts as an uncoupling agent of phosphorylation in mitochondria *in vitro* and *in vivo* (Hoch, 1962). The biochemical action of thyroxine depends on its concentration and its reaction within the target sites. High concentrations (50-100 μg/day) inactivates insulin and thrombin *in vitro* and depresses protein synthesis (Nikkila and Pitkanin, 1959; Sokolof *et al.* 1961), while lower concentrations (5-10μg/day) increases protein synthesis in thyroidectomized rats. Thyroxine stimulates glycolysis, but in general, it depresses glycogen metabolism (Baker and Lewis, 1956).

In chicken, it has been noted (Sturkie, 1951) that administration of thyroxine will decrease plasma protein at the expense of albumins while globulins remain normal. Skrivanek (1963) proved that thyroxine inhibits the growth of feathers in tissue cultures of the epidermis.

MATERIALS AND METHODS

Hind limb buds of white leghorn chick embryos at stage 18 (72 hours) were excised and transplanted singly onto the chorioallantoic (CA) membrane of host chick embryos at stage 34-35 (about 8½ days). A total of 850 transplants were performed. Immediately after transplantation, 0.08 ml. of thyroxine was deposited on the transplanted buds with a microdropper. There were seven groups of transplants corresponding to seven concentrations of thyroxine (200, 150, 100, 75, 50, 30, and 10 μg/ml.). After transplantation the recipient eggs were again incubated at 38°C. The transplanted limb buds were recovered at intervals of 6 hours, 1, 2, 3, 4, and 5 days after operation and examined with regard to length, weight and volume. Some transplants were kept more than five days to observe feather development. Control transplants were done by adding 0.08 ml. of sterilized saline solution to the transplant. All solutions were sterilized by Seitz filtration and the pH adjusted to 7.5.

For general examination the recovered limb buds were fixed in 4 percent neutral formalin for one day, washed in water, dehydrated, then embedded in paraffin (M. P. 55°C). Sections were cut at 4 μ and double stained with eosine and hematoxylin, or light green and acid fuchsin. For electron microscopy the recovered limb buds were fixed in cold (4°C) 4 percent gluteraldehyde (cacodylate buffer) for 3 hours, washed in the buffer, post-fixed in 1 percent osmium tetroxide for 1.5 hours, then dehydrated and embedded in epon-araldite after Anderson and Ellis (1965). Thick sections were cut at 0.5 μ on an LKB ultramicrotome and stained with acid fuchsin. Thin sections were cut at 60-80 Å and stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and examined with a Hitachi HU-11E-1 electron microscope.

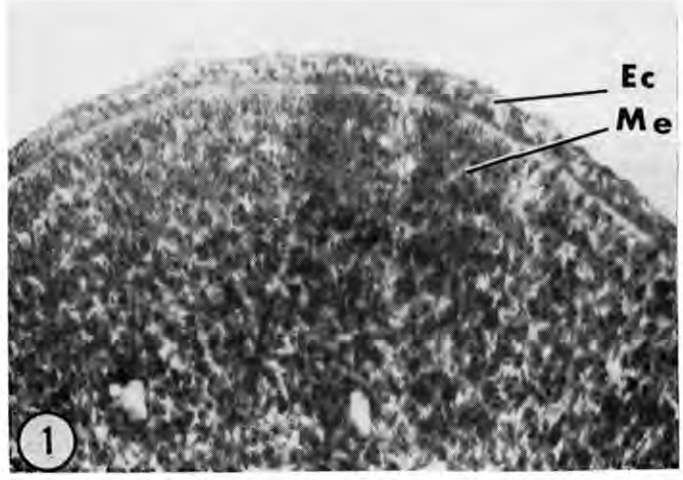

Figure 1. Chick limb bud, showing ectoderm (Ec) and mesoderm (Me).

[1] The study is part of a master's thesis, 1970. The author is indebted to Dr. John R. Baker for supervising.

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To demonstrate acid phosphatase, tissues were fixed in cold acetone and sectioned at 10-12 μ in a cryostat. The dry frozen sections were mounted on cover slips and stained after Gomori (1952). Sections were then washed in water, counterstained in Mayer's carmalum for 1 minute, dehydrated, cleared in xylene and then mounted on microscopic slides.

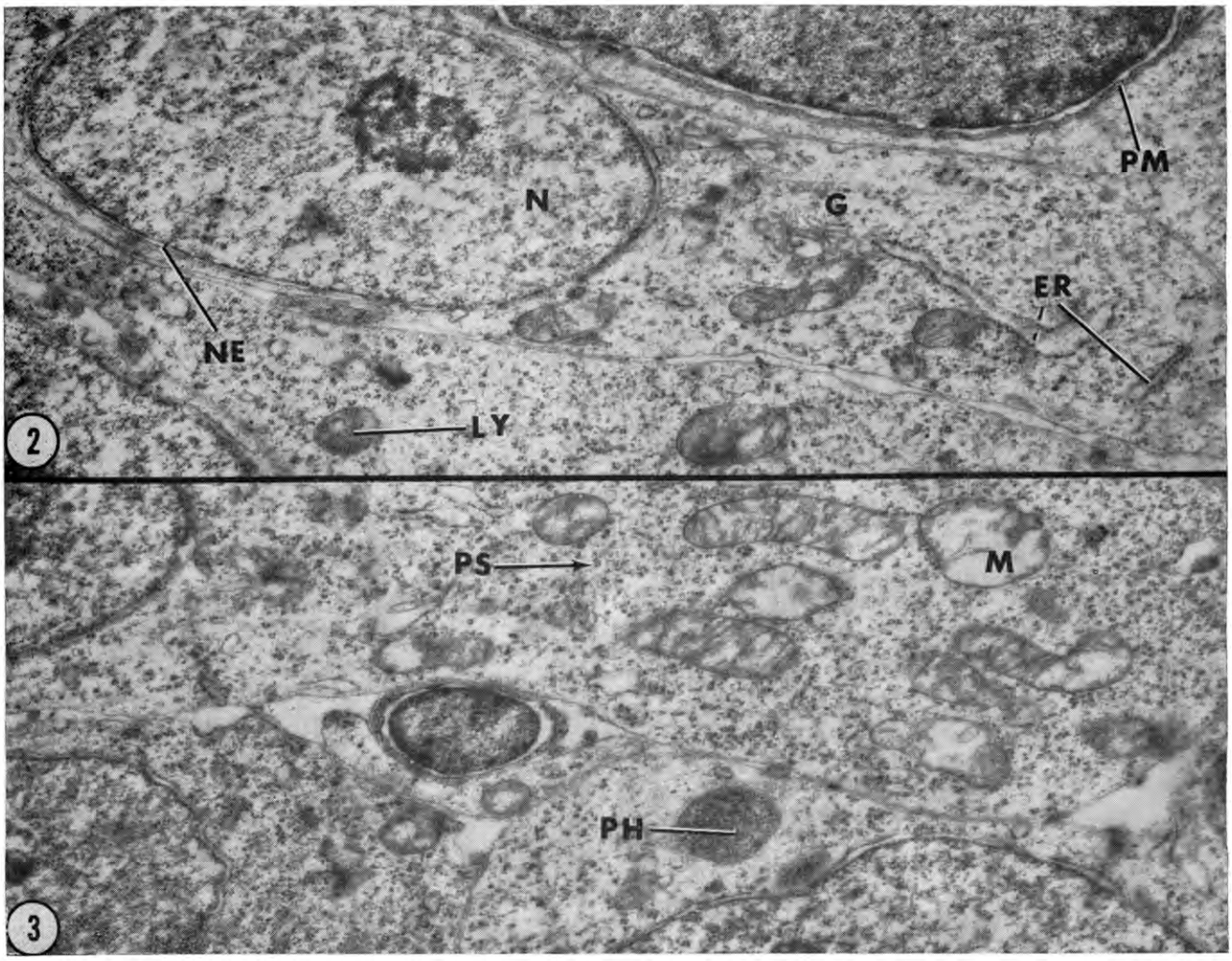
To demonstrate RNA, tissues were fixed in absolute alcohol containing 5 percent glacial acetic acid. Deparaffinized sections were hydrated, rinsed in phosphate buffer (pH 3), then stained in toluidine blue (same buffer) for 15 minutes after Thompson (1968). After a quick rinse in the buffer, sections were passed in xylene and mounted in permount. As a control, nucleic acids were extracted in 5 percent trichloroacetic acid at 60°C for 90 minutes before staining in toluidine blue.

TABLE 1. NUMBER OF FEATHER GERMS IN THE TRANSPLANTED LIMB BUDS

Concentration (μg/ml)	Average no. of feather germs/cm²	
	Treated	Control
75	54	215
50	68	
30	87	
10	98	

OBSERVATIONS

In developing chick-limb buds the ectoderm forms a cap above the mesoderm (Fig. 1) and the boundary between mesoderm and ectoderm is marked by a continuous basement membrane. During normal development there is more RNA at the ectodermal region of the limb bud and in the mesoderm RNA accumulates towards the basement membrane (Fig. 6). Small numbers of scattered lysosomes (1-4 per cell) and phagosome-like structures could be seen in the cytoplasm of mesodermal cells (Fig. 2, 3) and only traces of the en-



Figures 2 and 3. Electron micrographs showing the cellular structure of untreated limb bud in sections at the mesodermal region. The nucleus (N) at one side of the cell, the nuclear envelope (NE) with micropores. Mitochondria (M), polysomes (PS), lysosomes (LY), phagosome-like structure (PH), endoplasmic reticulum (ER), Golgi apparatus (G), cell process (CP), and the plasma membrane (PM). X20,800.

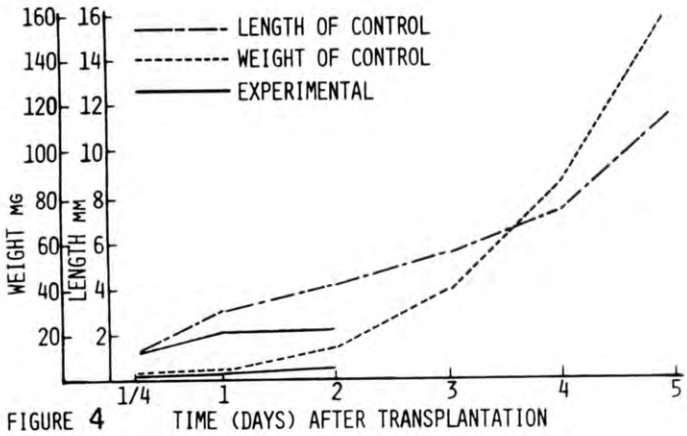


Figure 4. Growth of transplanted limb buds treated with 100 μg/ml thyroxine compared to the control.

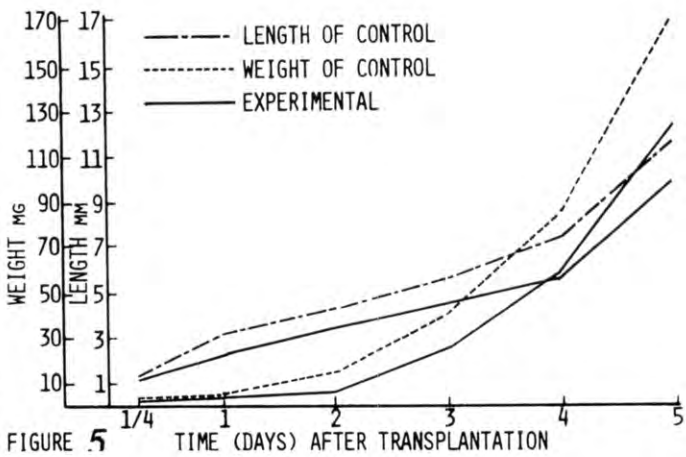


Figure 5. Growth of transplanted limb buds treated with 70 μg/ml thyroxine compared to the control.

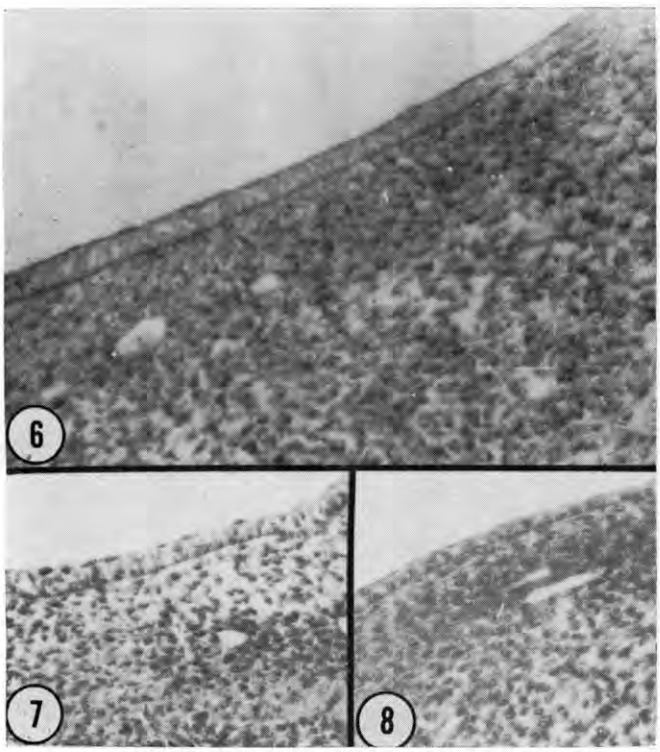


Figure 6. Portion of sagittal section of untreated limb bud recovered after 2 days and stained with toluidine blue. Note RNA distribution and its accumulation towards basement membrane. X400.

Figure 7. Portion of sagittal section of limb bud treated with 50 μg/ml. thyroxine, recovered after 2 days and stained with toluidine blue. Note decrease in the amount of RNA as revealed by reduction in stain. X400.

Figure 8. Portion of sagittal section of limb bud treated with 10 μg/ml. thyroxine, recovered after 2 days and stained with toluidine blue. Note slight decrease in amount of RNA. X400.

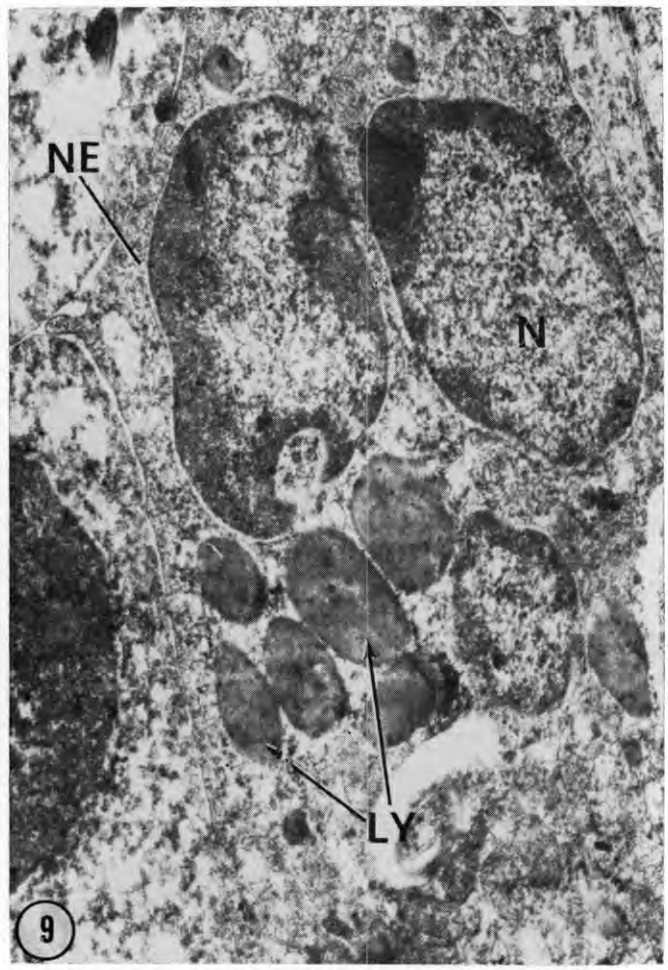


Figure 9. Electron micrograph showing increased number and aggregation of lysosomes (Ly) in mesodermal cell of limb bud treated with 75 μg/ml. thyroxine and recovered after 2 days. Nucleus (N), nuclear envelope (NE). Note depletion of cytoplasmic structures. X30,000.

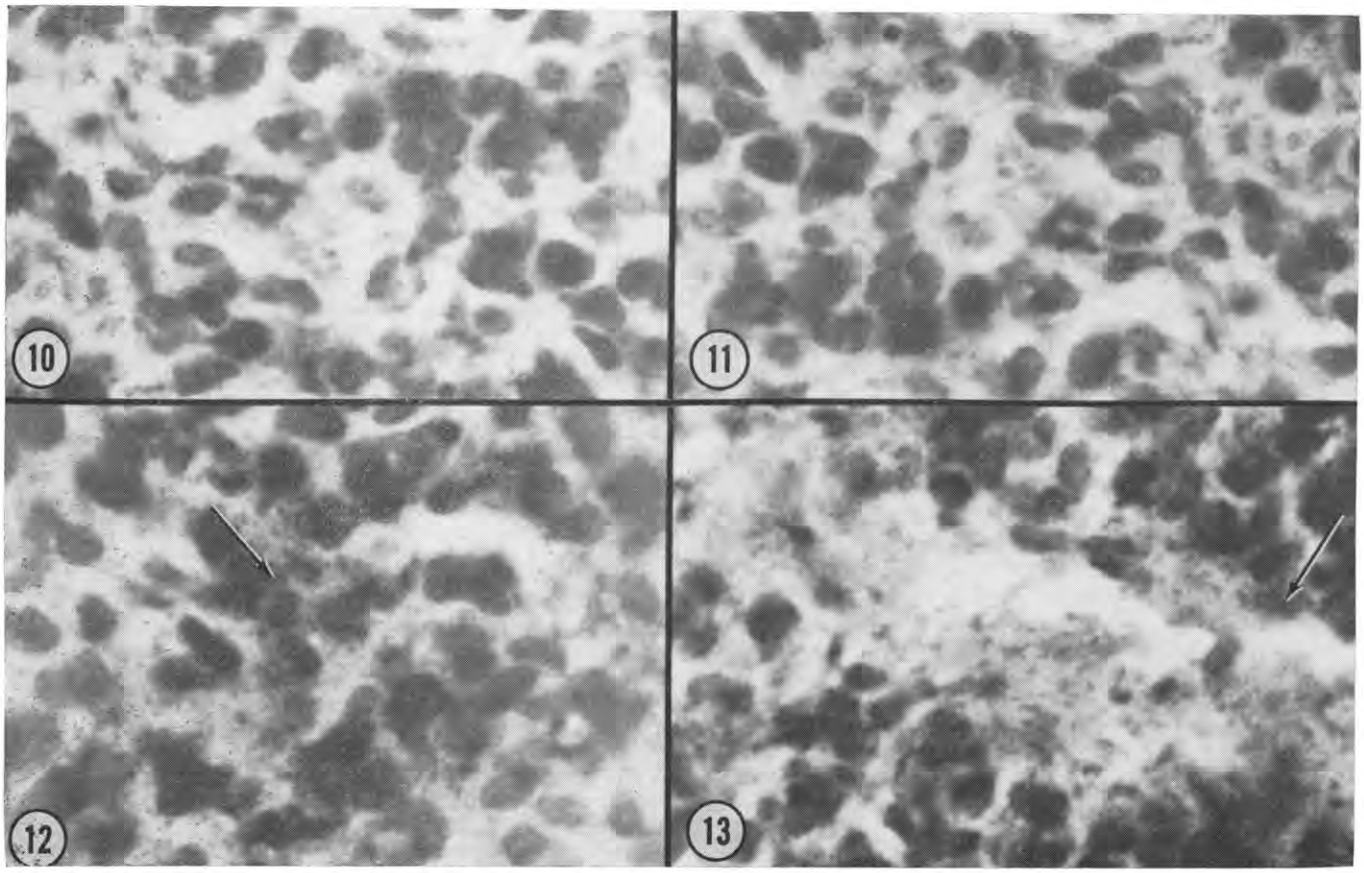


Figure 10-13. Cryostat sections of dry-frozen limb buds recovered 2 days after transplantation. Fig. 10, section of untreated (control) limb bud. Fig. 11, section of limb bud treated with 10 μg/ml. thyroxine. Fig. 12, section of limb bud treated with 50 μg/ml. and Fig. 13 is a section of limb bud treated with 75 μg/ml. Note the increase in acid phosphatase (arrow) with the increase of thyroxine concentration. X1,000.

zyme acid phosphatase could be detected (Fig. 10).

Effect of thyroxine on the development of transplanted limb buds was directly related to the concentration. Treatment with high concentrations (200, 150, and 100 μg/ml.) caused a drastic growth inhibition. In the case of 200 μg/ml. the transplanted limb buds did not develop at all and in many cases the host embryo died also. With 150 μg/ml. the transplanted limb buds were completely inhibited two days after treatment while with 100 μg/ml. inhibition occurred three days after the treatment (Fig. 4). The growth inhibition caused by the lower concentrations (70, 50, 30, 10 μg/ml.) was expressed as reduction in weight, size, and volume of the limb bud. This reduction continued throughout the period of transplantation (Fig. 5). From reference to Table 1, the limb buds which were maintained on the CA membrane until feather formation revealed a 1-2 days delay and a decrease in the number of feather germs even with the 10 μg/ml. concentration.

As revealed by electron microscopy, there was a noticeable increase in number of lysosomes in mesodermal cells after thyroxine treatment. The number of lysosomes in treated limb buds varies from 6-11 per cell (Fig. 9) compared to 1-4 per cell in normal (control) developing limb buds. It was also observed that lysosomes tended to aggregate towards the center of the cell. Accompanied with this increasing number of lysosomes there was a relative increase in acid phosphatase reaction (Fig. 11-13) and a depletion of cytoplasmic structures.

Thyroxine treatment caused a sharp decrease in the amount of RNA in mesodermal cells. This decrease was located mainly towards the basement membrane. It is apparent that the decrease in RNA was directly related to the concentration of thyroxine. While a slight decrease occurred in treatment with 10 μg/ml. concentration (Fig. 8) a more noticeable reduction was observed after treatment with other concentrations (Fig. 7).

DISCUSSION

The first to study the effect of thyroxine on chicken embryos was Willier (1924). He demonstrated that an excess of thyroxine obtained by grafting adult chicken thyroid tissue onto the embryo of 9 days (stage 35) or by stimulation of the embryonic thyroid gland by thyrotropin, caused stunting of the host embryo. No attempt was made to examine the effect of thyroxine on the cellular structure of chicken embryos.

The growth inhibition of transplanted limb buds treated with thyroxine may be a direct result of the observed decrease of RNA in the mesodermal cells. That the decrease in RNA was mainly towards the basement membrane may confirm that RNA of the mesoderm plays an inductive role to the ectodermal cells (Brachet, 1947; Thompson, 1963). It has been shown (Brachet, 1947; Davidson, 1970) that there is a continuing increase of RNA and protein synthesis during embryonic development. Thus, a reduction in protein synthesis, which is essential for growing embryonic cells, is an indirect result of treatment with thyroxine. The reduction in number of feather germs might also be a result of this decrease in RNA. Thompson (1963) had shown that RNA is important for normal development of integumentary derivatives of chick embryo.

The increased acid phosphatase in limb buds treated with 75 and 50 μg/ml. thyroxine concentrations (Fig. 11-13) reflects some effect of thyroxine on the activity of lysosomes. These were increased in number and aggregated towards the center of the cell. In some cases there was an increase in size of lysosomes which suggests that they may have fused. Straus (1963, 1964) reported a joining of lysosomes with phagosomes in the kidney and liver of rats after the administration of horseradish peroxidase. The increase in acid phosphatase reaction accompanied by decrease in RNA suggests an interesting correlation between the two. Thyroxine has been reported to affect membrane permeability (Lehninger and Schneider, 1959), so it might affect the membrane permeability of lysosomes and cause the release of their enzymes, of which ribonuclease and acid phosphatase may act to break down RNA.

If acid phosphatase is indeed correlated with RNA (increase in acid phosphatase with decrease in RNA), as it seems based on this study and previous studies (DeDuve, 1959), its relation to embryonic development provides an interesting possibility for further investigation.

LITERATURE CITED

ANDERSON, W. A. and R. A. ELLIS. 1965. Ultrastructure of *Trypanosome lewisi* flagellum, microtubules, and kinetoplast. *J. Protozool.* 12:483-499.

BAKER, S. B. and W. J. LEWIS. 1956. Metabolic actions of acetic acid analogs of thyroxine triiodothyronine. *Soc. Exp. Biol. Med. Proc.* 91:650-654.

BRACHET, J. 1947. Nucleic acid in the cell and the embryo. *Soc. Exp. Biol. Med. Symp.* 1:207-224.

DAVIDSON, E. H. 1970. Gene activity in early development. Academic Press, New York.

DEDUVE, C. 1959. Lysosomes, a new group of cytoplasmic particles. In T. Hayashi (ed.), Subcellular particles. Ronald Press, New York.

DEHAAN, R. L. and J. D. EBERT. 1964. Morphogenesis. *Ann. Rev. Phys.* 26:15-46.

GOMORI, G. 1952. Microscopic histochemistry. Univ. of Chicago Press.

HAMBURGER, V. and H. HAMILTON. 1951. A series of normal stages in the development of the chick. *J. Morphol.* 88:49-60.

HOCH, F. L. 1962. Biochemical actions of thyroid hormones. *Phys. Rev.* 42:605-674.

LEHNINGER, A. L. and M. S. SCHNEIDER. 1959. The swelling of rat liver mitochondria by thyroxine. *J. Biophys. Biochem. Cytol.* 5:109-116.

MARTINDALE, F. M. 1941. Initiation and early development of thyrotropic function in the incubating chick. *Anat. Record* 79:373-393.

MILAIRE, J. 1963. Etude morphologique et cytochimique du development des membres chez la souris et chez la taupe. *Arch. Biol.* 74:429-512.

NIKKILA, E. A. and E. PITKANIN. 1959. Liver enzyme pattern in thyrotoxicosis. *Acta Endocrinol.* 31:573-580.

SKRIVANEK, J. D. 1963. Effect of certain mitochondrial enzyme inhibitors on development of the down feathers. M.S. thesis, Iowa State Univ., Ames.

SOKOLOF, L. S., S. KAUFMAN, and H. V. GELBOIN. 1961. Thyroxine stimulation of soluble ribonucleic acid bound amino acid transfer to microsomal protein. *Biochem. Biophys. Acta* 5:410-412.

STRAUS, W. 1963. Comparative observations on lysosomes and phagosomes in kidney and liver of rats after administration of horseradish peroxidase, pp. 151-175. In A. V. S. deReuck and M. P. Cameron (ed.), Ciba Foundation Symposium on Lysosomes. Little, Brown and Co., Boston.

STRAUS, W. 1964. Occurrence of phagosomes and phago-lysosomes in different segments of the nephron in relation to reabsorption, transport, digestion and extrusion of intravenously injected horseradish peroxidase. *J. Cell Biol.* 21:295-408.

STURKIE, P. D. 1951. Effects of estrogen and thyroxine upon plasma proteins and blood volume in the fowl. *Endocrinol.* 49:565-570.

THOMSON, J. L. 1963. Development of integumentary derivatives in the chick embryo as studied by histochemical and grafting techniques. Ph. D. thesis, Iowa State Univ., Ames.

THOMPSON, S. W. 1966. Selected Histochemical and Histopathological Methods. Charles C. Thomas, pub., Springfield, Illinois.

VENABLE, J. H. and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* 25:407-408.

WARNER, J. R. 1967. Organization and function of cellular RNA, pp. 1-60. In R. A. Malt (ed.), Macromolecular synthesis and growth. Little, Brown and Co., Boston.

WILLIER, B. H. 1924. The endocrine glands and the development of the chick. 1. The effect of thyroid grafts. *Amer. J. Anat.* 33:67-103.

WINDELL, C. C. and J. R. TATA. 1966. Additive effect of thyroid hormone, growth hormone and testosterone on DNA-dependent RNA in rat liver nuclei. *Biochem. J.* 98:621-629.

ZWILLING, E. 1961. Limb morphogenesis. 1. Advances in morphogenesis. Academic Press, New York.