THE EFFECT OF
17-HYDROXY-CORTICOSTERONE (HYDROCORTISONE)
ON
CHICK EMBRYO THYROID GLANDS

by

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HYDROCORTISONE AND THYROID

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PREFACE

A part of the present study compared the growth-retardation effect of different concentrations of hydrocortisone on chick embryos injected on the fourth day of development (before the development of a functional endocrine system) with that injected on the eighth day of development (when there is doubtful endocrine activity) and related it to differences in the thyroid gland histology.

Together with the *in vivo* procedures, *in vitro* procedures were carried out to study the effect of hydrocortisone on isolated thyroid glands. Eight-day chick embryo thyroid glands were explanted using the hanging drop method. The medium used consisted of one drop of 13-day chick embryo extract and one drop of cock plasma in which was suspended hydrocortisone in concentrations of 30μg to 40μg per 100cc of total medium.

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ABSTRACT

Hydrocortisone was injected into the yolk sac of 4-day and 8-day embryos. Results have shown a marked interference with growth, development, and response of the embryos as a whole. Retardation of growth correlated with the dose used and the duration of the treatment. High mortality rate was manifested with higher doses and with lower doses administered at an earlier incubation age.

In all of the surving treated embryos with different concentrations of hydrocortisone, there was noted a decrease in the size of the experimental thyroids as compared to the controls. Microscopic study of thyroids from eggs injected with 4mg hydrocortisone on the 8th day have shown a marked decrease in the number of follicles with enlargement of these follicles. Correlated with the increase in size of the follicles was an increase in the number of cells per follicle in the experimentals as compared to the controls, In some late stages with the 4mg concentration, results have shown a degenaration of the follicles. Microscopic study of thyroids from eggs injected with 0.1mg hydrocortisone on the 4th day, have shown an insignificant decrease in the number of follicles and the size of these follicles as compared to the controls.

Simultaneously, in vitro experiments were carried out. The hanging drop method was employed and the medium consisted of equal amounts of cock plasma and 13-day chick embryo extract. 30µg and 40µg hydrocortisone concentrations per 100cc of total experimental medium were used respectively. Nine day embryonic thyroids were

cultured for a maximum period of 6 days and then studied microscopically. Follicle formation with accumulation of colloid in both the controls as well as the experimentals was observed. No identifiable difference between the experimental and the control thyroids was noted. For a more definite in vitro results further experiments with higher concentrations of hydrocortisone should be performed.

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I- INTRODUCTION

A- Thyroid Control of the Adrenal Cortex.

The thyroid gland and the adrenal cortex are two of the endocrine glands that are thought to exhibit interrelation besides being controlled individually by the hypophysis.

The first to observe an interaction between these two endocrine glands was Hoskins, (Hoskins, 1910). He reported that the thyroid administration in guinae pigs produced hypertrophy of the adrenal glands. Since that time numerous publications appeared on the subject of adreno-cortical thyroid interaction.

Evans and associates (1939) were able to show a pronounced atrophy of the adrenal glands in thyroidectomized animals.

Lebland and associates (1944), Baumann and Marine (1945), Zarrow (1949), and Money (1951), reported a decrease in the size of the adrenals of the rats which had been thyroidectomized or fed thiouracil (antithyroid hormone).

Deane and Greep (1947) reported an increase in the zona fasciculata in thyroid administered rats.

Kik-Nes (1954) reported that the daily administration of L-thyroxine to thyroidectomized dogs had restored normal adreno-cortical activity.

Levin and associates (1955) reported a 25% increase in the adrenal size as a result of hyperthyroidism produced by thyroid feeding.

All of these reported findings were based on experimental

studies. As a result of these findings, one can deduce that the thyroid excerts a stimulatory action on the adrenal cortex.

However, this is not so in all cases, for the data based on clinical observations are inconsistent. These clinical observations were brought up by the following investigators.

Holst (1935) observed hyperplasia of the adrenals in clinical hyperthyroid patients. While, Le Compte (1948) reported a decrease of the adrenal size in clinical hyperthyroidism. Still, Means (1948) observed no changes in the size of the adrenals in clinical hyperthyroidism.

Therefore, as observed from clinical work, the thyroid does not act always in stimulating the adrenal cortex. As a result of clinical and experimental studies, there comes up a difficulty in the interpretation of data. Therefore, we conclude that though in experimental work the thyroid appears always as stimulating the adrenal cortex, according to clinical data the results are either stimulatory, inhibitory, or show no effect.

B- Adreno-Cortical Control of the Thyroid Gland

1- Addison's Disease and Thyroid Insufficiency

Bloodworth and associates (1954) studied 35 patients with Addison's disease. They reported that anatomic changes in the thyroid gland reveal a high incidence of thyroid atrophy and laboratory evidence of thyroid hypofunction and insufficiency. In all of the reported cases of Addison's disease associated with thyroid insufficiency, Bloodworth reported that the evidence of laboratory study indicates that hypothyroidism appeared after Addison's disease.

His studies were based on Basal Metabolic Rate measurements. Histology of the thyroid gland in Addison's disease patients, as reported by Bloodworth (1954) revealed extensive lymphocyte infiltration of the thyroid gland, and destruction of acini in most cases.

2- Corticotrophin and Cortisone Inhibit the Production of Thyroid Hormone

Hills and associates (1950), Wolfson and associates (1950), and Money and associates (1951) studied several clinical and experimental patients and animals. All noted that corticotrophin together with cortisone inhibit the production of the thyroid hormone. The basis for their clinical and experimental work had been radio-active iodine. These results are contradictory to Bloodworth's based on Addison's disease patients. While, here cortisone appears to inhibit the thyroid gland, Bloodworth's results show that lack of cortisone tends to inhibit the thyroid gland.

3- Adrenocortical Extract and Retardation in Chick Embryo Growth Rate

Much work has been done to clarify the interrelation between the thyroid gland and the adrenal cortex, but the actual mechanism of interdependence is still obscure and the present findings are still conflicting.

Looking for new methods of approach to try and solve the problem, I came over Landauer's report. Landauer (1947) attempted to study the effect of corticosteroids on chick embryos to reveal their action. As a result of his studies, he reported a retardation of the growth rate of the developing chick embryo (0, 1 or 5 day) following

adreno-cortical injections into the yolk sac.

Karnosfsky et al., (1951) reported that cortisone does not affect the growth rate until after the eighth day of development.

Evans (1953), and Siegel (1957) reported that cortisone acetate inhibits growth and development of the chick embryo beyond the 8th and 10th day of incubation. Since the thyroid gland starts its activity on the eighth day of development (Woodside, 1937), this growth inhibiting effect, I thought, might be due to secondary thyroid gland inhibition.

All studies done to determine the effect of the corticoids on the chick embryo had shown alterations of the gross anatomy of the developing chick embryo. However, the histological changes had not been studied except by Siegel, (Siegel, 1957). He studied the effect of cortisone acetate on body organs microscopically and noted:-

a- Chorioallantoic Membrane: The chorioallantoic membranes were thinner than normal in the cortisone-treated embryos.

b- Skin: The epithelium became atrophic and underwent premature keratinization. The medulla was dilated and hypocellular. Blood vessels were fewer and less developed. Elaboration of the dermis was delayed.

c- Bones: Ossification of bones and formation of bone marrow were delayed.

d- Skeletal Muscle and Fat: Development of the skeletal muscle and subcutaneous fat lagged several days behind that of the controls.

e- Eyes: In addition to the disproportionately large eyes

found in all the treated embryos, 18-day embryos injected with 3.75mg of cortisone acetate had retinal lesions.

f- Lungs: There was a profound inhibition of the development of the bronchial tree.

g- Pituitary Gland: There was no significant histological changes that could be attributed to cortisone.

h- Thyroid: In the 18-day treated embryos the thyroids resembled those of the 10 to 12 day controls in that they showed some small follicles with little or no colloid.

i- Liver: Hepatic damage appeared early in the treated embryos and in those given the smallest dose of 0.5mg of cortisone acetate. The cytoplasm was vacuolated. Inflammatory cells were present with necrosis.

j- Kidneys: Subnuclear vacuoles in the convoluted tubules and swelling of the epithelial cells of Bowman's capsule were noted. Mononuclear cells with a vacuolated cytoplasm were found free within Bowman's capsule.

k- Gonads: No definite changes were noted in the ovaries. No tubular formation of the solid cords of the testis occurred as compared to the untreated embryos.

l- Heart: Areas of necrosis in the epicardium, associated with an infiltration by acute inflammatory cells were noted in the treated embryos.

C- Culture of the Thyroid Gland In Vitro

The technique for culture of organs in vitro was developed by T.P. Strangeways and Honor B. Fell (1926). Since that time the method

has been used for morphological studies. It was only till the last ten years that the technique has been adopted by the physiologists. Lately, much research has been devoted to study the physiological activity of hormones on isolated organ cultures.

By means of the organ culture technique, one can demonstrate the direct action of a hormone on a gland (Schaberg, 1955), or the direct action of a hormone on any other body tissue (Caillard, 1952 and 1957, Kahn, 1954, Sidman, 1956 and Elias, 1957). Carpenter (1942 and 1954) cultivated 8-day chick embryo thyroids in vitro. His results have shown that the formation of colloid occurs at the same rate or even quicker in vitro than in vivo. He observed that the explants do not increase in size, but they even diminish. This he explains by the loss of connective tissue at renewal of the medium.

Thyroid explants in organ culture were found to produce colloid and thyroxine and were always accompanied by follicular structure (Gonzales, 1954 and 1956).

Therefore, chick thyroid rudiments in organ culture undergo the same pattern of structural (Carpenter, 1942) and functional (Gonzales, 1954 and 1956) differentiation as in vivo.

II- MATERIALS AND METHODS

A- In Vivo Experiments

1- General Procedure

Eggs of the cross between Rhode Island Red males and Barbed Plymouth Rock females were brought from the Agriculture farm of A.U.B. and kept at a temperature of 15°C until used but not longer than four days. Incubation was at a temperature of 37.5°C in a forced draft incubator. Eggs were rotated by hand half a turn once per day. Injections were given into the yolk sac of about 4- or 8-day incubated eggs. Experimental eggs were each treated with 0.1mg, 1mg, 2mg, or 4mg of Hydrocortisone (alcoholic)* suspended in 0.5cc of distilled water. Control eggs were each treated with 0.5cc of pure distilled water.

2- Hormone Solution

Hydrocortisone (Compound F) or 17-Hydroxy-Corticosteroid was used in my experiments because it is the major free circulating adrenocortical hormone in human plasma before and after the administration of any Adreno-cortico-tropic Hormone (ACTH), (Harper, 1957). In the plasma of the human, the bovine, the swine, and the dog, it was shown that the perfusion of ACTH promotes the production of a number of corticosteroids (Hayano, 1951 , Hechter, 1953 , Jeanloz, 1953 ,and Levy, 1953). Under the influence of enzyme systems present in the tissue, corticosteroidogenesis proceeds in a chain reaction which divides into several pathways (Pincus, 1952). Gassner (1951) reported that the major circulating hormone of the

* Sigma Chemical Company, 3500 Dekalb St., St. Louis 18, Missouri, U.S.A.

corticoids is hydrocortisone. This concept received further support from the fact that this corticoid is the principal one present in adrenal venous blood after the intact animal has been treated with ACTH (Farrell, 1953). Cortisone (Compound F) and aldosterone are present in the plasma in relatively small concentrations. In the resting states and in the human, the plasma contains 5 to 15 μg per 100cc of hydrocortisone, the major corticoid of the blood (Harper, 1957).

The sterile hydrocortisone was homogenized in a sterilized hand homogenizer and with sterilized distilled water just before injection. The suspension of hydrocortisone and water was brought to 37^{o}C temperature before injection by placing it in the incubator for about 15 minutes.

3- Egg Injection

Dr. Garabedian's method for egg injection was used (Garabedian, 1961). Injections were carried out by 1.25 inch long, 22-gauge needle in a combination with a 1mm syringe. At the time of injection, the egg was candled from the blunt end to locate the air sac. The shell on the blunt end was drilled with an egg driller over the middle of the air sac area, puncturing only the shell and keeping the thick outer fibrous membrance intact.

After that the egg was candled through the smaller pole to locate the embryo. A mark was made with the pencil on the shell just over the located embryo.

Now, the egg was handled carefully (so that the embryo might not move from its place) and placed in a container with the drilled

blunt end upper-most.

The drilled area was disinfected by iodine and then cleaned well from iodine by 70% ethyl alcohol. The needle was then passed through the drilled hole slanting about 20° from the perpendicular angle to the table's working surface and in the direction exactly opposite to the located embryo. Injection of the hydrocortisone and distilled water into the yolk sac was very slow so as not to disturb the embryo inside the egg. In most cases aspiration with the needle after injecting the egg was carried out to make sure that the tip of the needle was in the yolk sac. After injection, the egg was sealed by a small drop of melted paraffin applied by a camel's hair brush over the drilled hole. Each needle and syringe were used for the injection of about three eggs and not more to maintain egg content sterility.

4- Embryo Autopsy

Experimental and control eggs were removed together from the incubator at the end of the experimental period. Each egg was opened, the embryo or chick removed, studied grossly, dried well on filter paper and weighed on a chain balance to the nearest mg. The length of the embryo from the tip of the beak to the tip of the toe was measured and recorded to the nearest mm. The embryo was then opened in the region of the neck to reveal the areolar tissue in which is embedded the thyroid gland that occurs as two reddish-purple oval bodies (no isthmus is present) on the base of the neck, internal to the jugular veins and in the angles formed by the divergence in the subclavian and common carotid arteries (Bradley, 1915 and 1960, and Lillie, 1927).

Dead embryos were examined grossly, but not microscopically, because of the rapid deterioration of embryonic tissues under incubation. The glands were separated from the surrounding tissue examined grossly, measured and immediately fixed in 10% formatin.

5- Histological Preparations

To simplify manipulation of the tiny soft glands and to ensure against loss through processing, the glands were attached before fixation with a thin film of egg albumen to a small lens paper on which was recorded the experimental or control number of the glands.

Glands were kept over-night in 10% formalin, washed in running tap water for half an hour, dehydrated by gradual stages of ethyl alcohol, cleared with xylol and embedded in paraffin. Serial, 10μ thick sections, were cut and stained with Harris Hematoxylin and Eosin. Other sections were taken from between serial sections for special Periodic Acid Schiff (PAS) stain study.

6- Mode of Histological Study

Hematoxylin and Eosin stained serial sections were studied in the following manner:

a- The 3 largest thyroid gland sections selected from the middle of the gland were counted as to the number of follicles per diameter. Each of the sections studied was separated by at least ten sections from any other on which a count was made (Adams and Buss, 1952).

b- The diameter of 18 largest follicles per gland (6 from each of the three largest sections) were recorded in μ units.

c- The number of cells in the 18 largest follicles was counted and recorded.

B- In Vitro Experiments

1- General Procedure

White Leghorn eggs were collected and stored at 18°C until used but were not kept longer than four to five days before incubation.

Eight-day eggs were removed from the incubator, cleaned with 75% ethyl alcohol and let dry for operation. They were aseptically opened, the embryos cut out, and placed in Hank's balanced salt solution (BSS), (Merchant et al., 1960). The two thyroid lobes were dissected out and washed with BSS before culturing to remove the debris. The thyroid at this stage consisted of thick, somewhat convoluted cords of cells separated by blood spaces and a very small amount of connective tissue and ranged from 0.34 to 0.4mm in medium sections. Thyroids were cultured in vitro for a maximum period of six days by the hanging drop method, using Carpenter's method (Carpenter, 1939 and 1942). The experimental medium consisted of cock plasma and 13-day chick embryo extract. Hydrocortisone was suspended in concentrations of 30µg and 40µg per 100cc of total medium respectively.

Control thyroid glands were cultured in vitro in which hydrocortisone was lacking.

Normal thyroids from 10, 12, 14 and 16 day old embryos were removed and fixed for comparison with the cultured.

2- Cock Plasma

Cock blood was drawn before use from the wing vein and from an eight month old cock. The needle and syringe were rinsed with

Heparin* (0.02mg%) prior to use to prevent clotting. Plasma was obtained by centrifuging for 20 minutes at 2500 r.p.m. (Merchant et al., 1960).

3- Embryo Extract

The homogenized 13-day embryo was centrifuged for 20 minutes at a speed of 2500 r.p.m. (Merchant et al., 1960). To the supernatant fluid an equal volume of BSS in which was suspended the hydrocortisone was added.

4- Hanging Drop Method

A round cover-slip was stuck to a 44 x 60mm cover-glass by a drop of sterile water. Care was taken so that no air bubbles form between the two cover-slips. The two thyroid lobes were placed with a drop of embryo extract to the center of the round cover-slip. One drop of plasma was added and the mixture was stirred rappidly with a small glass rod. When a firm clot enclosing the thyroid was formed, the depression slide was inverted over the culture centering the explants in the well. Now the slide was flipped to obtain a hanging drop. The large cover-slip was then sealed to the depression slide with a mixture of paraffin and vaseline.

The culture was incubated at a temperature of 36°C and examined daily. Thyroids were carefully removed every two days, washed in Hank's BSS and transferred to fresh media (Merchant et al., 1960).

5- Histological Study

At different culturing intervals, living thyroid glands, while

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* Heparin, Boots Pure Drug Company, Ltd., Date of Manufacture, 13-10-55.

still embedded in the medium, were stuck to lens paper to facilitate manipulation and fixed in Bouin's solution. 10μ thick serial sections were prepared and stained with Hematoxylin and Eosin to study the effect of hydrocortisone on the developing isolated thyroids. Other sections were taken from between serial sections and stained with PAS for special colloid study.

III- RESULTS

A- In Vivo Experiments

1- A dose of 4mg Hydrocortisone per embryo given at about the eighth day of incubation.

Three separate experiments at different time intervals were performed. Sixty eggs were devoted to this part of study of which half were used as experimentals and half as controls.

On opening the hydrocortisone treated eggs on the incubation age of 14 days and 10 hours (eggs E1 to E10) and 15 days and 5 hours (eggs E11 to E30), abnormal embryos were revealed. Interference with embryonic growth and development of the embryos was the result. A smaller body size of the treated embryos as compared to the controls was noted, but alterations in the head size were less observed. The retardation of growth was symmetrical except for the eyes which were disproportionately large and sometimes protruding from the orbit. The limbs and body were underdeveloped. The body wall was delayed in closure. Treated embryos were still, as contrasted with the controls that showed normal reflex movements. Feathers were scant and underdeveloped being quite short or absent completely in some areas, and mostly in the head region. In some cases almost the whole body was depleted of feathers. There were more deaths among the experimental embryos than the controls. The average embryo weight and length were decreased. The average thyroid gland size was lower in the experimentals as compared to controls. (See Tables IA, IB, IIA, IIB, IIIA, IIIB and VI).

Microscopic study of the thyroid glands have shown a great enlargement in the size of the follicles resulting in a decreased number of follicles per gland. Together with the enlargement of follicles in the experimentals, there was a simultaneous greater number of cells per follicle , apparently proportional to the increased size of the follicles, being more in the experimentals because of the related larger follicular size. The larger and fewer experimental follicles were more congested with colloid than the numerous smaller follicles of the controls that contained colloid proportional to their size (see Fig. 4 and Fig. 8).

Some glands among those given the higher doses have shown degeneration of the follicles, (see Fig. 5 and Fig. 6). Another one gland have shown an amazing enlargement and distortion in the shape of the follicles which had become elongated and some-what branched (see Fig. 7).

2- Doses of 4mg, 2mg and 1mg. hydrocortisone per embryo given at about the 4th day of incubation

One group of eggs was injected with 4mg hydrocortisone per embryo at the age of 4 days and 8 hours. 36 eggs were used, half of these acted as experimentals (E31 to E46) and half as controls (C31 to C46). Eggs opened on the 8th day revealed that none survived. Dead embryos probably died at an early stage one or two days after the hydrocortisone injection. All of the controls were alive and of normal development when opened on the same day as the experimentals (see Table VI).

Two groups of eggs were injected with 2mg hydrocortisone per embryo at the age of 4 days, 2 hours, and 4 days respectively. Here

again 36 eggs were used, half of which served as experimentals (E47 to E55 and E56 to E65) and half as controls (C47 to C55, C56 to C65). Eggs were opened on the 8th day and 6th hour, and 8th day and 8th hour respectively. Also here none of the experimental embryos survived. All experimental embryos appeared to have died about one or two days after the injection of the hydrocortisone. Almost all of the control embryos were alive and of normal appearance (see Table VI).

Two groups of eggs the total number of which was 72 were run separately. Half of each group have served as experimentals and were treated at the age of 4 days with 1mg of hydrocortisone per embryo. Eggs opened on the eighth day have shown no living embryos. All embryos have apparantly died one or two days after hydrocortisone injection. Almost all the control embryos have stayed alive (see Table VI).

3- Short treatment with a dose of 0.1mg hydrocortisone per embryo given at about the 4th day of incubation

The 5 experimental and the 3 control eggs were opened at the age of 9 days and 18 hours. The three surviving experimental embryos appeared to be inhibited in weight and length, but compared to inhibitions caused by the higher doses of 4mg hydrocortisone per embryo, this could be considered only a slight inhibition (see Tables IVA, IVB and VI). In all the three embryos, body proportions, eye development and hair development appeared to be normal (see Fig. 2).

4- Longer treatment with a dose of 0.1mg hydrocortisone per embryo given at about the 4th day of incubation.

A total of 24 eggs were used for this experiment. Nineteen eggs have served as experimentals (E107 to E125), and were injected on the 4th day and 8th hour with 0.1mg hydrocortisone per embryo.

Only 5 eggs were used as controls (C105 to C109). Eggs were opened for autopsy on the 13th day of incubation. Even with this low amount of hydrocortisone injected, a survival rate of 6/19 among the experimentals resulted (see Tables VA, VB and VI). Nine of the dead experimental embryos appeared to have died at an early stage i.e. the second or third day after injection. Four embryos were large and some-what developed, probably had died about one day or so before autopsy.

Upon examining the six surviving experimental embryos, inhibition in the gross size of the embryo length, and weight was noted as compared to the controls (see Tables VA, VB and VI). All gross characteristics noted in Exp.I, II and III were also seen here but to a lesser extent. Characteristics such as body underdevelopment, and eye deformity were seen here. Feather underdevelopment was restricted to the head.

Grossly the thyroid glands of the experimentals were smaller in size, having an average diameter of 0.5mm as compared to an average diameter of 0.9mm in controls. Microscopic study of the thyroid glands have shown a decrease in the number of follicles per gland. Also noted was a decrease in the size of the largest glandular follicles (see Fig. 11 and 12). One gland have shown a marked increase in the interfollicular embryonic blood spaces (see Fig. 9). Colloid in the experimental thyroids looked altogether scarce as compared to the controls (see Fig. 10 and Fig. 11). The number of cells per follicle in experimental and control thyroids was almost identical (see Table VI).

B- Result of in Vitro Work

Eight day embryonic thyroids were cultured in vitro in a medium supplemented with 30µg and 40µg hydrocortisone per 100cc.

The gross size of the thyroids appeared to stay the same. In some cases, it was shown even to regress to a smaller size. Cultures were studied daily under the low power of the microscope. Living cultures were easily judged by their firm attachement to the glass by means of fine cytoplasmic extensions and by the absence of large vacuoles. It was interesting to note the colloid droplets in the substance of the living cultured glands with the medium power of the microscope.

Glands were fixed while still embedded in the culturing medium at culturing intervals of 2, 4 and 6 days. Prepared sections stained with PAS were studied for colloid formation. In all the successful cultures of both the experimentals and the controls, follicles with colloid formation appeared. However, no difference in the size of the follicles or in the amount of colloid appeared between the experimentals and the controls and among the 2nd, 4th and 6th day cultures (see Fig. 16, Fig. 17, Fig. 18, Fig. 19 and Fig. 20).

DISCUSSION

It is evident from the present experiments that the administration of hydrocortisone to developing chick embryos produces a retardation in the growth and differentiation of the embryos. Previous experiments with cortisone and cortisone acetate had also shown under-development and dwarfing of embryos (Karnofsky et al., 1950 and 1951, Moscona et al., 1960 and Pickman et al., 1964).

The effect of cortisone on various body tissues was not clearly understood. It had been particulary difficult to differentiate between the direct and the indirect effects of this hormone. Karnofsky (1951) found that cortisone does not affect the growth rate of the chick embryo until after the 8th day of development. Karnofsky had postulated that cortisone may interfere with some new substrate which normally appears during the mid-period of embryogenesis (eighth to twelfth day) coincident with the initiation of the functional phase of the embryonic systems.

The eighth to twelfth day period of development is a critical one which involves remarkable changes in embryonic metabolism (Needham, 1950). The thyroid starts activity between the eighth and tenth day (Woodside, 1937). The pituitary starts to act between the eighth and tenth day (Dawson, 1949). The embryonic liver deposits glycogen and cholesterol between the eighth and tenth day (Dalton, 1931 and 1936). There is a sharp rise in liver peptidase between the eighth and tenth day (Dumm et al., 1949). Nitrogenous wastes which were first excreted as ammonia and then urea, appear as uric acid between the eighth and

tenth day. The islands of Langerhans show histological evidence of activity and estrogenic hormone may be recovered from the embryo at ten days (Needham, 1950).

As soon as the various organs assume their different active characteristics, a complex interplay between the various hormones begins to act in the embryo just as it does in the adult. Fugo had shown that 18-day hypophysectomized chick embryos had characteristics similar to those produced upon corticosteroid administration, such as smaller body proportions, poor developed feathers and hypoplastic thyroid, with deficient colloid formation (Fugo 1940). He concluded that the effect of cortisone on the chick embryos might be secondary to a primary action on the thyroid or on the pituitary. Or cortisone might have an antagonistic effect on the circulating thyroid and pituitary growth hormones.

Greenberg (1955) had shown that the administration of cortisone slows the growth rate in young rats, and that if thyroid extract was given the growthrate became normal.

Wilhelmj (1955) had shown that some of the effects of cortisone in dogs can be neutralized by properly calculated doses of pituitary growth hormones.

Breibat (1954) brought the evidence favouring the direct effect of cortisone on tissue, particularly the mesenchyme.

Comparison Between the Effects of the Various Doses and the Possible Interpretation of the Results.

Grossly, surviving embryos studied, those treated with 4mg on about the 8th day and those treated with 0.1mg on about the 4th day have shown similar characteristics. Microscopically, the thyroid

glands in the two cases have shown a decrease in the number of follicles, per gland. However, the average size of the follicles in the two cases was dissimilar. While, hydrocortisone administered at about the 8th day produced larger follicles than normal with a high pooled standard deviation, it produced, when administered at about the 4th day, smaller follicles with no significant pooled standard deviation but with less colloid (see Figs. 4, 8, 11 and 12 and Table VI).

It seems that the mode of action of the hydrocortisone in the two cases was probably different due to the different developmentat stages at which it acted.

This diverse action can also be due to the cyclic changes that occur in the thyroid. Boyd states, "The thyroid gland is one of the most labile organs in the body. It is continually being played upon by various influences (endocrine etc.) and responding to the varying demands of thyroixine.,..... the thyroid gland structure is not fixed, any more than the breast or the endometrial tissue", (Boyd, 1961).

Another possibility is the decrease in the release of thyroid hormone to the body tissues via the blood stream resulting in the engorgement of the follicles with colloid as seen in embryos treated with hydrocortisone on the 8th day.

Furthermore, it is possible that hydrocortisone could act indirectly through the pituitary resulting in understimulation of the thyroid gland which was manifested in fewer follicles and less colloid in the early stages and in enlargement of the follicles in the later stages.

The effect of hydrocortisone in both the 4- and 8-day administered groups cannot be attributed to stimulation of the thyroid gland, as there is a decrease in the size of this gland in almost all treated embryos, accompanied by a decrease in the number of follicles, and degeneration of some follicles in the 8-day group. This decrease cannot be interpretted except as an inhibition of the gland in both cases. The enlargement of follicles could be due to compensation to the direct inhibition of the gland manifested initially in fewer cells and less colloid.

The case were follicles appeared in branching forms could be explained according to the Pathological phenomenon of compensatory hypertrophy and hyperplasia, "compensatory hypertrophy and hyperplasia occurs in some cases and in order that the follicles may accomodate the increased number of cells, the follicular space become enlarged and soon the proliferated epithelium is seen to proliferate into this space in the form of a process", (Boyd, 1961).

CONCLUSION

In the present investigation a clear inhibition of the general development of the chick embryo as well as an inhibition of the thyroid gland was noted upon the administration of hydrocortisone. Due to the number of possible interactions that might take place *in vivo*, it is difficult to differentiate between a direct effect of the administered hormone on body tissues and an indirect effect through other endocrine glands.

For a more definite elucidation of the action of hydrocortisone on the thyroid, it is suggested that *in vitro* experiments be conducted using higher concentrations than those employed in one part of this work.

SUMMARY

Hydrocortisone administered to the developing chick embryo produced a variety of effects depending upon the dose and time of injection:

1. There was delayed growth and general underdevelopment of the surviving embryos when treated on the 4th and 8th days.

2. 0.1mg hydrocortisone resulted in smaller thyroids, fewer follicles per gland, which were smaller and had less colloid than controls.

3. 1-4mg administered on the 4th day proved to be fatal.

4. 4mg given on the 8th day produced smaller thyroid glands with fewer and larger follicles. In some glands there was degeneration of numerous follicles while in others a distortion in the shape of some follicles was noted.

LITERATURE CITED

Adams, A. Elizabeth and Buss, Jo Anne M., The Effect of a Single Injection of an Antithyroid Drug on Hyperplasia in the Thyroid of the Chick Embryo, Endocrinology v.50, 234-253, (1952)

Allbright, F., Harvey Lectures, v.38, 123, (1943)

Baumann, E. J. and Marine, D., Involution of the Adrenal Cortex of Rats Treated with Thiouracil, Endocrinology, v.36, 405, (1945)

Bloodworth, J.M.B. Jr., Walter, M. Kirkendall and Carr, T. Lyle, Addison's Disease Associated with Thyroid Insufficiency and Atrophy, Journal of Clinical Endocrinology and Metabolism, v.14(5), 540-541, (1954)

Boyd, William, A Textbook of Pathology, Structure and Function in Diseases, (Seventh Edition), Lea and Febiger Publishers, Library of Congress, Philadelphia, 1000-1025, 1961

Bradley, O. Charnock, The Structure of the Fowl. A. and C. Black, LTD., 4, 5 and 6 Soho Square, London, W., 1915

_____. The Structure of the Fowl. (Revised by Tom Grahame), Oliver and Boyd, Edinburgh and London, 1960.

Breibart, S. and Engel, F.L., The Influence of Cortisone and Insulin on the Respiratory Activity of Rat Adipose Tissue in Vitro, Endocrinology, v.55, 70-76, (1954)

Carpenter, Esther, Differentiation of Chick Embryo Thyroids in Vitro, Anatomical Records, v.75, Abstracts, 101, (1939)

_____. Differentiation of Chick Embryo Thyroids in Tissue Culture, Journal of Experimental Zoology, v.89, 407-431, (1942)

Carpenter, E., Beattie, J. and Chambers, R.D., The Uptake of I^{131} by Embryonic Chick Thyroid Glands *In Vivo* and *In Vitro*, Journal of Experimental Zoology, v.127, 249-269, (1954)

Dalton, A.J., Cholesterol Storage and Bile Secretion in Chorio-Allantoic Grafts of Liver, Anatomical Records, v.67, 431-437, (1937a)

_____. The Functional Differentiation of the Hepatic Cells of the Chick Embryo, Anatomical Records, v.68, 393-405, (1937b)

Dawson, A.B., Histochemical Evidence of Early Differentiation of the Suprarenal Gland of the Chicken, Anatomical Records, v.105, 602, (1949)

Deane, H.W. and Greep, R.O., A Cytochemical Study of the Adrenal Cortex in Hypo- and Hyperthyroidism, Endocrinology, v.41, 243-257, (1947)

Dumm, M.E. and Levy, M., Chemistry of the Chick Embryo, Journal of Cell and Comparative Physiology, v.33, 373-382, (1949)

Elias, J.J., Cultivation of Adult Mouse Mammary Gland in Hormone-Enriched Synthetic Medium, Science, v.126, 842-844, (October, 1957)

Evans, H.K., Simpson, M.E. and Pencharg, R.J., Relation Between the Growth Promoting Effects, of the Pituitary and the Thyroid Hormone, Endocrinology, v.25, 175-182, (1939)

_____. Action of Cortisone on Developing Chick Embryo, Proceedings of the Society for Experimental Biology and Medicine, v.83, 31-34, (1953)

Farrell, G.L. and Lambus, B., Steroids in Adrenal Venous Blood of the Dog, Proceedings of the Society for Experimental Biology and

Medicine, v.84, 89-93, (1953)

Fugo, N.W., Effects of Hypophysectomy in the Chick Embryo, Journal of Experimental Zoology, v.85, 271-298, (1940)

Gaillard, P.J., Parathyroid Gland Tissue and Bone In Vitro, Experimental Cell Research Supply, v.3, 154-169, (1955)

_____. Morphogenesis in Aimal Tissue Cultures, Journal of the National Cancer Institute, v.19, 591-596, (October, 1957)

Garabedian, G. and Matossian, R., Laboratory Instructions in Bacteriology, Immonology, and Virology for Students of Medicine, Department of Bacteriology and Virology, School of Medicine, American University of Beirut, Beirut, Lebanon, Excercise 31, 4, 1961.

Gassner, F.X., Nelson, D.H., Reich, H., Rapala, R.T. and Samuels, L.T., Isolation of Androgenic Compound from Adrenal Venous Blood of Cows, Proceedings of the Society for Experimental Biology and Medicine, v.77, 829-831, (1951)

Gonzales, F., Evidence of the Functional Differentiation of Embryonic Chick Thyroid in Tissue Culture, Texas Reprints of Biology and Medicine, v.12, 828-832, (1954)

_____. The Functional Differentiation of Embryonic Chick Thyroid in Roller Tube Cultures, Experimental Cell Research, v.10, 181-187, (1956)

Greenberg, S.M. and Aterman, K., Thyroid-Cortisone Antagonism in Rats as Measured by Growth, Organ Weights and Food Utilization, Metabolism, v.4, 264-274, (1955)

Harper, H.A., Review of Physiological Chemistry, (Sixth Edition), 323-333

Lange Medical Publications, Los Altos, California,

Hayano, M., Dorman, R. I. and Yamada, E.Y., Conversion of Desoxycorticosterone to Glycogenic Material by Adrenal Homogenates, Journal of Biological Chemistry, v.193, 175-181, (1951)

Hechter, O., Solomon, M.M. and Caspi, E., Corticosteroid Metabolism in Liver: Studies on Perfused Rat Livers, Endocrinology, v.53, 202-215, (1953)

Hiffer, S. Robert, The Stability of Embryonic Chick Thyroid Cells In Vitro as Judged by Morphological and Physiological Criteria, Reprinted from Developmental Biology, U.S.A. Academic Press, 1962, v.4, 1-21, (February 1962)

Hills, R.S., Jr., Reiss, R.S., Forsham, P.H. and Thorn, G.W., The Effect of Adreno-Corticotropin and Cortisone on Thyroid Function, Thyroid Adreno-Cortical Interrelation, Journal of Clinical Endocrinology, v.10, 1375-1400, (1950)

Holst, J., Pathologische Anatomie der Organe ausser der Schilddruse bei der Basedow'sden Krankheit, Zweite Internationale Kropfkonferenz, Bern (1933), Verhandlungsbericht, p.62, (1935)

Hoskins, R.G., Thyroid Secretion as a Factor in Adrenal Activity, Journal of American Medical Association, v.55, 1724-1725, (1910)

Jeanloz, R.W., et al., Chemical Transformations of Steroids by Adrenal Perfusion, Journal of Biological Chemistry, v.203, 453-461, (1953)

Kahn, R.H., Effect of Locally Applied Vitamin A and Estrogen on the Rat Vagina, American Journal of Anatomy, v.95, 309-335, (1954)

_____. Effect of Oestrogen and Vitamin A on Vaginal Cornification in Tissue Culture, Nature, v.174, 317, (1954)

_____. Organ Culture in Experimental Biology, Reprinted from University of Michigan Medical Bulletin, Ann Arbor, Michigan, v.24, 242-252, (June, 1958)

Karnosfsky, D.A., Stock, C.C., Ridgeway, L.P. and Patterson, P.A., The Toxicity of Vitamin B_6, 4-Desoxy-Pyridoxine, and 4-Methoxy-Methylpyridoxine, Alone and in Combination, to the Chick Embryo, Journal of Biological Chemistry, v.182, 471-478, (1950)

_____. Growth-Inhibiting Effect of Cortisone Acetate on the Chick Embryo, Endocrinology, v.48, 596-616, (1951)

Kik-Nes, Kristen, and Brizzee, Kenneth R., Adrenocortical Activity and Metabolism of 17-Hydroxycorticosteroids in Thyroidectomized Dogs, American Journal of Physiology, v.184(2), 371-375, (1956)

Landauer, W., Potentiating Effects of Adrenal Cortical Extract on Insulin-Induced Abnormalities of Chick Development, Enodocrinology, v.41, 489-493, (1947)

Lebland, C.P. and Hoff, H.E., Effects of Sulfonomides and Thiourea Derivatives on Heart Rate and Organ Morphology, Endocrinology, v.35, 229-233, (1944)

Le Compte, P.M., Width of Adrenal Cortex in Lymphatic Leukemia, Lymphosarcoma, and Hyperthyroidism, Journal of Clinical Endocrinology, v.9, 158-162, (1949)

Levin, Marvin E. and Daughaday, William H, Thyroid Adreno-Cortical Interrelations, Journal of Clinical Endocrinology and Medicine, v.15(12), 1499-1511, (1955)

Levy, H., et al., Chemical Transformations of Steroids by Adrenal Perfusion, Journal of Biological Chemistry, v.203, 433-451,(1953)

Lillie, Frank R., The Development of the Chick. (2nd Edition Revised), New York, Henry Holt and Company, 1927.

Means, J.H., ed., The Thyroid and Its Diseases. Philadelphia, J.B. Lippincott, Company, 1948.

Merchant, Donal J., Kahn, Raymond H., Murphy, William H., Jr., Handbook of Cell and Organ Culture, Minneapolis, Burgess Publishers Company, 44-46, 149-152 and 159-163, 1960.

Money, W.L., Kraintz, L., Fager, J., Kirschner, L. and Rawson, R.W., The Effects of Various Steriods on the Collection of Radio-Active Iodine by the Thyroid Gland of the Rat, Endocrinology, v.48, 682-690, (1951)

Moscona, Malka H. and Karnosfsky, David A., Cortisone Induced Modifications in the Development of the Chick Embryo, Endocrinology, v.66, 533-549, (1960)

Needham, J., Biochemistry and Morphogenesis. Cambridge University Press, London, 607-610, 1950.

Pickman, Donald S., Ridley, Alice, Orgel, Michael and Blumenthal, Herman T., Effect of Cortisone on Growth of Chick Embryos During Early Embryogenesis, Endocrinology, v.64, 790-794, (1959)

Pincus, G., Some Basic Hormone Problems, Journal of Clinical Endocrinology and Metabolism, v.12, 1187-1196, (1952)

Schaberg, A., Regeneration of the Adrenal Cortex In Vitro, Anatomical Records, v.122, 205-221, (1955)

Sidman, R.L., The Direct Effect of Insulin on Organ Cultures of Brown Fat, Anatomical Records, v.124, 723-734, (April, 1956)

Siegel, B.V., Smith, M.J., and Gerstl, B., Effects of Cortisone on the Developing Chick Embryo, Archaic of Pathology, v.63, 562-570,(1957)

Strangeways, T.S.T. and Fell, H.B., Experimental Studies on the Differentiation of Embryonic Tissues Growing In Vivo and In Vitro, Proceedings of the Royal Society of Medicine, v.99, 340-366, (1926)

Wilhelmj, C.M., Gunderson, D.E., Shuput, D. and McCarthy, H.H., A Study of Certain Antagonistic Actions of Pituitary Growth Hormone and Cortisone, Journal of Laboratory and Clinical Medicine, v.45, 516-525, (1955)

Wolfson, W.Q., Beietwaltes, W.H., Robinson, I.C., Duff, J.R., Jones, Knorpp, C.T. and Eya, M., Corticogenic Hypothyroidism, its Regular Occurrence and Clinical Significance During Prolonged Therapeutic Administration of ACTH or Cortisone, Journal of Laboratory and Clinical Medicine, v.36, 1005-1006, (1950)

Woodside, G.L., Experimentally Induced Hyper-thyroidism in the Chick Embryo, Anatomical Records, v.67, 423-430, (1937)

Zarrow, M.X. and Money, W.L., Involution of the Adrenal Cortex of Rats Treated with Thiouracil, Endocrinology, v.44, 345-358, (1949)

TABLES

Abreviations

Av.	-	Average
D.	-	Diameter
Eg.	-	Egg, Eggs
Emb.	-	Embryo
Fol.	-	Follicle, Follicles
Gl.	-	Gland
HC	-	Hydrocortisone
Inj.	-	Injection
Lng.	-	Length
S.	-	Section, Sections
Surv.	-	Survival
Unf.	-	Unfertile
Wt.	-	Weight

TABLE IA

EXPERIMENT I (EXPERIMENTAL)

Evaluation of thyroid glands and follicles studied on the 14th day and 10th hour of incubation following administration of 4mg of hydrocortisone on the 7th day and 23rd hour.

No. of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.*	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
E1	4.722	5.7	0.7	23	26	12
E2	6.365	6.5	0.7	23	42	15
E3	(No Embryo)					
E4	4.175	5.1	0.6	(Gland Looks To Be Degenerated)		
E5	7.139	7.0	0.7	27	38	13
E6	4.484	5.5	0.6	(Gland Looks To Be Degenerated)		
E7	4.430	6.4	1.1	24	43	13
E8	(Dead)					
E9	(Dead)					
E10	4.850	5.0	0.5	(I Doubt If It Is Thyroid Tissue)		

* The three diameters of an oblong gland where taken at different angles to represent the nearest diameter of that of a circle.

TABLE IB

EXPERIMENT I (CONTROL)

Evaluation of the thyroid glands and follicles of the control specimens of Experiment I treated with distilled water.

No. of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
C1	(No Embryo)					
C2	(No Embryo)					
C3	7.971	8.6	0.7	30	17	9
C4	8.995	8.7	0.8	40	19	8
C5	8.063	8.6	1.3	34	12	7
C6	(No Embryo)					
C7	10.360	9.3	0.8	36	18	9
C8	7.370	8.3	1.0	41	21	8
C9	11.386	9.3	0.9	39	15	8
C10	9.212	9.0	0.7	25	16	7

TABLE IIA

EXPERIMENT II (EXPERIMENTAL)

Evaluation of thyroid glands and follicles studied on the 15th day and 5th hour of incubation following administration of 4mg hydrocortisone on the 8th day and 5th hour.

No. of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
E11	(Dead)					
E12	6.270	7.6	1.1	36	29	12
E13	5.843	7.5	0.8	27	24	13
E14	(Dead)					
E15	(Dead)					
E16	4.031	5.7	0.6	25	39	16
E17	3.631	5.7	0.7	24	28	13
E18	4.553	6.6	0.7	19	51	23
E19	6.738	7.8	0.8	35	23	11
E20	(Dead)					

TABLE IIB

EXPERIMENT II (CONTROL)

Evaluation of the thyroid glands and follicles of the control specimens of Experiment II treated with distilled water.

No. of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
C11	(Dead)					
C12	12.631	11.7	1.2	36	18	7
C13	15.000	11.5	1.2	41	30	10
C14	(Dead)					
C15	14.222	12.6	1.5	39	23	16
C16	10.360	10.9	1.2	40	29	13
C17	14.180	11.2	1.2	40	25	11
C18	13.584	11.1	1.2	47	18	8
C19	(No Embryo)					
C20	(No Embryo)					

TABLE IIIA

EXPERIMENT III (EXPERIMENTAL)

Evaluation of thyroid glands and follicles studied on the 15th day and 5th hour of incubation following administration of 4mg hydrocortisone on the 8th day and 5th hour.

No. of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
E21	3.865	6.0	0.6	19	31	12
E22	(Dead)					
E23	(No Embryo)					
E24	(Dead)					
E25	(Dead)					
E26	(Dead)					
E27	3.800	5.5	0.6	19	54	42
E28	2.925	4.6	0.6	16	26	11
E29	(Dead)					
E30	(No Embryo)					

TABLE IIIB

EXPERIMENT III (CONTROL)

Evaluation of the thyroid glands and follicles of the control specimens of Experiment III treated with distilled water.

No. of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
C21	(No Embryo)					
C22	10.115	10.2	0.9	41	20	11
C23	11.410	10.0	1.2	43	20	11
C24	8.445	8.3	1.2	43	19	9
C25	9.810	8.8	1.0	41	24	11
C26	6.290	8.2	1.2	39	10	6
C27	7.355	8.6	0.6	40	20	11
C28	10.735	9.3	0.9	39	21	10
C29	7.455	8.7	1.2	45	23	10
C30	8.055	9.0	0.8	39	20	10

TABLE IVA

EXPERIMENT IX (EXPERIMENTAL)

Evaluation of thyroid glands and follicles studied on the 9th day and 18th hour of incubation following administration of 0.1mg hydrocortisone on the 4th day and 18th hour.

No. of Egg.	Weight of Embryo	Length of Embryo	Size of Gland
E102	(Dead)		
E103	(Dead)		
E104	1.335 gm	3.9 cm	0.7 mm
E105	1.480 gm	4.1 cm	0.6 mm
E106	1.427 gm	4.0 cm	0.8 mm

TABLE IVB

EXPERIMENT IX (CONTROL)

Evaluation of the thyroid glands and follicles of the control specimens of Experiment IV treated with distilled water.

No. of Egg.	Weight of Embryo	Length of Embryo	Size of Gland
C102	(Dead)		
C103	2.050 gm	4.7 cm	0.5 mm
C104	1.880 gm	4.5 cm	0.5 mm

TABLE VA

EXPERIMENT X (EXPERIMENTAL)

Evaluation of thyroid glands and follicles studied on the 13th day of incubation following administration of 0.1mg hydrocortisone on the 4th day and 18th hour.

No. of Egg	Wt. of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
E107	(Dead)					
E108	3.900	6.3	0.5	26	15	6
E109	3.640	6.1	0.5	30	10	5
E110	3.385	6.3	0.5	34	12	6
E111	2.585	6.5	0.6	24	11	5
E112	2.663	5.8	0.6	30	14	6
E113	3.663	6.5	0.4	(Lost While Processing)		
E114-E125(Dead)						

TABLE VB

EXPERIMENT X (CONTROL)

Evaluation of the thyroid glands and follicles of the control specimens of Experiment V treated with distilled water.

No.of Egg	Wt.of Emb. in gm.	Lng.of Emb. in cm.	Size of Gl. in mm.	Av.No.of Fol.per D.in 3 Largest S.	Av.D.of 18 Largest Fol. per Gl. in μ	Av.No.of Cells in the 18 Fol.
C105	5.417	8.4	0.5	26	15	5
C106	6.810	8.6	1.0	39	28	8
C107	5.435	8.6	0.8	38	17	7
C108	6.190	8.6	1.2	47	17	8
C109	(Dead)					

TABLE VI

Comprehensive table summarizing tables IA, IB to VA, VB.

No. of Exp.	No. of Eggs	Mg Hc per Egg	No. of Eg.	No. of Unf. Eg.	Age at Inj.	Age at Aut.	Surv. Rate	Av. Emb. Weight in gm.	Av. Emb. Lng. in cm.	Av. Gl. Size in mm.	Av.No. of Fol. per Av. D.	Av. D. of 18 Largest Fol. per Gl. in μ	Av.No.of Cls in t 18 F.
Exp.I	(C1 -C10)	0	10	3	7d 23h	14d 10h	7/7	7.480 (7.370-11.386)+	8.8 (8.3- 9.3)+	0.9±0.2* (0.7-1.3)+	35±5.8* (25-41)+	17±06.4** (10-28)+	1.51** (14)+
Exp.I	(E1 -E10)	4	10	0	7d 23h	14d 10h	7/10	5.219 (4.430- 7.139)	6.0 (5.1- 7.0)	0.7±0.3 (0.6-1.1)	24±2.5 (23-27)	36±19.5 (24-60)	2.89 (19)
Exp.II	(C11 -C20)	0	10	2	8d 5h	15d 5h	6/8	11.949 (10.360-15.000)	11.5 (10.9-12.6)	1.2±0.1 (1.2-1.5)	41±3.6 (36-47)	24±04.3 (14-32)	1.87 (17)
Exp.II	(E11 -E20)	4	10	0	8d 5h	15d 5h	6/10	5.178 (3.631- 6.738)	6.8 (5.7-7.8)	0.8±0.17 (0.6-1.1)	28±6.6 (1.9-36)	29.4±32 (13-72)	2.93 (1.27)
Exp.III	(C21 -C30)	0	10	1	8d 5h	15d 5h	9/9	7.982 (6.290-11.410)	9.2 (8.2-10.2)	1.0±0.2 (0.8-1.2)	41±6.8 (39-45)	20±06.3 (7-25)	3.54 (18)
Exp.III	(E21 -E30)	4	10	2	8d 5h	15d 5h	3/8	3.530 (2.925- 3.865)	5.3 (4.6- 6.0)	0.6±0 (0)	18±1.7 (16-19)	37±41.4 (24-73)	4.51 (68)
Exp.IV	(C31 -C46)	0	16	3	4d	8d	13/13						
Exp.IV	(E31 -E46)	4	16	2	4d	8d	0/14						
Exp.V	(C47 -C55)	0	9	0	4d 2h	8d 6h	7/9						
Exp.V	(E47 -E55)	2	9	0	4d 2h	8d 6h	0/9						
Exp.VI	(C56 -C65)	0	9	0	4d	8d 8h	8/9						
Exp.VI	(E56 -E65)	2	9	0	4d	8d 8h	0/9						
Exp.VII	(C66 -C84)	0	18	2	4d	8d	16/16						
Exp.VII	(E66 -E84)	1	18	1	4d	8d	0/17						
Exp.VIII	(C85 -C102)	0	18	2	4d	8d	15/16						
Exp.VIII	(E85 -E102)	1	18	1	4d	8d	0/17						
Exp.IX	(C102-C104)	0	3	2	4d 18h	9d 18h	2/3	1.965 (1.880- 2.050)	4.6 (4.5- 4.7)	0.5±0 (0)			
Exp.IX	(E102-E106)	0.1	5	0	4d 18h	9d 18h	3/5	1.414 (1.335- 1.480)	4.0 (3.9- 4.1)	0.7±0 (0.6-0.8)			
Exp.X	(C105-C109)	0	5	0	4d 18h	13d	4/5	5.963 (5.417- 6.810)	8.5 (8.4- 8.6)	0.9±0.27 (0.5-1.2)	38±8.7 (26-47)	19±2.7 (13-32)	±1.7 (1-11)
Exp.X	(E107-E125)	0.1	19	0	4d 18h	13d	6/19	3.306 (2.585- 3.900)	6.2 (5.8- 6.5)	0.5±0.1 (0.4-0.6)	31±4.6 (24-34)	13±03.18 (8-18)	±0.64 (1-7)

\+ - Range of Variation
* - Standard Deviation
** - Pooled Standard Deviation

PLATE I

Explanation of Figure

Figure 1. (Experiment I) Shown are 14-day and 10-hour old chick embryos. Lower row are experimentals from eggs injected into the yolk sac at the 7th day and 23rd hour of incubation with 4mg hydrocortisone per embryo. Above row are controls injected with 0.5cc of distilled water each.

PLATE I

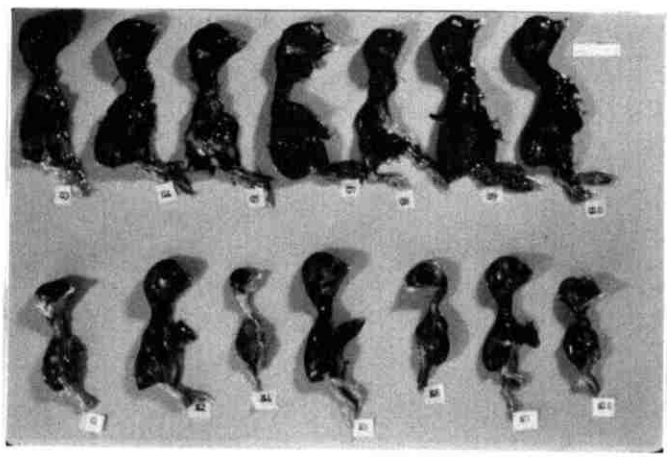


Figure 1.

PLATE II

Explanation of Figures

Figure 2. (Experiment IX) Shown are 9-day and 18-hour old chick embryos. The three smaller ones on the left are experimentals from eggs injected into the yolk sac with 0.1mg hydrocortisone per embryo at the age of 4 days and 18 hours. The two on the right are controls injected with 0.5cc distilled water each.

Figure 3. (Experiment X) Shown are 13-day old chick embryos. The four on the left are experimentals from eggs injected into the yolk sac at the 4th day and 18th hour with 0.1mg hydrocortisone per embryo. The three on the right are controls injected with 0.5cc distilled water each.

PLATE II

Figure 2.

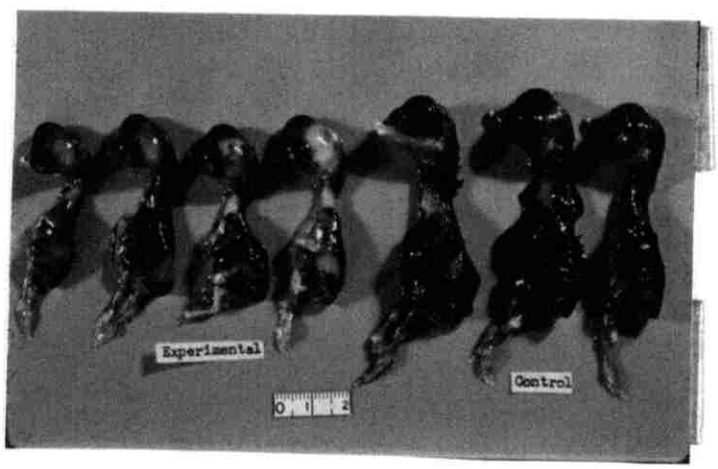


Figure 3.

PLATE III

Explanation of Figures

Figure 4. (E18) A thyroid section showing larger than normal follicles from a 15-day and 5-hour chick embryo thyroid after 4mg hydrocortisone administration on the 8th day and 5th hour of incubation. Periodic Acid Schiff and Hematoxylin x 560.

Figure 5 &6. (E21 and E27) Thyroid sections showing degeneration of follicles from 15-day and 5-hour chick embryo thyroids after the administration of 4mg hydrocortisone per each on the 8th day and 5th hour of incubation. Hematoxylin and Eosin x 560.

Figure 7. (E28) A thyroid section showing large follicles with distorted shapes from a 15-day and 5-hour chick embryo thyroid after the administration of 4mg of hydrocortisone on the 8th day and 5th hour of incubation. Hematoxylin and Eosin x 560.

Figure 8. (C15) A thyroid section showing normal thyroid tissue from a 15-day and 5-hour chick embryo after the injection of 0.5cc of distilled water into the yolk sac on the 8th day and 5th hour of incubation. Periodic Acid Schiff and Hematoxylin x 560.

Note: PAS stained sections from eggs E21, E27 and E28 were not possible due to unfavourable circumstances.

PLATE III

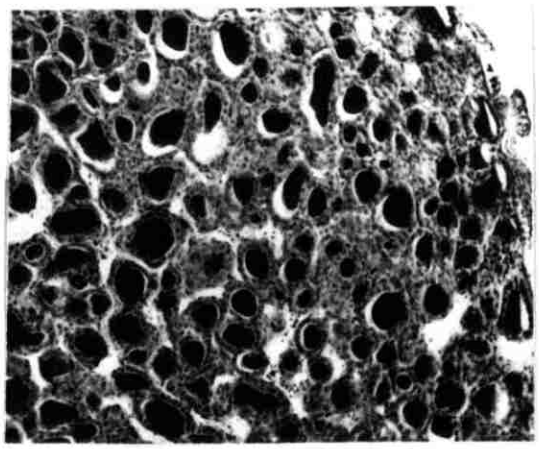
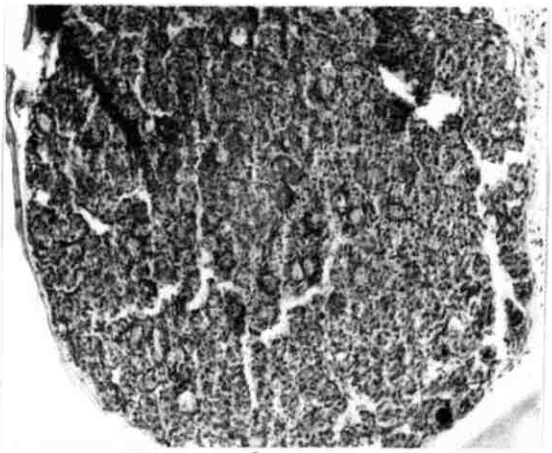


Figure 4.

Figure 5.



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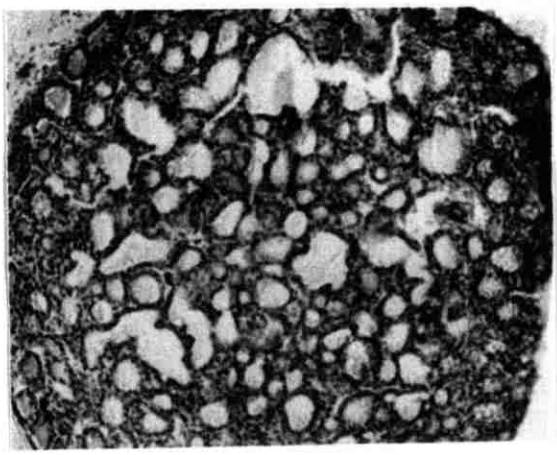

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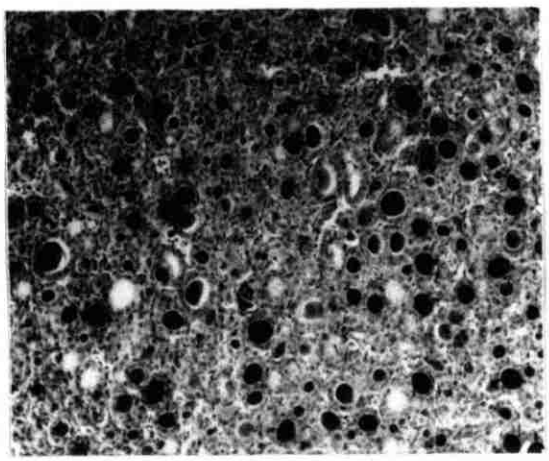


Figure 8.

PLATE IV

Explanation of Figures

Figure 9. (E108) A thyroid section showing a lot of embryonic blood cells running between the follicles from a 13-day old chick embryo after 0.1mg hydrocortisone administration on the 4th day and 18th hour of incubation. Periodic Acid Schiff and Hematoxylin x 560.

Figure 10. (E108) As Figure 9 but under higher power. Oil Imersion x 5600.

Figure 11. (E111) A thyroid section showing lower than normal colloid amount from a 13-day old chick embryo after 0.1mg hydrocortisone administration on the 4th day and 18th hour of incubation. Periodic Acid Schiff and Hematoxylin x 560.

Figure 12. (C106) A normal thyroid section from a 13-day old chick embryo after the injection of 0.5cc of distilled water given on the 4th day and 18th hour of incubation. Periodic Acid Schiff and Hematoxylin x 560.

PLATE IV

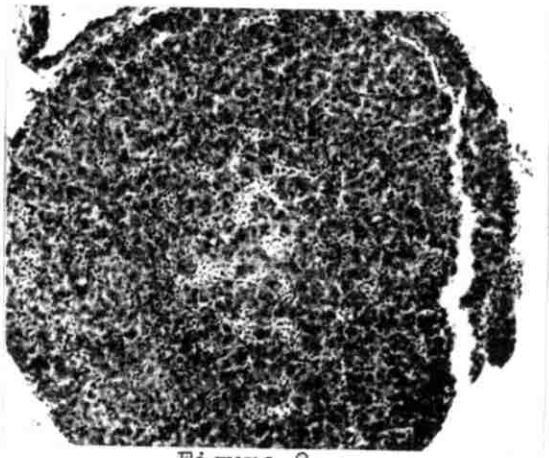


Figure 9.

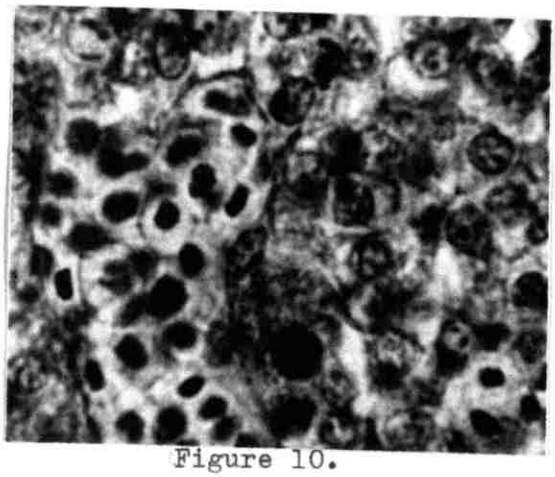


Figure 10.

Figure 11.

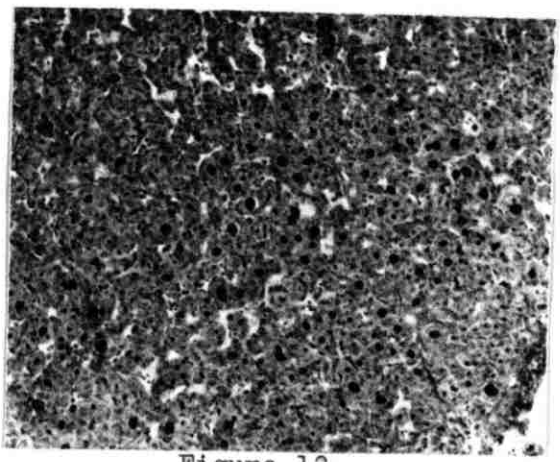


Figure 12.

PLATE V

Explanation of Figures

Figure 13. A thyroid section from a 9-day old normal chick embryo. Hematoxylin and Eosin x 2240.

Figure 14. A thyroid section from a 14-day old normal chick embryo. Hematoxylin and Eosin x 2240.

Figure 15. A thyroid section from a 16-day old normal chick embryo. Hematoxylin and Eosin x 2240.

PLATE V

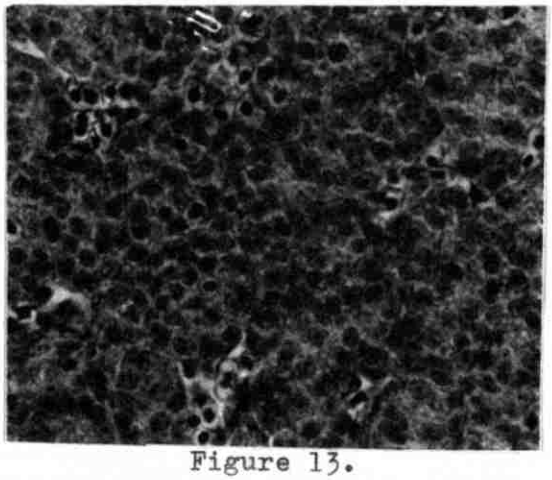
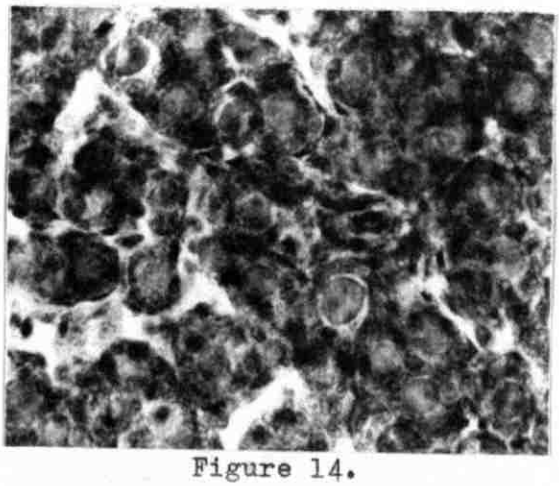


Figure 13.



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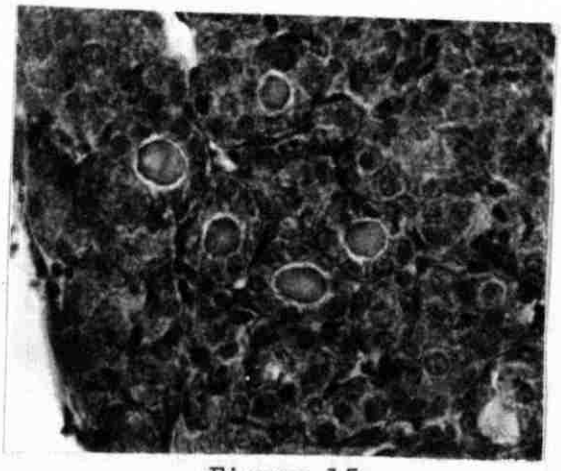

Figure 15.

PLATE VI

Explanation of Figures

Figure 16. A section from a 9-day old chick embryo thyroid that was cultured for two days in 30 γ hydrocortisone per 100cc of total medium showing colloid. Periodic Acid Schiff and Hematoxylin x 2240.

Figure 17. A section from a 9-day old chick embryo thyroid that was cultured for six days in 30 γ per 100cc of total medium showing colloid. Periodic Acid Schiff and Hematoxylin x 2240.

Figure 18. A section from a 9-day old chick embryo thyroid that was cultured for two days in normal medium showing colloid. Periodic Acid Schiff and Hematoxylin x 2240.

PLATE VI

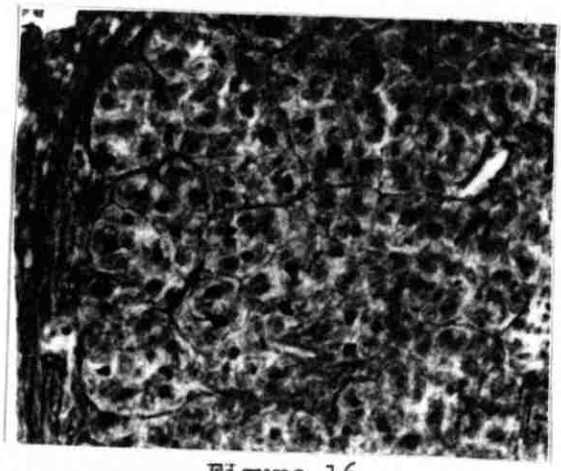

Figure 16.

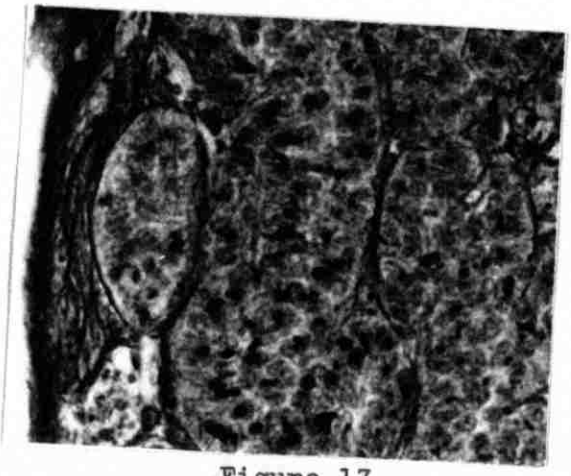

Figure 17.

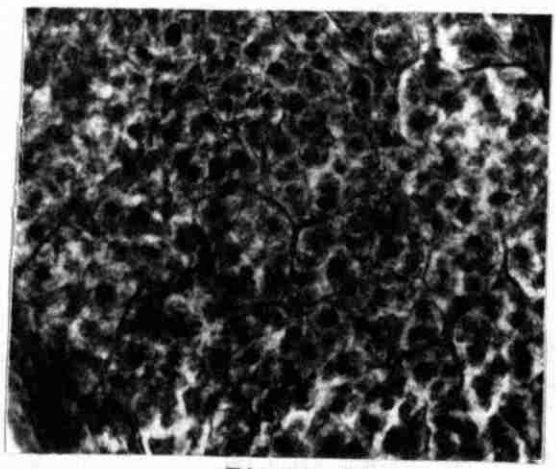

Figure 18.

PLATE VII

POSSIBLE MODE OF ACTION OF HYDROCORTISONE