A STUDY OF THE EFFECTS OF THYROXINE ON THE EARLY EMBRYONIC DEVELOPMENT OF *Brachydanio rerio* (Hamilton and Buchanan)

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By

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A STUDY OF THE EFFECTS OF THYROXINE ON THE EARLY EMBRYONIC DEVELOPMENT OF *Brachydanio rerio* (Hamilton and Buchanan)

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INTRODUCTION

On entering the field of physiological embryology, one is amazed at the tremendous amount of data accumulated in the last half century. Especially in the area of endocrinology has the experimental study been intense. However, the effects of hormones on the early development of fish embryos appears to be an almost virgin field. Few attempts have been made to determine the physiological effects of internal secretions before the glands of secretion themselves have become active.

The objective of the study reported herein was an attempt to alter, by retardation or acceleration, the early development of the embryo of the zebra fish, Brachydanio rerio. To gain an insight into mitosis and pigmentation, thyroxine solutions of different concentrations were applied for varying lengths of time to 1216 fish embryos in the early stages of development.

REVIEW OF THE LITERATURE

The functional activity of the thyroid gland and its hormone has fascinated a multitude of observers. The hormone is called iodothyroglobulin. Thyroxine is an effective amino acid constituent of this protein molecule, and iodine forms an essential atom of the amino acid component. It is believed that the Chinese, many centuries before Christ, had learned by trial and error that substances now known to contain iodine exerted beneficial effects upon the thyroid (Turner, 1950). Burnt sponge and seaweed were added to the diet during the time of Hippocrates (460-370 B.C.) in order to relieve enlarged thyroids. Juvenal's words "Quis tumidum guttur miratur in Alpibus" bespeak recognition of the thyroid for over two thousand years. Aristotle (384-322 B.C.) spoke of the thyroid gland. "Pliny the Elder (23-79 A.D.) suggested that impure water might be the cause of goiter. Galen (131-201) described the thyroid and believed it provided a lubricating fluid for the larynx." The Chinese, again about 600 A.D., treated cretinism with sheep thyroids whereas Roger of Palermo in 1180 used the ashes of ground sponges and seaweed. Vesalius in 1543 gave a full description of the gland, and Thomas Wharton (1614-1673) supplied its current name (Hamblen, 1945).

The discovery of iodine in 1811 by Courtois led shortly to its use in the treatment of goiter by Coindet. Shortly before 1900, the view that iodine is an essential atom of the protein molecule synthesized by the thyroid began to take form. Since 1900, investigations have produced a mass of literature on the physiologic importance of the thyroid hormone (Hamblen, 1945; Turner, 1948).

The first isolation, by Kendall in 1914, of crystalline thyroxine from thyroid tissue accelerated further interest resulting in added information to the already voluminous literature. Magnus-Levy's classical observations reported in 1895, subsequently confirmed by laboratory experimentation, led to the concept that the thyroid regulated the rate of oxidation (Needham, 1950). Fleischmann (1951) observed that the effects of the thyroid hormone is restricted to the Phylum Chordata. The thyroid gland of the adult lamprey consisting of isolated follicles represents the most primitive anatomical condition of the gland (Leach, 1946; Goldsmith, 1949). The theory that the endostyle in the larval or ammocoetes stage is a primitive thyroid mechanism concerned with iodine metabolism has recently been corroborated through studies using radioactive iodine (I^{131}) as a tracer. Specific cells of the endostyle of ammocoetes of the free-living lamprey Entosphenus lamottei are capable of storing radioactive iodine. "It seems

significant that endostylar tissue actively removes inorganic iodine from its fluid environment and stores it intracellularly even before it has begun its differentiation into thyroid tissue" (Gorbman and Creaser, 1942). Metamorphosis from the larval or ammocoetes stage to the lamprey is not accompanied by an increase in oxygen consumption. It has been further shown that ammocoetes do not respond to administration of mammalian thyroid (Fleischmann, 1951). Neither the rate of metamorphosis nor that of metabolism is affected. The theory has been proposed that the thyroid in the lower vertebrates is concerned mainly with growth and maturation (Leach, 1946).

The endostyle found in the lowest chordate animals -- tunicates and acraniates -- was long believed to be homologous and analogous to the vertebrate thyroid gland. This idea has been discarded more recently for morphological reasons (Hyman, 1942) and because of the fact that neither the endostyle of the tunicate, Perophora annectens (Gorbman, 1941), nor that of the acraniate, Amphioxus (Gorbman and Creaser, 1942) is capable of storing radioactive iodine.

In most fishes the thyroid consists of follicles scattered in the connective tissue along the ventral aorta and afferent branchial arteries (Fleischmann, 1951).

Grobstein and Bellamy (1939) reported a decreased growth rate and altered body proportions as a result of feeding sexually immature fishes desiccated mammalian thyroid. It should be recalled, however, that a decreased growth rate does not necessarily signify a retardation of differentiation according to Needham (1950). Hoadley (1929) has shown by transplantation of eye cups of the chick embryo to the chorio-allantoic membrane that a suppression of cell division was produced, but histogenesis continued. Differentiation, said Hoadley, "is not primarily dependent upon any mechanism involved in specific cell-divisions, but to a large extent takes place independently of these. Inasmuch as this is true, and inasmuch as typical development depends not only on the subordinate differentiation of the constituent parts of the embryo, but also on the spatial relations between them and their size, the truth of the following statement is evident - 'typical development is the result of the usual balance between morphogenetic (form producing) and histogenetic (cell differentiation) processes'."

Turner (1948) citing Gundenatch (1912) stated, "While thyroid feeding accelerates differentiation, growth is definitely retarded". Studies seem to indicate (Allen, 1938) that the hastening of metamorphosis (in Rana catesbiana) is not due merely to a stimulation of metabolic rate.

Allen (1918) in a statement of the converse principle of Gudernatsch's work maintained that the ablation of the thyroid rudiment prevents the metamorphosis of amphibian larvae. Such thyroidectomized tadpoles, though not metamorphosing into adults, grow large and eventually develop lungs and reproductive organs.

"Growth, on the other hand, without differentiation is likely to occur whenever there is any failure of the formation or liberation of primary or secondary organizers" (Needham, 1950). The discussion of Kyle (1921) may be cited as an example of this. He found that flat fishes occasionally fail to turn over, and thus retain their bilateral symmetry throughout their lives.

It is also noted that metabolism without growth and differentiation in embryonic cells has occurred. It is not so generally known that something analogous to diapause occurs in mammalian development. According to the work of many observers, reviewed by Hamlett (1935), the embryos of many mammals pass a considerable proportion of their intra-uterine life in a state of suspended development. There can be little doubt but that some metabolism is proceeding also during this "diapause". In reptiles, embryonic hibernation is found in the case of the tuatara lizard, Sphendon (Dendy, 1898), and the pond tortoise, Emys (Boulenger, 1898). "It is said that the blackbird, for instance, lays one egg each day for a week, but although sitting has begun from the first day, all the eggs hatch out together" (Needham, 1950).

Richardson (1933, 1940, 1945) on regeneration in Triturus found that an excess of thyroxine retards regeneration, while absence of the thyroid increases the rate of regeneration. Richardson's work is further corroborated by that of Manner (unpublished manuscript) who stated, "The removal of the thyroid results in an increase in the rate of regeneration. This leads us to conclude that thyroxine is an inhibitor to growth. If we hold our assumption, then we must also hold that the regeneration rate increases inversely to the metabolic rate." Manner also reports the retardation of the formation of new cells as a result of the presence of thyroxine.

It is pertinent also to indicate an imbalance between the rates of growth and respiration. X-radiation, for example, at a certain level will abolish growth, but cause no change in the intensity of respiration or fermentation; while at another level it will exercise an inhibiting influence on metabolic processes as well (Needham, 1950).

Needham (1950), citing various authors (Schlenk, 1933, and S. Smith, 1936, on Salmo; Trifonova, 1934, 1935, on Perca; Amberson and Armstrong, 1933, and Phillips, 1940, on Fundulus; Bezler, 1939, on cyprinids) stated, "As would be expected from the growth of the embryo at the expense of inert yolk, the oxygen consumption of the whole egg rises regularly throughout development." In this same discussion, Needham gives the succession of energy sources in teleostean fishes as carbohydrate preceding protein and preceding fat.

It has been reported by Etkin, Root and Mofshin (1940) and Matthews and Smith (1947) that fish do not respond with a clearcut increase in metabolism after thyroid feeding. Fleischmann (1951) stated that most fish are insensitive to thyroxine. Root and Etkin (1937) found that thyroxine injected in doses of 10 mg. per kilogram body weight daily for four to five days had no effect on the oxygen consumption of the toadfish, Opsanus tau. Smith and Everett (1943) confirmed the opinion that thyroid preparations fail to enhance oxygen consumption or somatic growth in the guppy, Lebistes reticulatus.

The problem of metamorphosis in fishes is a complex one. Sklower (1928) and Hagen (1936), cited by Needham (1950) described marked hyperplasia of the thyroid in the larvae of the eel at the time of transformation from leptocephalus to elver. A similar change occurs in the thyroid of the flatfish, Pleuronectes platessa, during metamorphosis (Sklower, 1930). In the tropical goby, Periophthalmus chrysospilos, metamorphosis can be accelerated by thyroid feeding. It is of interest that this fish normally leads an amphibian life. After administration of desiccated thyroid it lived less in water and more on land than the untreated controls. (Fleischmann, 1951).

"The most classical case of a specific morphogenetic stimulation produced by a glandular hormone is no doubt the effect of thyroxin upon amphibian metamorphosis discovered by Gudernatsch

and investigated by many workers, such as Huxley, 1925. After the establishment of the circulation and the development of the thyroid gland, thyroxin is poured into the blood at the time of metamorphosis. The processes of growth and differentiation which form the hind-limbs are thus activated, the resorption of the tail and gills takes place, and a host of other changes, such as performation of the operculum (in anurans) and the development of the outer ear, follow. All these changes are not due to the specific action of thyroxin, though they take place much earlier than they would normally if thyroxin is fed. Many of them involve local specific stimuli of the nature of third- and fourth-grade organizers. It is likely, however, that most of them are to some extent under the thyroid control." (Needham, 1950).

Salmon (1938) proposed the following while working with rats. "It may well be that the body tissues as the end organs of hormonal actions need to be primed with thyroid before they can respond to pituitary growth hormones."

Etkin (1935), on the other hand, reported that neither the size of the animal nor the presence or absence of the thyroid gland in later tadpole stages plays any significant role in the nature of the response.

Needham (1950) gave in a diagram the effects of thyroxin as an accelerator of metabolism and an accelerator of amphibian metamorphosis (Gudernatch, 1912; Huxley, 1925). It is also known

that in mammals thyroxine considerably raises the basal metabolic rate, but has little or no effect upon the isolated tissue-slice (Dodds, 1935). Thyroxine also increases the gaseous exchange and probably the heat production of the tadpole (Huxley, 1925; Schwartzbach and Uhlenluth, 1936).

In the amphibian, Rana pipiens, investigators have found that the rate of metamorphosis in tadpoles is, within broad limits, proportional to the concentration of the thyroxine solution (Schwanger, 1951). Etkin (1935) stated that the rate of response to thyroxine in anuran metamorphosis is a function of the concentration. As the concentration is decreased, the time required for a given event at first rises slowly, then more abruptly, also (Etkin, 1950) that the acquisition of sensitivity in Rana pipiens appears to occur in all tissues at about the same time.

Stein (1951) in a preliminary study of the effects of temporary immersion in thyroxine on larvae of Rana pipiens, indicated that the metamorphic effect of thyroxine is proportional to the length of time the embryos were subjected to thyroxine. He further stated that the mechanism by which thyroxine is absorbed and stored in the embryo is obscure. The animals' thyroid glands are still in an undifferentiated state during the periods of exposure so there is no reason to suppose that they are the foci of storage of the

hormone as they are later in the life time of the animals. It is possible that the proteins of the embryo generally collect thyroxine which is an amino acid and store it for later use.

Etkin (1935) stated that there was little difference in sensitivity among fully grown tadpoles of Rana cantibrigensis although differences which appeared indicated a slower response on the part of the fully-grown animals. This was further corroborated by Schwanger (1951) in a preliminary investigation on Rana pipiens. Schwanger, using half-grown tadpoles (18 to 20 mm. in length) and tadpoles designated as small (10 to 10 1/2 mm. in length) found little difference in thyroxine sensitivity due to age. Allen, B. M. (1932) working with Rana arvalis, on the other hand, found that the influence of thyroxine in solution is proportional to the age of the tadpole, the larger animals showing the more rapid metamorphosis.

Newly-born guppies, either placed in water to which thyroxine was added daily or fed dessicated thyroid powder from birth showed, over a period of 50 to 90 days, no change in their growth rates as compared to non-treated fish . Also, male guppies, when fed dessicated thyroid powder for a week or more, showed no change in their rate of oxygen consumption. (Smith and Everett, 1943).

Beyer (1951), working with eggs from a barred columbian stock of Gallus domesticus, reported no significant increase in the

metabolic rate of the chick embryo during any part of its embryonic development as a result of injected thyroxine at the preincubation stage.

With regard to the effects of thyroxine on the cardiovascular system, tachycardia is not dependent upon nervous connections but appears to be due to a direct and persistent effect of the hormone upon the cardiac musculature. "The excised heart of an animal given thyroxine in excess, beats at the increased rate when perfused, or transplanted to the body of another animal. Also, fragments of heart muscle of a two-day-old chick embryo pulsate at a more rapid rate when thyroxine is added to the nutrient fluid. The high pulse pressure seen in hyperthyroidism is due to the general vasodilation combined with an increased stroke volume. The increased circulation rate is chiefly the result of the higher metabolic rate". (Best and Taylor, 1945).

Lerman, Clark and Means (1933, 1934) arrived at essentially the same conclusions. These writers found that deficiencies or excesses of thyroxine are paralleled by profound impairments in the circulatory mechanism; that most of the vascular abnormalities probably result secondarily from the lowered or heightened metabolic rate; and that in hyperthyroidism, the heart enlarges, and its rate and amplitude are diminshed.

The investigations of Gibson and Harris (1939) and Thompson (1926) led them to conclude that under conditions of hyperthyroidism,

the mass movement of blood is less than normal. If an excess thyroxine is present, the heart rate is quickened, the peripheral vessels dilate, and an increased mass movement of blood ensues. In thyrotoxicosis there is a tendency for the blood volume to be increased above normal, while the reverse condition prevails in cases of thyroid deficiency. (Gibson and Harris, 1939; Thompson, 1926).

During normal development, the rate of heart beat seems to change fairly regularly. In fish embryos, it increases from 16-20 in Pristeurus melanostoma five mm. in length, to 27-29 at 10 mm. in length and 43-45 at 17 mm. in length (S. Paton, 1909). The heart rate of trout at hatching has been recorded by Preyer and Kollicker (1921) at 50 to 72 and by Babak and Hepner (1912) to be 100. Similar increases in heart rates were reported by Anderson (1929) and Preyer and Kolliker (1921) on embryos of the lizard Lacerta agilis (Needham, 1931). Romanoff and Vizbara (1929) studying young chicks also observed an increase in heart rate with age. The foregoing results seem to be in agreement with Prosser's (1950) general statement regarding fish embryonic heart rates, "The rate of heart beat in a fish embryo increases as the embryo nears the time of hatching."

"The subject of melanin pigmentation is still in an unsatisfactory state, since apart from histological and morphological studies, which,

alone are incapable of advancing it much (e.g. Thumann, 1931, on fishes; Dorris, 1938, on the chick; du Shane, 1934, on amphibia; Peck, 1931, 1934, on the rabbit; Makarov, 1929, 1931, on the chick embryo retina), we know practically nothing of the nature or origin of the substrate of tyrosinase in embryos, nor what mechanism controls the formation and deposition of melanin" (Needham, 1950).

From a genetic standpoint, it should be indicated that melanin formation is due to the action of certain genes in the normal chromosomal make-up of the organism.

"The enzyme, tyrosinase, converts tyrosine into 3,4-dihydroxy-phenylalanine, or dopa, which is an intermediary in the production of the insoluble brown or black melanin pigments of the skin, hair, iris, and choroid, and pigmented layer of the retina. The melanins are colloidal polymerized oxidation products of 3,4-dihydroxyphenyl-alanine, which is closely related to adrenaline and may be a precursor of this hormone. Melanins can also be formed from adrenaline and tyramine by phenol oxidases. Typical melanin contains an indole nucleus formed by union of the alpha-amino nitrogen with the benzene ring; other dark pigments classified as melanins do not contain nitrogen. In epidermal melanoblasts, melanin formation is accelerated by dopase; this enzyme is also in the kidney and the liver." (Everett, 1946).

The presence of a melanophore dispersing hormone (intermedin) should be mentioned. "It has been found that the hypophyseal cells

of the chick begin to release detectable amounts of intermedin on the fifth day of incubation, much in advance of the onset of any cytologic differentiation that can be interpreted as indicative of secretory competence. Intermedin is present in the hypophyses of all classes of vertebrates and it is instrumental in regulating the chromatophores of fishes, amphibians and reptiles. Though present in plentiful amounts in the hypophyses of birds and mammals, this hormone appears to have no effect upon the pigmentation of these forms". (Turner, 1950).

The origin of melanophores in frogs and birds has shown to be the tissues of the neural crest (du Shane, 1948; Bourne, 1951). This has been further corroborated by the work of Saunders (1949) on chick embryos. Our knowledge of their origin in mammals is less complete, but experimental evidence is consistent with what we may call the neural crest hypothesis (du Shane, 1948). Borcea (1909), cited by du Shane (1948), reported that the migratory cells in the embryos of several species of bony fishes detached themselves from the junction of the neural tube and epidermis to migrate either laterad between the somites and epidermis or ventrad between nerve cord and somites. In one species, pigment granules developed in the migratory cells only after they had reached their definitive position. In others, the cells were already loaded with pigment at the time of their separation from the epidermis and nerve cord.

At that time, 1909, the common concept of pigment cells was that they were modified connective tissue cells. In view of this, it is curious that Borcea published no more than a two-page paper, without illustration, in support of his novel interpretation and that he did not specifically identify the source of these cells as the neural crest. There is, even today, no other account of pigment development in fishes which points to a neural crest origin for chromatophores. The only possible exception, perhaps, is that of the transplantation experiments of Lopashov cited by Openheimer (1949), and her atypical pigment-cell differentiation in embryonic Fundulus grafts and isolates. Lopashov, according to Openheimer, confirmed the neural crest origin of pigment-cells in fishes of three species. Openheimer's production of chromatophores by grafts and isolates does not negate the neural crest hypothesis, but suggests that under certain experimental conditions that pigment-cells can be differentiated by cells which normally do not contribute to the teleostean counterpart of the neural crest.

The effects of thyroxine on pigmentation in birds has received considerable study. Not all species of birds are equally sensitive to thyroxine administration. Domesticated birds, such as chickens and pigeons, are more sensitive to exogenous thyroid hormone than are wild birds, such as the common crow, Corvus corone (Zawadowsky

and Rochlina, 1927). Even large doses of thyroid do not produce molting in crows, although considerable depigmentation of feathers occurs. (Fleischmann, 1951).

The mechanism of action of thyroxine on the plumage has been studied by Lillie and Juhn (1932). They injected thyroxine into fowls at definite time intervals after plucking. In this way, regenerating feathers of known age were exposed to the hormone, and a record was obtained, in the definitive feather, of the physiological events taking place during tis formation from the feather germ. With increasing doses of thyroxine, black pigment appeared. Barbule formation and pigment deposit followed each other in order of increasing thyroxine concentration. Barbule formation may occur alone, but pigment formation is always associated with barbule formation. It should be noted in this connection, however, that thyroxine administered to a white leghorn cock produces no modification in color from the normal white (Juhn, 1933).

According to Turner (1950), thyroid activity produces marked alteration in the pigmentation of the feathers of chickens, but these changes appear to be quite variable. In some forms the color change is in the direction of loss of pigmentation, but in others excessive thyroid causes increased pigmentation of the plumage.

There is evidence that feathers on the various parts of the body respond differently to thyroid excess. In the English sparrow (Miller, 1935), for example, the brown contour feathers and the black breast feathers fail to deposit brown pigment normally, and

the feathers appear slate grey. On the other hand, the light grey feathers of the abdominal region of the male, regenerating after thyroxine injection, become excessively pigmented and appear darker than normal.

Perhaps the most interesting work on pigments in ontogenesis is that of Faris (1926) on a peculiar brownish pigment found in the tissues of amphibia. Some of its characteristics would cause it to be classed as a melanin. Working with the embryos of Ambystoma microstomum, Faris counted the pigment bodies in the myotomes, and found that they increased in number with the age of the embryo. The amount of pigment increased as the yolk decreased, and varied directly with the degree of differentiation and development of function. On the other hand, simple growth, or proliferation of cells, was not accompanied by any increase in the amount of pigment. Later, when the myotomes and central nervous system were penetrated with blood-vessels, the pigment began to decrease in amount, and eventually disappeared completely before the onset of metamorphosis. Faris concluded that there was a definite relation between pigment production and "differential metabolism", but not "growth metabolism".

Fleischmann (1951) cited Iljin (1932) as follows: "The rabbit the only mammal in which changes of pigmentation due to exogenous thyroid hormones have been reported. Iljin found that desiccated thyroid fed in large doses (0.56 g. daily) produced growth of white hair instead of black on the ears of the black Russian rabbit".

In Fundulus embryos, Wyman (1924) reported, "Thyroid powder had no effect on melanophores while the embryo was still in the egg membranes, but caused contraction of the melanophores of the larva of the embryos which had been removed from the shell." Wyman described the embryo as a developing Fundulus which had not emerged from the egg membranes.

Langrebe (1941), working with the brown river trout and the silver sea trout, both regarded as the same species, Salmo trutta, that the brown trout turned silvery after one month of bi-weekly injections of thyroid extracts equivalent to one gram of fresh tissue. Also, he reported the disappearance of parr marks in Salmon after two months of bi-weekly injections of thyroid extracts. The age of langrebe's experimental fish was 18 months.

Thiourea and related substances have been shown to affect thyroid function: in the amphibians, Gordon, Goldsmith and Charipper, (1943), Hughes and Astwood (1944); in the chick, Mixner, Reineke and Turner (1944); Astwood, Sullivan, Bissell and Tyslowitz (1943); in the rabbit, Baumann, Metzger and Marine (1944); and in the hyperthyroid human, Astwood (1943), Williams and Bissell (1943), Himsworth (1943). Treatment with this drug is followed by an enlargement of the thyroid gland which is accompanied by a fall in the metabolic rate. These effects are ascribed to an influence by thiourea upon the synthesis of normal thyroid hormone (Keston, Goldsmith, Gordon, and Charipper, 1944; Franklin, Lerner, and Chaikoff, 1944). In the young rat, administration of thiouracil

(Hughes, 1944) or thiourea (Goldsmith, Gordon and Charipper, 1944) results in retardation of growth.

In immature fish, Platypoecilis maculatus ✕ Xiphorus hellerii, treated with a 0.033 percent solution of thiourea, growth was inhibited and there was a failure in the development of secondary sex characters. It appears that thiourea produces these effects by interfering with thyroid hormone production in much the same manner as reported for the mammal. (Goldsmith, Nigrelli, Gordon, Charipper, and Gordon, 1944).

MATERIALS AND METHODS

In many respects the zebra fish, Brachydanio rerio, is admirably suited for the production of eggs for embryological research. A native of India (Ceylon, Bengal, and Madras) the danio is a tropical representative of the family Cyprinidae.

This species is a small (maximum length, two and one-half inches), highly colored, cannabalistic fish which will spawn every month in the year. The longevity of the danio is approximately two years. The spawning act of this fish is easily recognized by the intense activity of both sexes. Several males in pursuit of a female heralds the presence of precleavage eggs in the bottoms of aquaria. Spawning begins within one hour after sunrise and may continue for an hour thereafter. Usually a prolific female lays 50 to 100 eggs. Each female will spawn every three to five weeks. Since all the females do not spawn on the same day, 100 to 300 eggs may be collected each day when a sizable number of breeding fish is maintained. The writer has maintained 150 to 200 breeding fish throughout the study. This numberwas distributed into six tanks, each tank containing from 35 to 40 fish.

The transparent, teleoecithal egg of the danio yields to detailed study in toto. The oil globule is absent and the yolk is homogeneous. The eggs are non-adhesive, heavier than water, and

measure an average of 625 microns in diameter. The clear, colorless, watery substance between the egg proper and the chorion allows maximum vision from any desired aspect.

The danio egg is characterized by pronounced protoplasmic streaming through the yolk material in its precleavage stage. The duration of precleavage is 45 minutes at 27 degrees Centigrade and ensuing cleavages occur at intervals from 17-20 minutes after the first cleavage (Roosen-Runge, 1939; confirmed by Jones and Penrod, unpublished data). The meroblastic cleavage produces large easily recognizable blastomeres. Each cleavage is recognized first by a slight indentation in the protoplasmic mass, designated as the cleavage furrow. The furrow becomes progressively deeper until after a lapse of three minutes two distinct daughter cells may be distinguished. The tenth cleavage is attained in three hours at 27 degrees Centigrade (Roosen-Runge, 1939). The rapidity of cleavages and morphogenesis produces a hatched, free-swimming fry, with a small amount of yolk, withinan average of 72 hours after fertilization (27 degrees Centigrade).

Throughout the period of study the breeding fish were kept at maximum egg production by feeding a variety of foods four or five times daily. Commercially prepared fish foods were used as the basic diet. This diet was supplemented daily with live food (dwarf white worms and brine shrimp), strained (infant) canned food (liver and veal), and hard boiled egg yolk.

All aquaria were kept at a constant temperature, 27 degrees Centigrade, by the use of a thermostat in one tank, which controlled the heaters in each of the other tanks. Dechlorinated water, tap water allowed to stand in open containers for 24 hours, was used as the initial water for breeding tanks. The water lost in the siphoning procedures was replaced each time with fresh tap water.

All tanks were placed in a north window and protected from draft and excess sunlight by covering the lower half of the window with cardboard. This cardboard covering proved to be an additional asset. The removal of the cardboard from the window shortly after sunrise and an artificial sunlamp seemed to stimulate spawning due to the abrupt increase in light intensity.

The aquarium water, six inches in depth, was constantly aerated by the use of a small pump. The bottom of the tanks were covered with a layer of marbles which protected the eggs from egg-eating fish.

The collection of eggs was accomplished by the use of a siphoning apparatus (Plate I and Fig. I) designed in the laboratory of Dr. Roy W. Jones. By systematically and slowly moving the siphoning tube over the entire bottom of the tank all eggs were collected. As shown in Plate I, a strainer with sufficiently small guage to retain the eggs was used to separate tank debris from the eggs. A small pipette and a wide field binocular dissecting

Plate I

Method of Siphoning Eggs from the Bottom
of the Aquarium

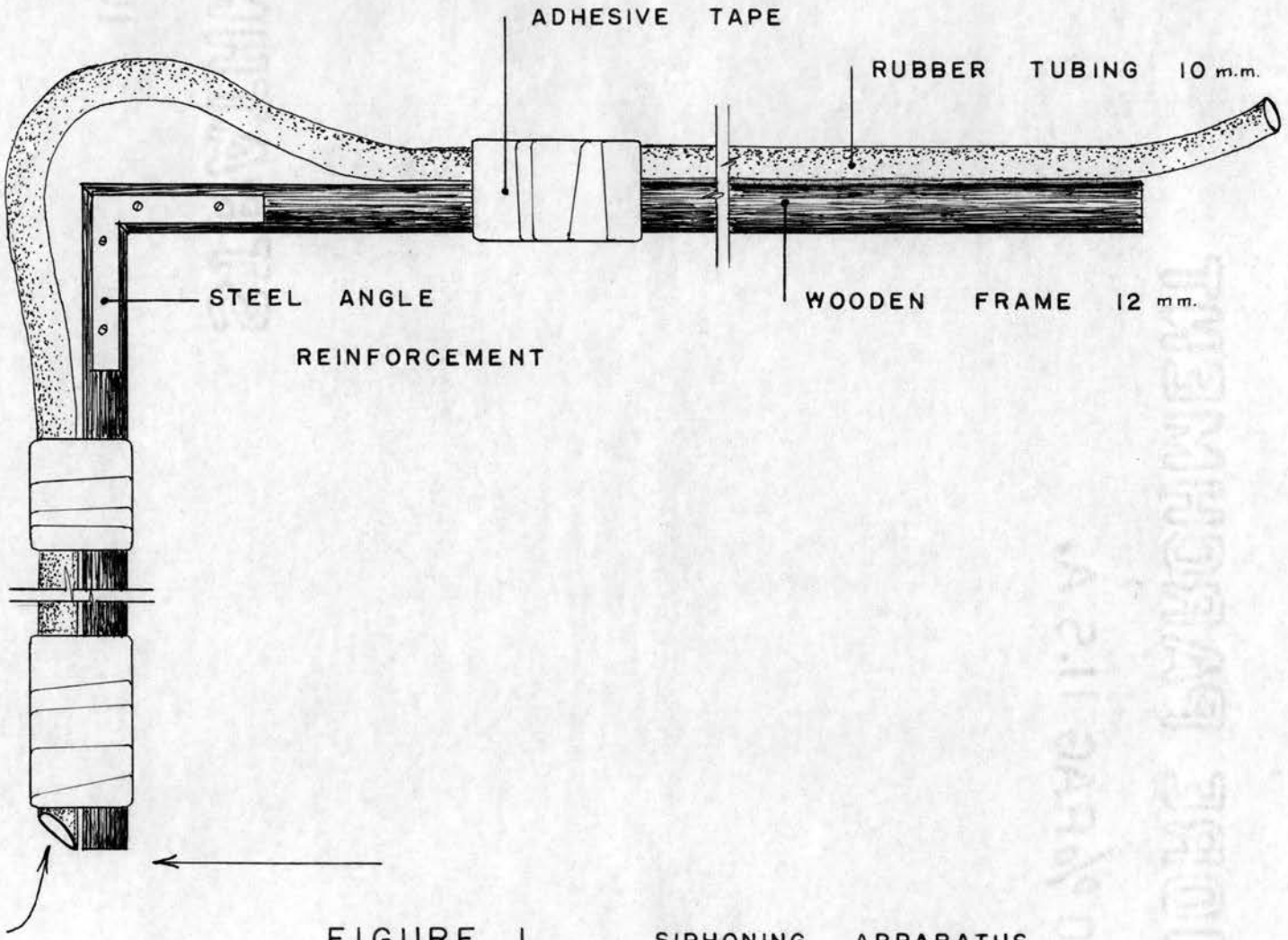


FIGURE 1. SIPHONING APPARATUS

microscope was used to remove the eggs from the strainer. Following removal from the strainer, all eggs were washed in three changes of aged tap water to remove debris and mold spores. The eggs, now ready for experimentation were placed in finger bowls four inches in diameter and containing 150 milliliters of thyroxine solution, thyroid powder solutions, thiouracil solution or dechlorinated water (control). To avoid overcrowding not more than 25 eggs were placed in each finger bowl. Separate pipettes were used with each different concentration of solution to prevent contamination.

The thyroxine used during the study was manufactured by the Delta Chemical Works, New York, New York. Fresh solutions were prepared every thirty days. Solutions with concentrations of 1:1,000,000, 1-10-25-50-100-500 ppm were prepared with aged tap water. Thyroid powder used in this study was furnished by Eli Lilly Co., Indianapolis. Solutions were prepared in the same manner as thyroxine and in concentrations of 25 and 50 parts per million.

Thiouracil, manufactured by the Nutritional Biochemical Corporation, Cleveland, Ohio, was used for the study herein reported. Solutions with concentrations of 50 ppm of thiouracil were prepared, with aged tap water, every thirty days.

All 35 mm microphotography used in this study was accomplished with a Leica, 1 C back, with a 1/3 x Mikro-Ibso attachment. (Plate II).

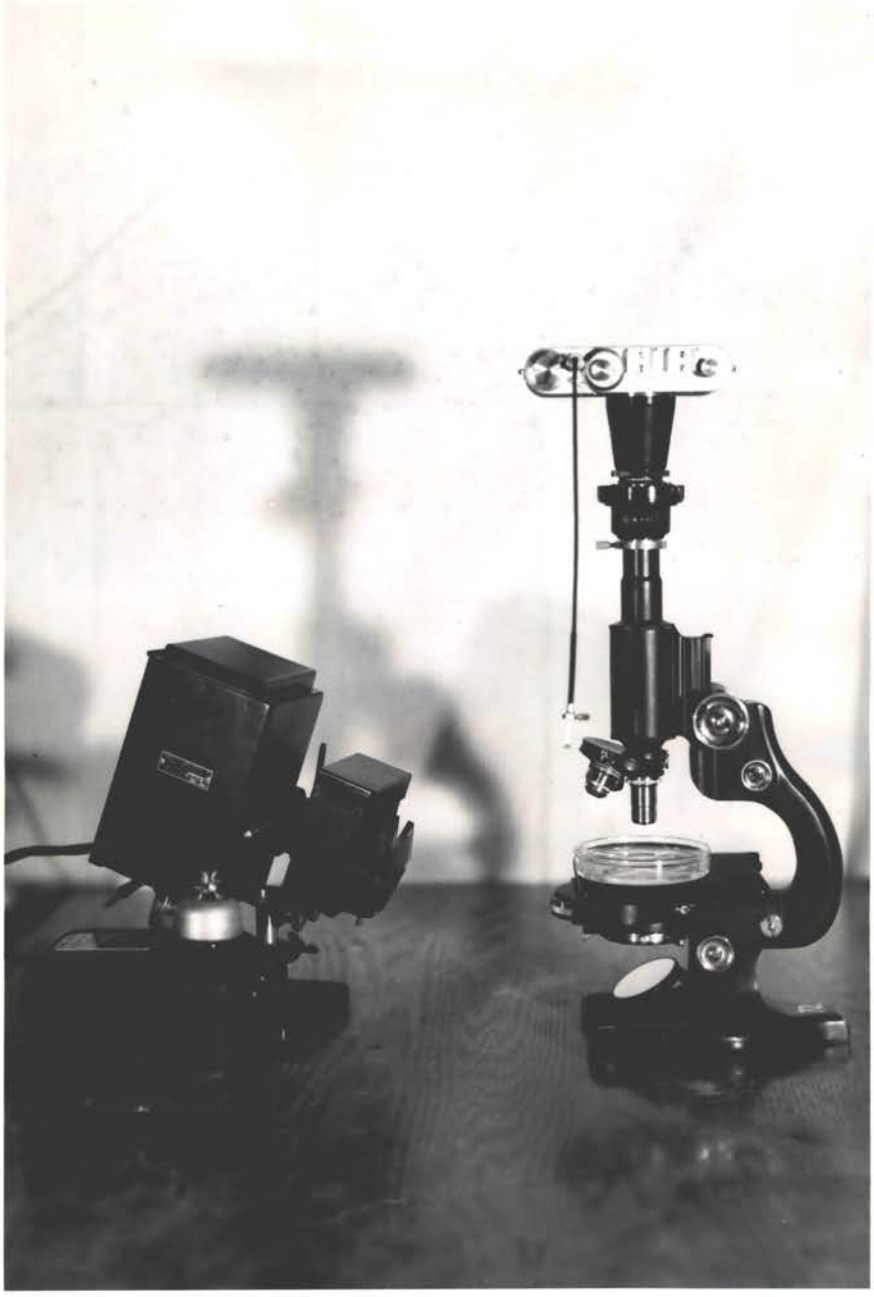


Plate II

35 mm. Phase Photomicrographic Equipment
(Phase Objective in Position)

The 16 mm. time-lapse photography was accomplished with Palliard Bolex Cine-Cameras and adjustor rings and a 1/3 x Mikro-Ibso attachments (Plate III). A Steven's Cine-Timer and a timer designed by the Research Division of the Oklahoma Institute of Technology, Oklahoma A. and M. College, was used in the time-lapse photography.

All embryos were photographed in Syracuse watch glasses or a sectional watch glass in the solutions in which they were incubated.

Film used in both 35 mm. or 16 mm. photomicrography was Panatomic X or Kodak Linagraph Shellburst Safety Film. Illumination was obtained by the use of a Botsch and Lomb ribbon filament lamp which has a three step transformer and heat absorbing water jacket. The use of variable intensity of light from the lamp, with the use of filters allows a choice of contrast or detail to be obtained. Table I may be consulted for all pertinent information concerning the photomicrography.

Heart rate counts were made by counting 50 beats and converting to beats per minute. The standard electric timer used for this work was sensitive to 1/10 second.

Pigment cell counts were made by counting the melanophores on the median dorsal line. All melanophores were counted for a distance of 0.652 mm. anteriorly from the most posterior portion of the caudal fin. The eye piece used in measurement was calibrated using a Spencer stage micrometer.

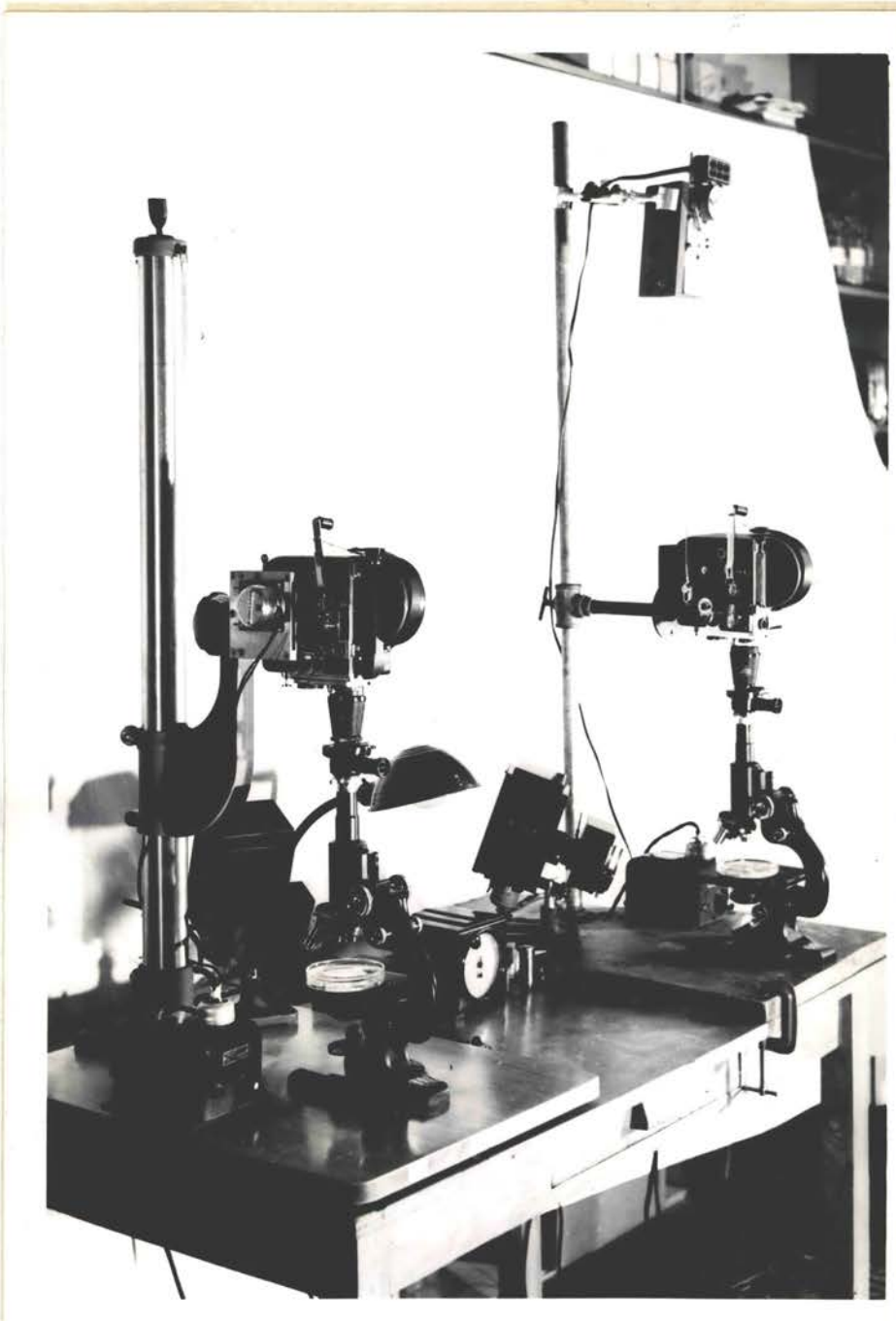


Plate III

Simultaneous Time-Lapse Photomicrographic Equipment

TABLE I

Exposure Table for 35 mm. Panatomic X Film

Ocular	Objective	Exposure Time	Lamp Filters	Transformer Position
10 X	25 mm. (6X)	1/25 second	Cobalt	Low
10 X	25 mm. (6X)	1/125 second	None	Low
10 X	25 mm. (6X)	1/50 second	0.3 N.D.F.*	Low
10 X	25 mm. (6X)	1/25 second	0.6 N.D.F.	Low
10 X	25 mm. (6X)	1/10 second	0.9 N.D.F.	Low
10 X	25 mm. (6X)	1/5 second	1.2 N.D.F.	Low
10 X	25 mm. (6X)	1/2 second	1.2 + 0.3 N.D.F.	Low

Note:

* 1. N.D.F. = Neutral Density Filter

2. Transormer position may be placed at medium by reducing the exposure time to one-half that given in table.

3. Transformer position may be placed at high by reducing the exposure to one-half the medium exposure calculated from the table.

4. Table may be used to calculate wratten color filters.

5. The lamp position in the above calculations is 5 1/4 inches from the base of the lamp to the base of the microscope.

6. 32 mm. (4X) objective may be substituted for the 25 mm. (6X) objective without changing the exposure time.

7. 40 mm. (3X) and 48 mm. (2.2X) objectives may be substituted for the 25 mm. objective by halving the exposure time.

8. All calculations are with top element of the condenser removed.

9. Calculations for 16 mm. time-lapse may be made from the table by keeping the exposure time constant at 1/25 second.

10. Calculations for other types of film may be made by use of the table and the rating of the film desired.

11. The Weston rating of Panatomic X is 24 daylight or 16 tungsten.

12. The Weston rating of Kodak Linagraph Shellburst Safety film is 100 daylight or 64 tungsten.

All embryos were fixed, at hatching, in Bouin's solution stained in Harris modification of Delafield's, embedded in parafin, and sectioned at 5 to 10 microns.

EXPERIMENTAL DATA AND OBSERVATIONS

Experiment No. I. (T.S. 105)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos-control	Chemical concentration	Length exposure
Pre-Cleavage to 32 Cell Stage	60		15	Aged water	To hatch
		15		Thyroxine, 1:1,000,000	24 hrs.
		15		Thyroxine, 1:10,000,000	24 hrs.
		15		Thyroxine, 100:1,000,000	24 hrs.

The embryos of Experiment No. I were allowed to develop for 24 hours in the thyroxine solution, after which time they were transferred to aged water and allowed to develop until hatching. The embryos were fixed at the time of hatching.

Experiment No. II. (T.S. 106)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos-control	Chemical concentration	Length exposure
Pre-Cleavage to 32 Cell Stage	32		16	Aged water	24 hrs.
		16		Thyroxine, 1:1,000,000	24 hrs.

The embryos of Experiment No. II were allowed to develop in thyroxine solution for 24 hours, after which time they were fixed.

Experiment No. III. (T.S. 107)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical concentration	Length exposure
Pre-Cleavage to 32 Cell Stage	20		10	Aged water	Continued to hatching
		10		Thyroxine, 25:1,000,000	21 hrs.

The embryos of Experiment No. III were exposed, by submersion, in the solution for 21 hours, after which time they were changed to aged water and allowed to develop to hatching. They were fixed in Bouin's at hatching.

Experiment No. IV. (T.S. 108)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length epxosure
Pre - Cleavage to 32 Cell Stage	20		10	Aged water	Continuous hatching
		10		Thyroxine, 100:1,000,000	3 minutes

The embryos were exposed by submersion in the solution, after 3 minutes they were changed to aged water and allowed to develop until hatching. Embryos were fixed in Bouin's.

Experiment No. V. (T.S. 109)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length exposure
Pre-Cleavage to 32 Cell Stage	84		11	Aged water	Continuous to hatching
			10	Aged water	12 hours
		11		Thyroxine, 1:10,000,000	12 hours
		10		Thyroxine, 1:10,000,000	12 hours
		11		Thyroxine, 1:1,000,000	12 hours
		10		Thyroxine, 1:1,000,000	12 hours
		11		Thyroxine, 1:100,000,000	12 hours
		10		Thyroxine, 100:1,000,000	12 hours

The embryos were exposed for 12 hours to solution, after which time 10 of each group were fixed in Bouin's, and 11 were allowed to develop in aged water to hatching and then fixed.

Experiment No. VI. (T.S. 110)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
Pre-	184		46	Aged water	125 hours
Cleavage		46		Thyroxine, 1:10,000,000	125 hours
to		46		Thyroxine, 1:1,000,000	125 hours
32 Cell		46		Thyroxine, 100:1,000,000	125 hours
Stage					

The embryos of this experiment were allowed to remain in thyroxine solution for a period of time after hatchings, then fixed in Bouin's.

Experiment No. VII. (T.S. 111)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
Pre-					Continuous
Cleavage	48		20	Aged water	to hatching
to					
16 Cell		28		Thyroxine, 100:1,000,000	3 minutes
Stage					

The treated embryos of this group were submerged in solution, changed to aged water and allowed to develop to hatching.

Experiment No. VIII. (T.S. 112)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
	240		30	Aged water	Continuous to hatching
			30	Aged water	3 hours
		30		Thyroxine, 1:10,000,000	3 hours
4 hours		30		Thyroxine, 1:10,000,000	3 hours
		30		Thyroxine, 1:1,000,000	3 hours
		30		Thyroxine, 1:1,000,000	3 hours
		30		Thyroxine, 100:1,000,000	3 hours
		30		Thyroxine, 100:1,000,000	3 hours

One-half of the embryos of each group were fixed at 3 hours, the remaining half were allowed to develop in aged water to hatching, then fixed.

Experiment No. IX. (T.S. 113)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
	140		20	Aged water	To hatching
		20		Thyroxine, 1:1,000,000	To hatching
Two to Eight Cell Stage		20		Thyroxine, 10:1,000,000	To hatching
		20		Thyroxine, 25:1,000,000	To hatching
		20		Thyroxine, 50:1,000,000	To hatching
		20		Thyroxine, 100:1,000,000	To hatching
		20		Thyroxine, 500:1,000,000	To hatching

Embryos of this experiment were submerged continuously throughout development to hatching in the various concentrations of thyroxine, and fixed at the time of hatching.

Experiment No. X. (T.S. 114)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
	120		20	Aged water	Continuous to hatching
Two		20		Thyroxine, 1:,000,000	10 min.
to		20		Thyroxine, 10:1,000,000	10 min.
Eight		20		Thyroxine, 25:1,000,000	10 min.
Cell		20		Thyroxine, 50:1,000,000	10 min.
Stage		20		Thyroxine, 100:1,000,000	10 min.

Embryos of this group were submerged in the various concentrations for 10 minutes, washed and changed to aged water and allowed to develop to hatching. Embryos were fixed at hatching.

Experiment No. XI. (T.S. 115)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
	120		20	Aged water	To hatching
Two		20		Thyroxine, 1:1,000,000	To hatching
to		20		Thyroxine, 10:1,000,000	To hatching
Eight		20		Thyroxine, 25:1,000,000	To hatching
Cell		20		Thyroxine, 50:1,000,000	To hatching
Stage		20		Thyroxine, 100:1,000,000	To hatching

Embryos of this group were exposed continuously until hatching in the various concentrations of thyroxine.

Experiment No. XII. (T.S. 116)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
	34		5	Aged water	To hatching
		5		Thyroxine, 1:1,000,000	To hatching
Two to Sixteen Cell Stage		5		Thyroxine, 10:1,000,000	To hatching
		5		Thyroxine, 25:1,000,000	To hatching
		5		Thyroxine, 50:1,000,000	To hatching
		9		Thyroxine, 100:1,000,000	To hatching

Embryos of this group had a constant exposure to the hormone until hatching.

Experiment No. XIII. (T.S. 117, Thio. 150)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
	26		10	Aged water	To hatching
24 hours		10		Thyroxine, 1:1,000,000	To hatching
		6		Thiouracil, 50:1,000,000	To hatching

The embryos of this group were submerged in the concentrations continuously until hatching.

Experiment No. XIV. (T.S. 118, Thio. 151)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
Pre-Cleavage to 64 Cell Stage (most 4 to 8 cell stage)	88		28	Aged water	To hatching
		20		Thyroxine, 1:1,000,000	To hatching
		20		Thyroxine, 1:10,000,000	To hatching
		20		Thiouracil, 50:1,000,000	To hatching

Embryos of this group were continuously exposed to the various solutions of thyroxine and thiouracil until hatching.

Experiment No. XV. (908-909)

Age of embryos	Total no. of embryos	No. of embryos treated	No. of embryos control	Chemical and concentration	Length of exposure
8 to 10 hours	90		30	Aged water	To hatching
		30		Thyroid powder, 25:1,000,000	To hatching
		30		Thyroid powder, 50:1,000,000	To hatching

The embryos of Experiment No. XV were allowed to develop in the thyroid powder solution until hatching, after which time they were fixed.

The embryos used in the preceding experiments demonstrated a uniformity of pigment retardation due to the concentrations of thyroxine as low as one part per ten million to two hundred parts per million. Development of pigmentation seemed to be inversely proportional to the concentration of the thyroxine solution (Plate No. IV, V, VI, VII, VIII). The length of time in which the embryos were exposed to the thyroxine solution seemed to produce little or no variation in effects. Neither the length of exposure nor the concentration seemed to cause a variation in the time of appearance of the melanophores. In both treated and control specimens the melanophores appeared at approximately the same time. Melanophores become visible in the danio at 35 to 40 hours of age at 27 degrees Centigrade.

The embryos submerged in the solution for as little as three minutes, showed at the time of hatching approximately the same amount of retardation as did those submerged for 72 hours in the same concentration. The morphological pattern of the melanophores was not visably altered, except that there was a lack of pigment concentration deposited and/or synthesized. Melanophore counts on embryos (Table II) upheld the writer's visual and photographic observations. The melanophore counts were made at the time of hatching.

Counts of melanophores on untreated control specimens was difficult because of apparent continuity and density of melanin or

TABLE II

Number of Melanophores per 0.652 mm.

Experiment	Number of embryos	Average	Low	High
No. VI. control	11	20.9	18	23
No. VI treated	5	11.6	10	13
No. I control	9	22.8	19	28
No. I treated	6	10.3	8	12

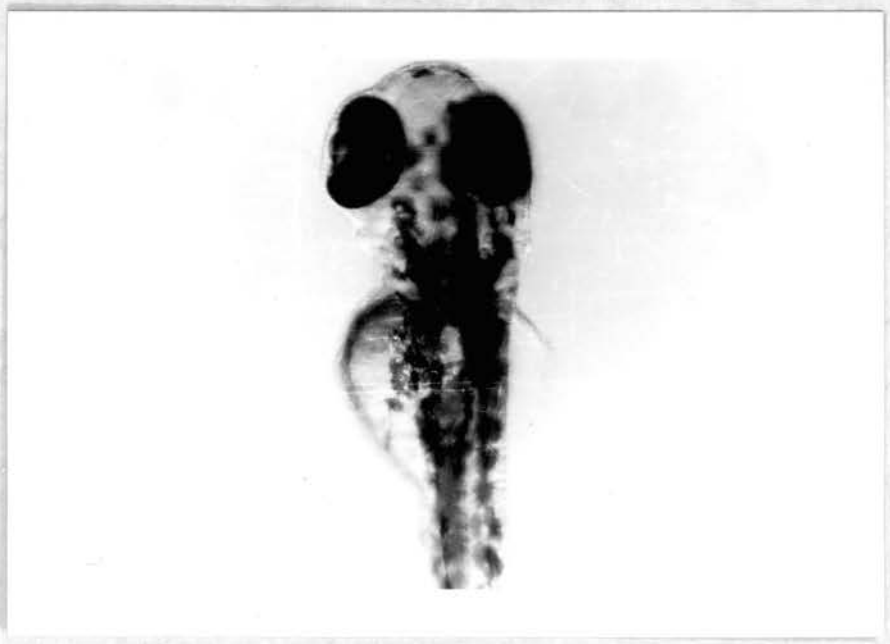


Plate IV

Control Specimen
Incubated in Aged Water

Plate V

Hormone Treated Specimen
Incubated in Thyroxine Solution 1:1,000,000
Exposure Time 10 minutes

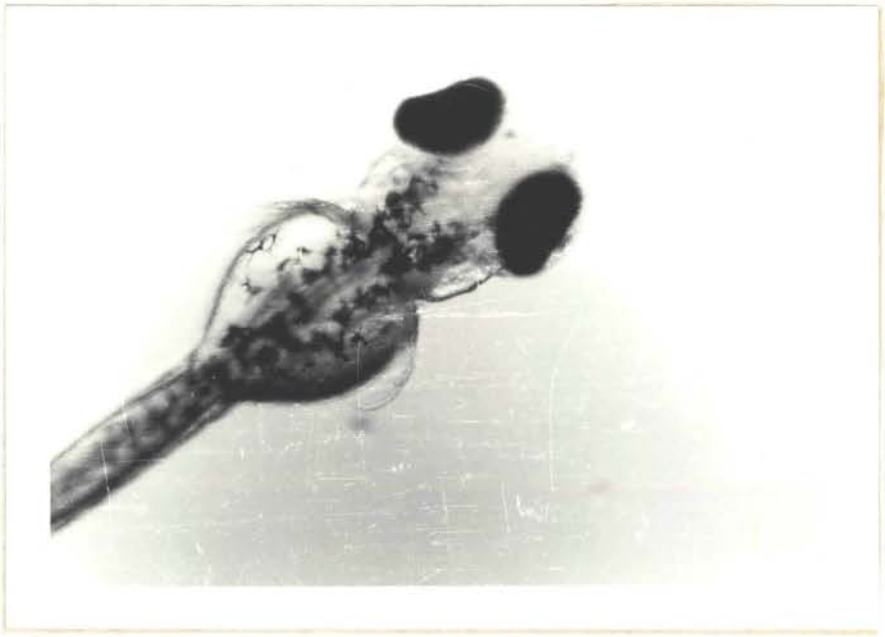


Plate VI

Hormone Treated Specimen
Incubated in Thyroxine Solution 10:1,000,000
Exposure Time 10 minutes

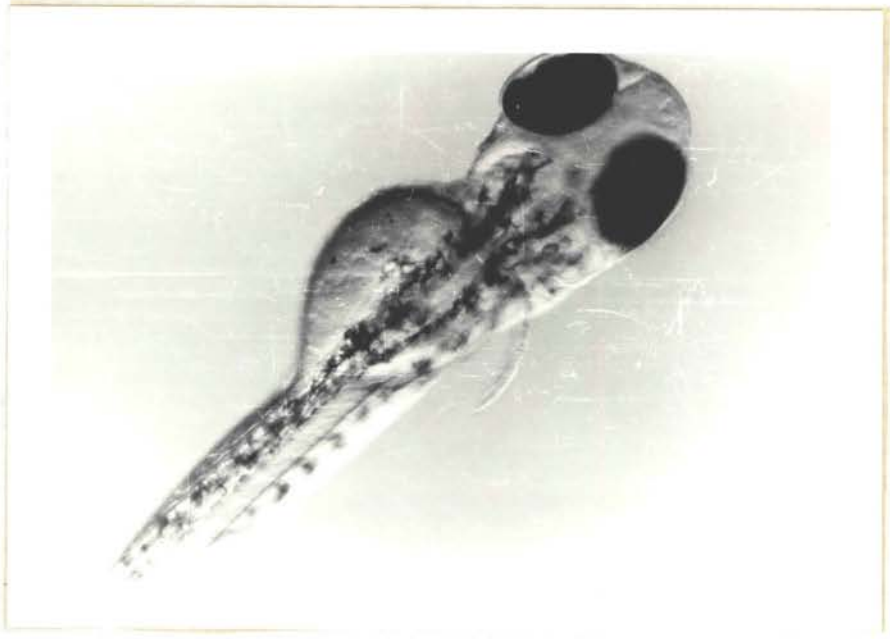


Plate VII
Hormone Treated Specimen
Incubated in Thyroxine Solution 50:1,000,000

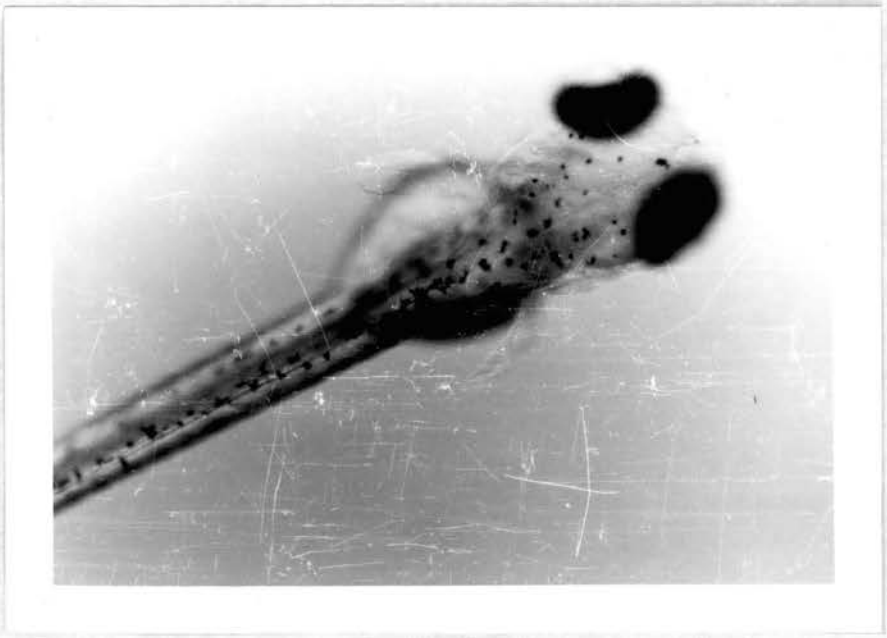


Plate VIII
Hormone Treated Specimen
Incubated in Thyroxine Solution 100:1,000,000
Exposure Time 10 minutes

melanophores. In such a case where no distinct melanophores were discernable, the regions of increased pyknosis were counted. This would possibly result in the melanophores counted being fewer than actually present as it leaves much melanin unaccounted. However, the difference between treated and untreated specimens was still quite evident in melanophore number. In the treated specimens whose melanophores were small and sparse, no such difficulty in counting was encountered. The uniformity in numbers of melanophores of the 31 embryos (20 control, 11 treated) counted was notable.

Morphogenesis, either retardation or acceleration, has been one of the more complex problems in this study. In observing the embryos, the lack of measurable morphological criteria has been a constant difficulty. The writer has observed, almost daily, a general retardation of growth. This retardation seems to be non-specific (except for pigmentation), i.e. the embryos were apparently morphologically normal except for an immeasurable dimminution in size as compared to the controls. There were, however, several structures, positions, and proportions which deserved consideration. The most striking evidence for morphological retardation was the position assumed by the treated as compared to the control specimens. At the time of hatching the control specimens assumed a position of rest with the ventral surface against the bottom, whereas the treated specimens would not rest with the ventral aspect down, but rested upon their sides (Plate IX).

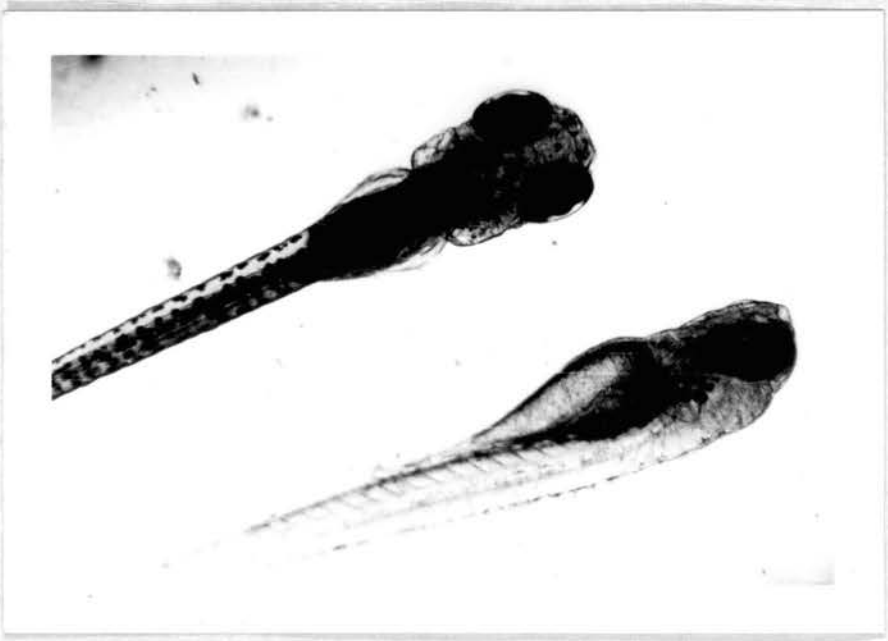


Plate IX

Hormone Treated Specimen and Control
Incubated in Thyroid Powder Solution 50:1,000,000
Exposure, Continuously to Hatching

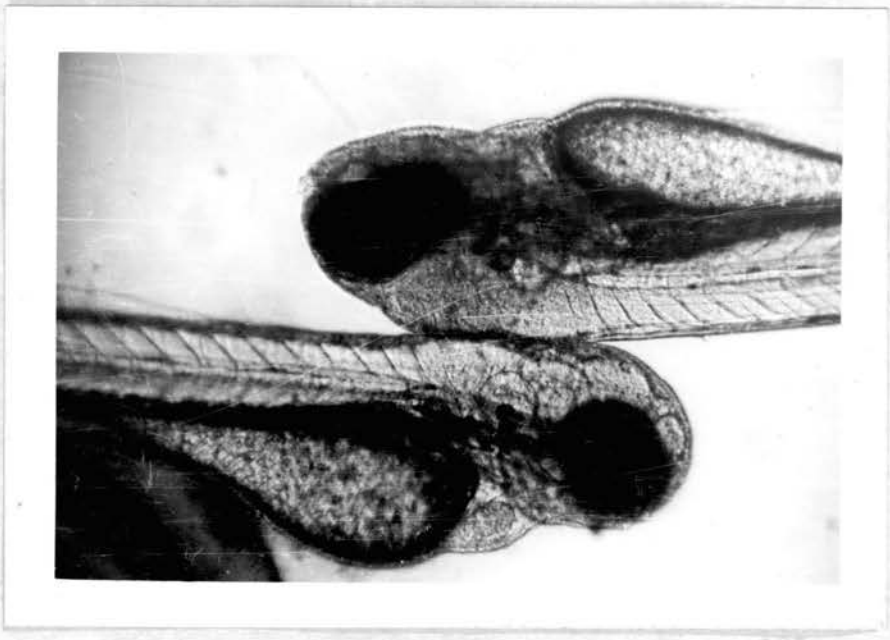


Plate X

Hormone Treated Specimens
Incubated in Thyroid Powder Solution 25:1,000,000
Exposure, Continuously to Hatching

The area anterior to the eyes seemed significant. Here there appeared to be a lack of development in the treated specimens comparable to that of the controls. (Plate IX, X, XI, XII). The brain appeared to be lacking in advancement. There were well formed mouth structures in the control, whereas in the treated specimens, while the mouth structures appeared normal, apparently they were not so far advanced in development.

In the eyes, there was, in the controls, a distinctness of structures (Plate IX, X, XI, XII) which was not found in the treated specimens. Upon closer observations of the eyes it was noted that the eye muscles of the controls at hatching were formed and functioning. In the treated specimens there was a noticable lack of movement as compared with the controls.

Other structures to be noted were differences in distinctness of heart structures, and also the auditory capsule. The opercular covering and gills were not so far advanced in the treated as in the non-treated. The air bladder was seemingly more distinct in the non-treated than in the treated specimens.

Second only to pigmentation was the effect of thyroxine upon the embryonic heart rate. While the heart beats became visible at approximately the same time in both treated and controls, the differences in heart rates became increasingly apparent.

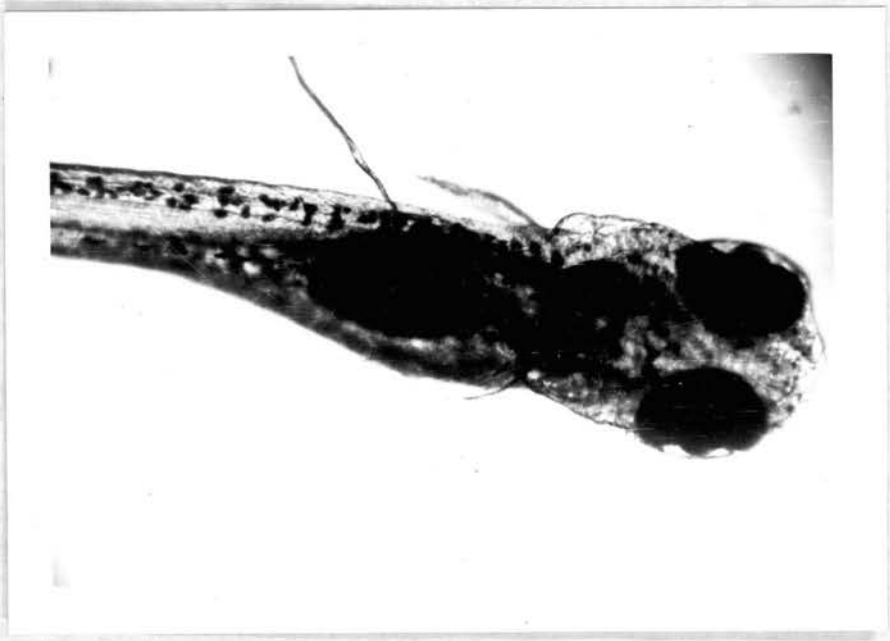


Plate XI

Control Specimen
Incubated in Aged Water

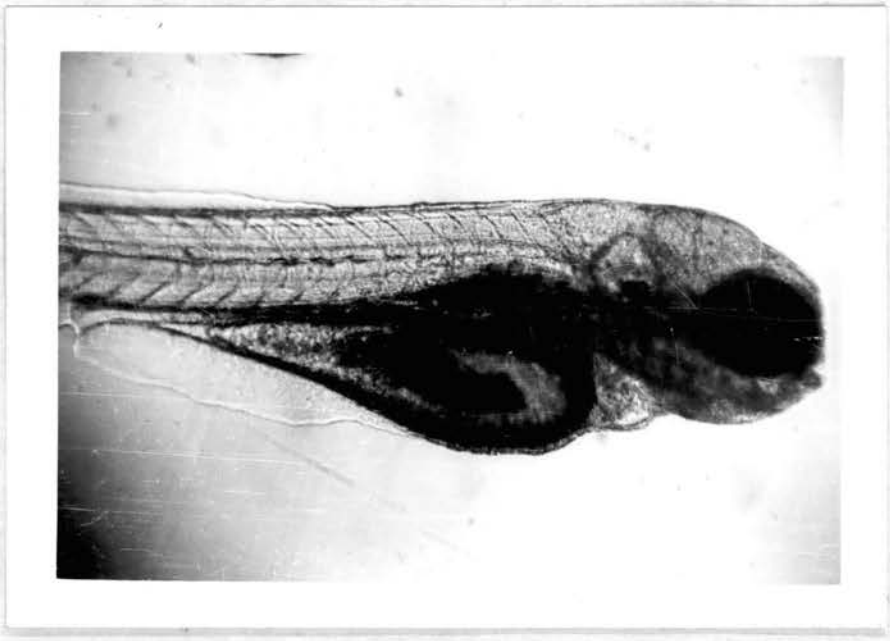


Plate XII

Hormone Treated Specimen
Incubated in Thyroid Powder Solution 50:1,000,000
Exposure, Continuously to Hatching

Figure II represents a total of 255 heart rate counts involving 88 embryos (28 control, 20 thyroxine treated with 1:1,000,000, 20 thyroxine treated with 1:10,000,000, and 20 embryos treated with thiouracil 50:1,000,000). These embryos were immersed in the solution for the duration of the experiment.

Approximately six counts were taken from each group at three-hour intervals from 31 hours to 61 hours of age. Selection of embryos from each group at every interval was taken at random. The heart rates were taken by first counting the beats for a few seconds to obtain the rhythm, then without hesitation, the timer was started and 50 beats were counted. The time required for 50 beats was converted to heart rate per minute.

At the age of 119 hours a count was again taken on the same groups and the rate was found to be still rising. At this time the rates of each group were as follows:

Control	200/min.
Thyroxine, 1:1,000,000	214/min.
Thyroxine, 1:10,000,000	208/min.
Thiouracil, 50:1,000,000	172/min.

Thyroxine in the concentrations used in this study apparently caused no variation in the time of hatching of the embryos, since both treated and control specimens hatched at approximately the same time.

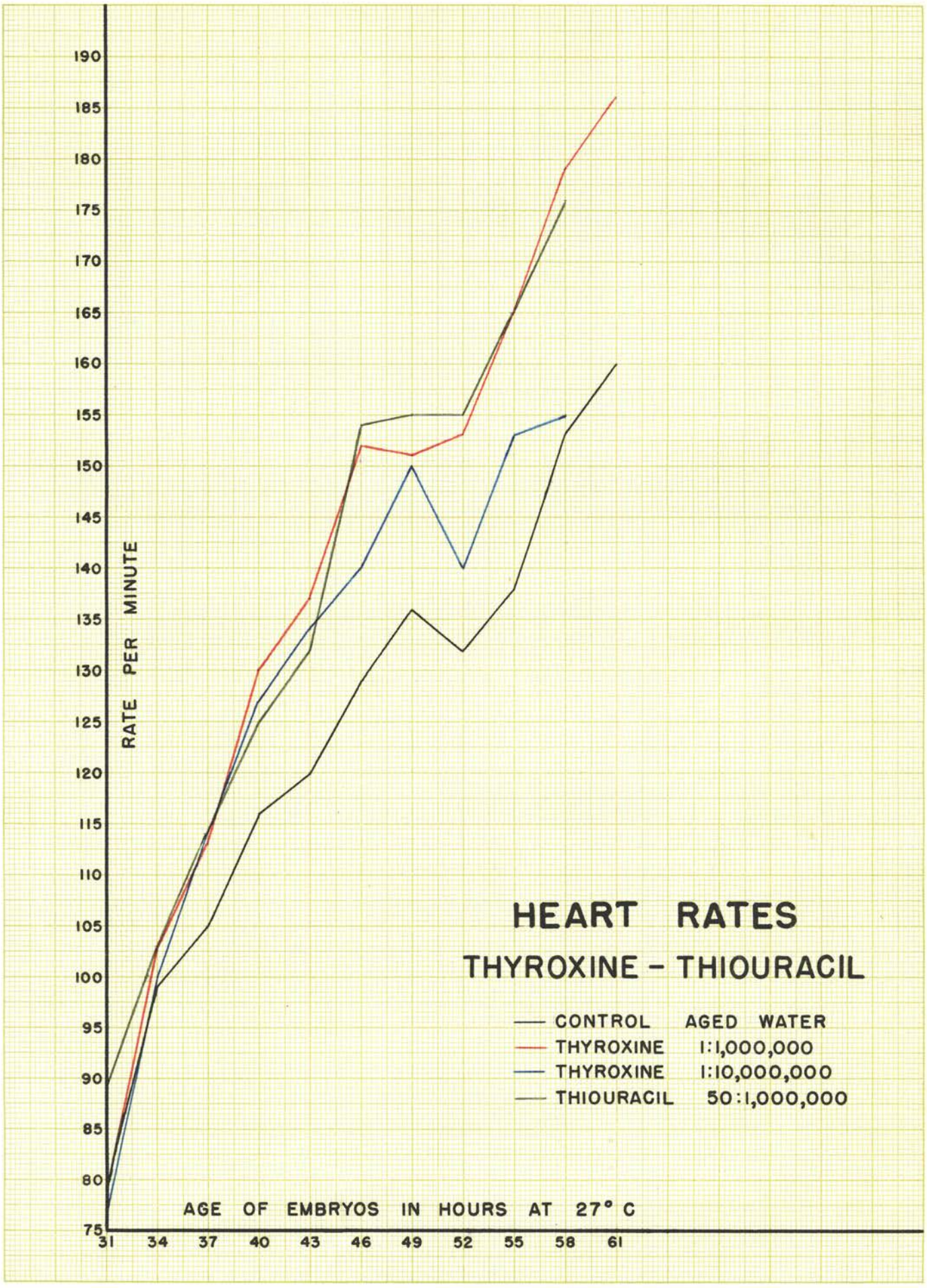
The percentage of kill in both treated and non-treated specimens was approximately 25 percent. It is concluded that the concentrations

of thyroxine used did not produce an abnormally high kill since death was as prevalent in the controls as in the treated specimens. Death of many of the embryos may be attributed to fungus.

Limited experimentation with thiouracil was undertaken for the purpose of comparison with thyroxine treated embryos. Exposure of embryos to this chemical in a 50:1,000,000 concentration apparently produced in some aspects opposite effects as did thyroxine.

Jones and Nickolls (personal communication) in a study of 131 thiouracil treated embryos observed an uniformly higher concentration of pigment in these specimens than in the controls. Using concentrations from 10:1,000,000 to 2000:1,000,000 they found little difference in the effects from 100:1,000,000 to 2,000: 1,000,000 but less effect in 10:1,000,000. However, a concentration as low as 10:1,000,000 produced a greater concentration of pigment and a more advanced embryo than appeared in the controls. The age of their embryos at the beginning of exposure was four hours. Concerning a 2,000:1,000,000 concentration, Jones stated: "Within 24 hours the brain and eye structures appeared to be more advanced, and at 72 hours there was recognizably more pigmentation and seemingly more advanced embryos than in the control specimens".

"The percentage of kill in all thiouracil-treated individuals ran almost as high as 30 percent. This high percentage was probably due to fungus and high temperature (30 to 33 degrees Centigrade). The kill was as prevalent in the controls as in the treated, hence apparently deaths were not due to the drug". (Jones, unpublished data.)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RATE PER MINUTE
31
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37
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43
46
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52
55
58
61
AGE OF EMBRYOS IN HOURS AT 27° C
HEART RATES
THYROXINE - THIOURACIL
CONTROL AGED WATER
THYROXINE 1:1,000,000
THYROXINE 1:10,000,000
THIOURACIL 50:1,000,000

DISCUSSION

There is voluminous literature concerning the thyroid gland and the effect of its hormone on many different types of animals. The number of types is exceeded only by the methods and circumstances under which they are investigated. Adult fishes have received a considerable amount of attention in this respect, but investigations on embryonic fishes have been relatively lacking.

The results herein reported may be correlated with previous work by other investigators. Wyman (1924) reported a contraction of melanophores in a dechorionated Fundulus embryo; however, thyroid powder was ineffective when the embryo was still in the egg membranes. Langrebe (1941) reported a loss of pigmentation as a result of feeding immature trout thyroid powder. Fleischmann (1951) on rabbit and crows, Turner (1950) on birds, and Miller (1935) on the English sparrow reported a similar lack of pigmentation due to excess thyroid hormone. On the other hand, Lillie and Juhn (1932) on regenerating feathers of the Brown Leghorn, Turner (1950) on birds, and Miller (1935) on the grey feathers of the abdominal region of the male English Sparrow found an increase in pigmentation due to excess thyroid hormone. The specific effect of thyroxine on different animals is obviously variable. It is unknown whether or not thyroxine inhibits melanoblast production in the fish, alters the membrane permeability of potential melanophores, inhibits

synthesis of tyrosine, inhibits the action of tyrosinase, or other intermediate metabolic steps in melanin formation. It would be interesting to know if perhaps thyroxine acts in a similar manner as the dyes (phenol indophenol, and orthocresol indophenol) used by Figge (1948). Figge produced a Necturus devoid of pigmentation and concluded that these dyes inhibited the action of tyrosinase.

"Heart rates", wrote Prosser (1950) "are directly related to rates of metabolism, and any alteration in metabolism is reflected in an alteration in heart rate. Gas exchange is not the only index of activity or energy production of an organism, but its correlation in a quantitative way with rate of metabolism and heat production has made it the generally accepted standard of the over-all measure of metabolic processes." If Prosser's conclusions are acceptable, we are led to conclude then that thyroxine treated embryos, with faster heart rates, have undergone an increase in oxygen consumption and hence an increase in metabolism. This seems to be in agreement with Lerman, Clark, and Means (1933, 1934); Gibson and Harris (1939); and Thompson (1926) on mammals, who observed that excesses of thyroxine raises the metabolic rate. Conversely, Etkin, Root and Mofshin (1940) on fish; Mattheus and Smith (1947) on fish; Root and Etkin (1937) on toadfish; Smith and Everett (1943) on guppies reported no change in oxygen consumption

due to thyroxine administration. Since none of the converse observations concerned embryonic development, valid material for comparison is wanting.

Normal heart rates during development have been studied by relatively few investigators. Paton on Pristeurus melanostoma; Preyer and Kollicker on trout and lizard; Babak and Hepner on trout; Anderson on the lizard; Romanov and Vizbara on the chick (Needham 1931), and Prosser (1950) on fish have observed the rate of heart beat to increase fairly regularly until hatching.

The writer is unable to explain the break at 46 hours to 52 hours of age in the danio heart rate. At this age there was no visible evidence to substantiate such a change. Diapause in embryonic development suggested by Hamlett (1935) on mammals; Dendy (1898) on the tuatara lizard; and Boulenger (1898) on the pond tortoise may be one of the many possible explanations.

Finally, retardation in danio morphogenesis due to excess thyroxine seems to be in agreement with Richardson's (1933, 1940, 1945) and Manner's (unpublished) observations on regeneration. On the other hand, Gundernatch stated that thyroid feeding accelerates differentiation in the amphibian.

SUMMARY AND CONCLUSIONS

The study of 1216 embryos of the zebra fish, Brachydanio rerio, reveals that these embryos are sensitive to thyroxine concentrations as low as 1:1,000,000, and that concentrations as high as 100:1,000,000 are not immediately lethal. Exposure for three minutes in the early cleavage stages appeared to be sufficient to produce typical effects in later development. Apparently the length of time in exposure produced little or no variations in effects. Development of pigmentation seemed to be inversely proportional to the concentration of solution, i.e., the higher the concentration the less the pigment produced in the embryo. Retardation of morphogenesis appeared to be general throughout the animal and not differential in any particular areas or organs. Metabolism appeared to be accelerated in embryos by thyroxine as low as 1:10,000,000.

Thiouracil in concentrations of 10:1,000,000 to 2,000:1,000,000 produced, in some aspects, the opposite effects of thyroxine, i.e. acceleration of pigment formation and morphogenesis.

The data presented leads the writer to conclude the following:

1. Thyroxine in solutions from 1:1,000,000 to 100:1,000,000 retards the production of melanophores and pigmentation in Brachydanio rerio.

2. Thyroxine stimulates the heart rate in the early embryonic stages of this fish.

3. Thyroxine produces apparent retardation in some phases of danio embryonic development.

4. Thiouracil apparently produces some changes converse to that of thyroxine, i.e. acceleration of pigment formation and morphogenesis in the early stages of development of the embryo.

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