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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

A STUDY OF THE EFFECT OF GOITROGENS AND THYROID COMPOUNDS ON RESPIRATION RATES IN PLANARIANS

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MARIE MAGDALEN JENKINS

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A STUDY OF THE EFFECT OF GOITROGENS AND THYROID

COMPOUNDS ON RESPIRATION RATES IN PLANARIANS

APPROVED BY

Harriet Harvey

Carl D. Riggs

Jacques Self

Cluff E. Hopla

Lawrence M. Rohobaugh

DISSERTATION COMMITTEE

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LIST OF ABBREVIATIONS USED

T_4	Thyroxine, or 3,5,3',5'-tetraiodothyronine
T_3	3,5,3'-triiodothyronine
T'_3	3,3',5'-triiodothyronine
T'_2	3,3'-diiodothyronine
DIT	3,5-diiodotyrosine
MIT	3-monoiodotyrosine
T_4A	3,5,3',5'-tetraiodothyroacetic acid
T_3A	3,5,3'-triiodothyroacetic acid
T_4P	3,5,3',5'-tetrathyropropionic acid
T_3P	3,5,3'-triiodothyropropionic acid
Ta	Thiourea
P	Phenylthiourea
Tl	Thiouracil

A STUDY OF THE EFFECT OF GOITROGENS AND THYROID COMPOUNDS ON RESPIRATION RATES IN PLANARIANS

CHAPTER I

INTRODUCTION

Although the thyroid gland was known to the early Greeks and Romans, it was not recognized as an endocrine gland regulating metabolic activity until the latter half of the nineteenth century. This discovery resulted in studies of thyroid morphology and physiology, particularly in man and other mammals, and soon yielded results of great therapeutic value.

Interest in the function of the gland was heightened by the isolation of the hormone, thyroxine (Kendall, 1915), its identification as L-3,5,3',5'-tetraiodothyronine (Harington, 1926), and its synthesis (Harington and Barger, 1927). At least three other hormones have since been identified: 3,5,3'-triiodothyronine (Gross and Pitt-Rivers, 1952; Roche et al., 1952); 3,3',5'-triiodothyronine and 3,3'-diiodothyronine (Roche et al., 1954; Roche et al., 1955).

All these hormones are to be found both in plasma and in thyroglobulin. The triiodothyronines are present only in very small amounts, but both thyroxine and diiodothyronine are more abundant. Of the four hormones, T_3 has by far the highest biological activity in mammals in accelerating the basal metabolic rate, being from five to ten times as potent

as T_4 according to the test used for the bioassay (Roche and Michel, 1956).

The mechanism of action of the thyroid hormones in the vertebrate body has been the subject of much speculation and of a great number of physiological and chemical investigations. Two techniques, chromatography and autoradiography of compounds labelled with the radioactive isotope of iodine, I^{131}, have been widely used in advancing our knowledge of thyroid metabolism. It is generally thought the organic binding of iodine involves the following reactions:

(1) oxidation of iodide to iodine,

(2) iodination of tyrosine to monoiodotyrosine and diiodotyrosine,

(3) oxidative coupling of diiodotyrosine to form thyroxine,

(4) formation of triiodothyronine.

Whether or not the partial deiodination of thyroxine is a step in the process has not been established. Also, we are without any real knowledge of the enzymes which oxidize iodide to iodine and those which potentiate the coupling of diiodotyrosine to thyroxine (Pitt-Rivers, 1958).

Since the discovery that the administration of certain chemicals would result in a goitrous condition characterized by thyroid hyperplasia and a distinct decrease in the basal metabolic rate (Astwood et al., 1943; MacKenzie and MacKenzie, 1943), it has been possible to investigate more fully the biochemical processes which occur within the gland itself. Two principal groups of goitrogenic compounds are known. The first and most active group consists of thiourea and related substances such as thiouracil and propylthiouracil. The second group includes chemicals such as the sulfonamides which have an aminobenzene ring structure, and certain other more or less related compounds. Unlike drugs which depress the

ability of the gland to take up and store iodine (thiocyanate and perchlorate), the goitrogens appear to interfere with one or the other of the mechanisms whereby the hormones are synthesized. Whether this interference with hormone synthesis is accomplished by blocking iodination of tyrosine or by inhibiting the oxidative coupling of iodotyrosines to form iodothyronines has not been determined (Astwood, 1955).

Most of the information that has been gathered concerning the nature of the thyroid hormones was derived from work with adult mammals. However, since Gudernatsch's discovery (1914) that metamorphosis in tadpoles was remarkably accelerated when they were fed thyroid, attention has been increasingly directed to the role of the thyroid in cold-blooded vertebrates, and to the effect of thyroid substances upon the lower animals. A number of extensive reviews on this subject are available (Schneider, 1939; Fleischmann, 1947; Goldsmith, 1949; Lynn and Wachowski, 1951).

The effect of the thyroid hormones in accelerating the metabolic rate in mammals is well-known. Whether or not a similar effect is to be found in cold-blooded vertebrates and invertebrates is controversial. In amphibia, early investigations appear to have revolved around the problem of how oxygen consumption was related to metamorphosis, the view being that metamorphic changes were possibly due to increased metabolism brought about by either endogenous or exogenous thyroid hormone. Support for this stand was found in the reported rise in oxygen consumption during metamorphic climax in Rana pipiens tadpoles (Helff, 1923, 1926) and axolotls (Bělehrádek and Huxley, 1928). Uhlenhuth (1921) stated that "metamorphosis seems to result if metabolism is increased in such a degree and manner that catabolism becomes higher than anabolism." Etkin's findings

(1934) that in Rana catesbeiana the metabolic rate per unit of dry weight dropped markedly during the metamorphic climax cast strong doubt on this theory. Confirmatory evidence for the latter view was supplied by Taylor's work (1939) which showed that, although oxygen consumption dropped in thyroidectomized salamanders (Triturus torosus), and rose, after a preliminary drop, in animals containing homotransplants of thyroid tissue, moulting was inhibited regardless of metabolic rate.

More recent reports have made it clear that the metamorphic action of thyroxine and related compounds need not be correlated with the capacity of these compounds to stimulate oxygen consumption in the mammal Roche et al., 1956). Both thyroxine and triiodothyronine have been found in anurans (Berg and Gorbman, unpublished; cited in: Berg et al., 1959), but whether or not these compounds are able to stimulate respiratory exchange in amphibia is still an open question. Sobels (1949) reported that iodinated casein preparations in concentrations too low to induce metamorphosis nevertheless led to a significant increase in oxygen consumption in Xenopus laevis larvae, and Lewis and Frieden (1959) found that a significant increase in the respiration of Rana grylio tadpoles occurred after treatment with either T_3 or T_4, regardless of what unit of tissue was used to express the results. Fletcher and Myant (1959), however, found that T_4, T_3 and T_4P, although potent in inducing metamorphosis in Xenopus laevis and Rana temporaria tadpoles, were without effect on oxidative metabolism, and attributed any rise or fall in metabolic rate to change in the body temperature of the animal.

The antagonistic action of the thiocarbamides on metamorphosis and thyroidal function in lower vertebrates has been established both in

amphibia and in reptiles. Metamorphosis was inhibited in tadpoles by thiourea (Gordon et al., 1943) and by thiouracil (Hughes and Astwood, 1944), and hyperplasia and reduction in colloid volume were apparent in leptodactylid toads treated with thiourea and phenylthiourea (Lynn, 1948). Thiourea delayed or inhibited hatching and yolk sac retraction in turtle embryos (Dimond, 1954) and both thiourea and thiouracil caused some increase in follicular cell height and loss of colloid in adult turtles (Adams and Craig, 1950). Adult lizards given thiourea and thiouracil showed a characteristic hypertrophy of the thyroid, with loss of colloid (Adams and Craig, 1951). However, no reports of the direct action of goitrogens on oxygen consumption in amphibians are available other than Steinmetz's findings (1952) that Rana clamitans larvae treated with thiouracil were larger than the controls and consumed more oxygen per unit weight.

Little is known about the effect of thyroid compounds on oxygen consumption in fishes and cyclostomes. Thyroxine or mammalian thyroid extracts have been reported to cause no significant change in respiration in the larval lamprey, Petromyzon fluviatilis (Horton, 1934), the toadfish, Opsanus tau (Root and Etkin, 1937), goldfish, Carassius (Etkin et al., 1940; Hasler and Meyer, 1942), the guppy, Lebistes (Smith and Everett, 1943), and Fundulus heteroclitus (Matthews and Smith, 1947). Extracts of the thyroid gland of Bermuda parrot fish (Pseudoscarus guacamaia), however, have been found to raise the oxygen consumption of Bermuda white grunts (Haemulou sp.), providing the injected fish were not too small (Smith and Matthews, 1947). Parrot fish thyroid extracts have also been reported to increase respiratory metabolism in white rats in a manner

similar to that obtained with synthetic thyroxine and dessicated mammalian thyroid powder (Smith and Brown, 1952). Hopper (1959), using Lebistes reticulatus as an experimental animal, was the first to present convincing evidence that increased oxygen consumption could result in fishes which were immersed for a period of time in water containing mammalian thyroid powder. He added that thyroxine had never been shown to be effective in this respect.

In view of existing evidence it seems quite probable that the thyroid gland of fishes and other lower chordates produces the same hormones as do the glands of higher vertebrates. Metamorphosis can be provoked in frog tadpoles by thyroidal material from the lamprey (Horton, 1934) and from selachians (Scyliorhinus canicula and S. stellare)and the teleost, Cyprinus carpio (Sembrat, 1927). Sembrat (1953) also reports endostyles of the lancelet, Branchiostoma lanceolatum, implanted in Ambystoma mexicanum, are able to effect metamorphic changes in the latter. Thyroxine has been found in the thyroid gland of the marine lamprey, Petromyzon marinus marinus (Fontaine et al., 1952), and in the shark, Scyliorhinus canicula (Gorbman et al., 1952), and both T_4 and T_3 have been demonstrated by chromatographic techniques in Protopterus annectens (Leloup, 1958).

That goitrogens are effective in inhibiting growth in some fishes has been demonstrated in Lebistes (Hopper, 1952; Smith et al., 1953), in aquarium fishes of five different species (Frieders, 1954), and in the goldfish, Carassius (Honma and Murakawa, 1957). This retardation was associated with a typical hyperplastic thyroid condition (Nigrelli et al., 1946; Frieders, 1954; Honma and Murakawa, 1957). Whether or not these chemicals have an effect on metabolic rate in fishes has not been

established. Matthews and Smith (1947), using on Fundulus heteroclitus a dosage of thiourea previously determined to be non-toxic, were unable to observe any depression of oxygen consumption, although they noted a very slight rise in about one-third of the experimental animals.

Iodinated proteins have been found throughout the invertebrate world (Roche, 1952; Gorbman et al., 1954) principally in the form of mono- and diiodotyrosine. However, in a number of insects (Limpel and Casida, 1957) and in Musculium, a fresh-water mollusk (Gorbman et al., 1954), a high percentage of the protein-bound iodine was found to be in the form of thyroxine. Both thyroxine and triiodothyronine have been identified in Eunicella verrucosa, a gorgonid (Roche et al., 1951; Roche and Jouan, 1956). Such protein-bound iodine is found principally in horny or fibrous structures, and to a lesser extent in epithelial tissue.

Neither iodotyrosines nor iodothyronines have ever been demonstrated to take part in any physiological processes in the invertebrate animal. Their presence can be explained on a chemical basis, however, in the light of the discovery (Ludwig and von Mutzenbecher, 1939) that thyroxine could be recovered from iodinated casein. It is now known that when any of a large number of proteins is iodinated, the same limited series of iodine compounds is formed, whether in the thyroid, in artificially iodinated proteins, or in the iodoproteins of invertebrates (Reineke, 1949).

In view of the dramatic effects of thyroid hormones on amphibian metamorphosis, a study of the possible effects of these hormones on metamorphosis in invertebrates has been undertaken by a number of workers. Goldsmith's review of these investigations (1949) revealed that no clear-cut, reproducible response of an invertebrate to any kind of thyroid

treatment had been unequivocally demonstrated. Much of the confusion appears to result from the fact that in most cases no attempt was made to control or determine the exact amount of thyroid substance given. That this can be of major importance was demonstrated by Ungar and Zerling (1933) who observed the development of sea-urchin (Psammechinus miliaris) eggs in a series of graded concentrations of crystalline thyroxine dissolved in sea water. They found weak dosages of thyroxine had a stimulating action on the early development of the embryo, which became more marked in the more dilute solutions. Higher concentrations slowed development, and the retardation became more pronounced as the concentration was increased.

Often, too, in the interpretation of results, no distinction has been made between the effect of thyroxine and that of whole thyroid gland. The importance of this discrimination was shown in Cori's work with Paramecium (1923) in which it was found that thyroid extract accelerated cell division strongly while thyroxine had but a slight effect. She suggested that thyroid extract must contain some active principle other than thyroxine. Torrey and his co-workers (1925) found a similar difference of effect: thyroxine, unlike total thyroid, depressed the division rate of Paramecium. Schneider (1939) offered the suggestion that these different results might be explained on the theory that thyroxine affects only catabolic processes of the cell, while thyroid substance apparently accelerates both anabolism and catabolism.

Investigations of the effect of thyroid compounds on oxygen consumption in invertebrates have been limited in part to studies of tissues or of one-celled organisms. Ashbel (1935) measured the respiration of fresh

and of dried and powdered tissues of various invertebrate phyla. He found that thyroxine was without effect but that administration of thyroid extract to fertilized eggs of an echinoderm and of various crustaceans raised the oxygen consumption up to 300 per cent. The action of the extract on dried and powdered eggs increased the oxygen consumption as high as 5,000 per cent in some cases. A positive effect of thyroxine on the respiration of the excised heart of Limulus was noted by Davis and Hastings (1936). Either with or without the ganglion of the median nerve attached to the heart, they found the respiration of the thyroxine-treated tissue, after a latent period, was remarkably above that of the control.

One of the earliest studies of factors influencing the rate of oxygen consumption in unicellular organisms was that of Leichsenring (1925) who determined the effect of a number of anesthetics, amino acids and carbohydrates on Paramecium and Colpoda. She found that thyroxine produced a rise of 13 per cent in oxygen consumption of Paramecium and concluded the chemical resembled, in this respect, its effect on the metabolism of higher animals. However, her data also showed that lactose, caprine (norleucine), glutamic acid and peptone increased oxygen consumption by 16, 18, 14 and 13 per cent respectively, so the effect of thyroxine appears to be neither unique nor specific. Since early experiments did not make use of axenic cultures, it is possible part of the stimulatory effect reported here may have been the result of nutritional conditions.

In an effort to eliminate modifications of results by the presence of nutritional materials, Wingo and Cameron (1952) tested the effect of thyroxine on bacteria-free cultures of Tetrahymena geleii. They found that oxygen uptake increased markedly at a concentration of 1.0 mg/liter,

after a latent period. Positive results in other unicellular organisms have also been reported: yeast respiration was stimulated by concentrations of thyroxine ranging from 10^{-2} to 10^{-10} molar (Gutenstein and Marx, 1957), and non-proliferating bacterial cells of Escherichia coli were provoked to an almost immediate augmentation of oxygen consumption by both T_3 and T_4, although the respiratory effect of T_4 was noticeably weaker (Roche et al., 1959).

There is a marked scarcity of data on the influence of thyroid compounds on the oxygen consumption of metazoan invertebrates. Srinivasan et al. (1955) reported that thyroxine added to the diet of rice moth (Corcyra cephalonica) larvae increased the oxygen requirement but that thyroglobulin was without effect. Bodine and Lu (1952) found that concentrations of T_4 ranging from 0.02 to 0.2 mg/ml had no effect on the endogenous oxygen uptake of either mitotically active or blocked intact grasshopper embryos, homogenates or nuclei. Earlier, Bodine and Fitzgerald (1949) had subjected blocked and active embryonic cells of Melanoplus differentialis to the action of thiourea, phenylthiourea and thiouracil. They found oxygen uptake to be stimulated by low concentrations of the three substances, but inhibited by higher. When the cells were washed and resuspended in Ringer's solution, oxygen consumption returned to normal levels. Jones and Wilson (1959), studying the effect of phenylthiourea on the respiratory metabolism of the silkworm, Philosamia cynthia, found a pronounced depressant action on the rate of oxygen consumption.

Few studies have been made of the action of either thyroid compounds or of goitrogens on physiological activities in planarians. Wulzen (1923) found that thyroid-fed Dugesia tigrina (Planaria maculata) lagged in

growth considerably behind those fed liver, thymus and adrenal gland, and slightly behind those fed muscle. Castle (1928) reported that Phagocata (Planaria) velata was attracted to and fed readily upon macerated sheep thyroid, but, in spite of the fact that their intestines seemed well-filled after each meal, they decreased in size even more rapidly than worms subjected to starvation. He concluded that the active principle of the thyroid increased the rat of catabolic processes to such an extent that the reserves and body tissues were broken down more readily than they could be replaced. Goldsmith (1935, 1937), studying the effect of endocrine feeding on regeneration and growth in Dugesia tigrina (Planaria maculata), noted no significant differences in the head regeneration time of the gland-fed animals. Thyroid-fed individuals, including worms newly emerged from capsules, increased in size, but to a lesser extent than the liver and pituitary-fed forms. Egg capsule production was markedly decreased in the thyroid-fed worms.

The influence of thyroxine on eye formation in Phagocata gracilis has been studied by Weimer et al. (1938). Pieces of planarians cut at different levels were allowed to regenerate in a saturated thyroxine solution, and the first appearance of eyespots, as well as the rate of formation, was noted. Both the pieces in thyroxine and those in the control culture water began the process of reconstitution after a lapse of approximately the same number of days. Once the process had begun, however, the rate of formation was much higher for the pieces in thyroxine.

The most recent work which includes some mention of the effect of thyroid compounds on planarians is that of Okugawa and Kawakatsu (1957). They studied breeding and fission frequencies in both sexual and asexual

races of Dugesia gonocephala fed on a variety of diets. Individuals of the sexual race given "thyroideum" (thyroid gland) had a very high fission frequency combined with rapid reduction in both size and number of reproductive organs; those of the asexual race given thyroid had both a low fission frequency and a low growth rate.

Various effects of goitrogens on planarians have been reported by several writers. Goldsmith (1946) observed that head regeneration in thiourea-treated planarians (Dugesia dorotocephala) proceeded at a retarded rate with a high incidence of atypical regenerates in higher concentrations. Kambara (1954) and Kido and Kishida (1960) reported eye depigmentation in Dugesia gonocephala subjected to thiourea at various concentrations and under different conditions. Jenkins (1959), using thiourea, phenylthiourea and thiouracil on Dugesia tigrina found these three goitrogens to be effective in retarding healing and growth. Phenylthiourea also completely inhibited eye-pigment formation.

As can be seen from the brief review of the literature given above, the great majority of studies in this field have been concerned with the effects of the thyroid compounds, or of the goitrogens, or of both, on growth and differentiation in the animals under observation. The direct effect of either group on oxygen consumption in lower vertebrates or invertebrates has been the subject of very few investigations. This is not surprising in view of the fact that techniques for determining oxygen consumption or metabolic rates, particularly in lower organisms, are tedious and time-consuming, and in many cases, special adaptations have to be made for particular experimental animals. Too, the oxygen consumption in these groups is often quite variable, depending upon environmental

conditions, and norms for comparison are at times difficult to obtain.

A number of investigations on the oxygen consumption of planarians under conditions approaching as nearly as possible their normal surroundings have been made by a number of skillful workers. One of the earliest to investigate this field was Libbie H. Hyman, who reported her findings in a series of articles published about forty years ago. Her work has been confirmed by several, while others have published results which differ from hers to a greater or lesser degree. This conflict appears to stem both from disagreement over interpretation of results obtained, and from questions concerning the accuracy of the apparatus or method used in the determination of the respiratory rate.

The Winkler method for measuring dissolved oxygen in water was used by Hyman (1919a). In a later paper (1932) she presented statistical evidence defending the accuracy of the Winkler method in reply to Shearer's criticism (1930) of her work based on his own manometric observations. Allee and Oesting (1934) presented a critical evaluation of the Winkler method, comparing it with other methods of determining oxygen consumption. They repeated some of the experiments of Hyman and others, and concluded that although some of Hyman's figures might be erroneously high, due to a nitrite error, such error "need not have affected Hyman's conclusions regarding oxygen consumption for the given experiments."

Fraps (1930) found the manometric method of Warburg adequate for measurement of oxygen consumption in planarians when certain modifications were applied. Bolen (1937), in his study of specific dynamic action in planarians, rejected both the Winkler and manometric methods in favor of Winkler micro-analysis. Wilder (1937) performed a series of comparative

experiments, using both the Winkler method and respirometers in order to evaluate the relative accuracy of the two. She found statistical agreement between the two methods at the base level for calculation of oxygen consumption. Løvtrup (1953) attempted to use the Warburg method but, finding that shaking destroyed the planarians, employed instead the Cartesian diver technique for her determinations.

In addition to the above investigations of normal respiration rates in planarians, studies have also been made of the effects of certain acids, alkalies and other chemicals on oxygen consumption. However, no experiments of this nature have included the use of either goitrogens or of thyroid compounds.

In view of these facts, it seemed worthwhile to test the effects of these two groups of chemicals on oxygen consumption in planarians, and also to test whether or not the techniques to be employed would provide accurate and consistent measurements on which valid observations and conclusions could be based.

The first experiment in this study was designed in part to obtain information in regard to the conditions under which the Warburg respirometer might be used as a research instrument for a study of planarian metabolism. Attention was directed both to technical details in manipulation of the apparatus and to the responses of the worms to the varying procedures to which they were subjected. Also, in order that results obtained following treatment with goitrogens or thyroid compounds might not be invalidated by fluctuations in oxygen consumption due to extraneous factors, the experiment was so arranged that a determination might be made of the oxygen consumption of planarians as affected by feeding, starvation

and changes in temperature.

Using the data obtained upon the completion of the procedure outlined above, two other sets of experiments were performed. The first was designed to test the effects of various goitrogens on oxygen consumption in planarians; in the second a study was made of the effect of thyroid compounds on respiratory rates in the experimental animals.

CHAPTER II

THE USE OF THE WARBURG RESPIROMETER IN DETERMINING OXYGEN CONSUMPTION

Planarians typically show a high increase in metabolic activity directly after feeding which later declines during starvation to a fairly constant level at which it remains for some time. Oxygen consumption rises later as the worms begin to make use of their own body tissues in the absence of other food (Hyman, 1919b, 1920).

The first experiment in this study had two objectives: 1) to find out how many days would elapse after feeding before the more or less constant level of metabolic activity would be established, and 2) to determine whether or not the constant level, in this species, would continue for a period of time sufficient for other experimental work.

The planarians used throughout the three sets of experiments comprising this study were collected during the latter part of the summer of 1959 from Buckhorn Springs, approximately five miles south of Sulphur, Oklahoma. About 2,000 large, sexually mature animals were obtained, together with several hundred egg capsules. The worms have been identified by Dr. Libbie H. Hyman as Dugesia dorotocephala (Woodworth, 1897). Judging from external appearance, habitat and physiological reactions, they appear to belong to the group formerly known as Planaria agilis (Hyman, 1920, 1929).

This group is now included in the species Dugesia dorotocephala by both Kenk (1935, 1944) and Hyman (1951).

Procedure

The worms were kept at a constant temperature of 17° C. for approximately two months before being used for experimentation. They were maintained in deep, enameled pans, each with an aerator, in water obtained from Crystal Lake, north of Norman, Oklahoma. They were removed to a warmer room (22-23° C.) every five days for feeding. They were given beef liver and allowed to feed two to three hours. After the liver was removed the pans were washed and the worms placed in fresh lake water. The worms remained sexually mature and produced egg capsules regularly throughout the course of the experiment.

For this experiment, two pans of stock worms were used. One group (pan # 1) was tested at 17° C., the other (pan # 2) at 22° C. Worms in pan # 1 were fed as usual and returned to the constant temperature room where they were left without food during the course of the experiment. Water was changed every five days and aerated continuously. Worms used from this pan were returned to a separate pan, so that the same individual would not be used twice during the experiment. The first determination of the oxygen consumption of these animals was made a few hours after feeding had been completed. Additional measurements were made every other day for sixteen days.

Worms in pan # 2 were left in the warm room overnight and fed the following day, after which the first determination of oxygen consumption at the higher temperature was made. Except for being kept at the warmer temperature, these animals were treated exactly as those in pan # 1.

On the day that the oxygen consumption of a group was to be measured, 25 of the largest worms were picked up with a wide-bore, large-bulb pipette and placed in a 600 ml beaker of aerated lake water. Large, mature worms were selected in order that a small number of heavy individuals could be used for each determination. This provided more accuracy in weighing, due to less surface/mass for mucus production, and greater ease in removing excess water. Each worm was transferred individually to the weighing pan by means of a small, moistened camel's hair brush, and weighed on a Roller-Smith torsion balance accurate to 0.2 mg. The weights thus obtained were used for subsequent calculation of the oxygen consumption of the worms, measured in microliters/gram/hour.

For each determination two ml of aerated lake water, pH 7.85 - 8.2, were placed in each of five Warburg reaction flasks. The worms were rapidly weighed and transferred to the flasks, and 0.2 ml of 10 per cent KOH and a small strip of fluted filter paper added to the center well. An additional flask was used as a thermobarometer. Worms tested at 17° C. were equilibrated for ten minutes. Those tested at 22° C. were equilibrated for twenty minutes to offset the effect of having been in the colder room during the process of being weighed and transferred to the flasks. After the period of equilibration was completed, readings were taken every half-hour for three hours.

Results

Data obtained were calculated by standard methods (Umbreit *et al.*, 1949) and the oxygen consumption of the planarians expressed in microliters of oxygen consumed per gram of wet weight per hour.

The results of the study of oxygen uptake of planarians maintained

TABLE I

OXYGEN CONSUMPTION OF PLANARIANS IN WATER AT 17° C.

	$\mu l\ O_2/gm/hr$								
	Days after feeding								
Flask	0	2	4	6	8	10	12	14	16
1	163	131	133	109	112	114	99	93	86
2	160	118	*	107	110	101	98	88	89
3	163	127	111	117	112	94	104	99	85
4	162	126	116	101	104	95	97	93	97
5	169	127	107	111	131	103	80	89	84
Avg.	163	126	117	109	114	101	96	92	88

* One worm died; data not used.

at 17° C. are given in Table I. The data show that, although there is a certain amount of variation, there was a definite trend from an initial higher rate of oxygen consumption shortly after feeding, through a decline of several days, to a fairly constant rate which continued until the end of the experiment.

In spite of the variation in averages shown in Table I, hourly consumption of oxygen in individual flasks was quite constant. Typical hourly readings are presented in Table II. The constant oxygen uptake over a period of three hours, exemplified in flask 2, occurred a number of times during the course of the experiment. Repeated values, such as are shown for flasks 3, 4 and 5, were of quite common occurrence.

Worms tested at 22° C. showed more hourly variation in oxygen uptake

TABLE II

HOURLY CHANGES IN OXYGEN CONSUMPTION OF PLANARIANS IN WATER AT 17° C.

	µl O_2/gm/hr				
	Flasks				
	1	2	3	4	5
Hour 1	107	98	106	101	85
Hour 2	87	98	99	88	72
Hour 3	101	98	106	101	85
Average	99	98	104	97	80
Weight of worms in mg	222	223.6	219.8	234.4	218.8

TABLE III

OXYGEN CONSUMPTION OF PLANARIANS IN WATER AT 22° C.

	µl O_2/gm/hr								
	Days after feeding								
Flask	0	2	4	6	8	10	12	14	16
1	164	150	149	152	145	130	138	135	135
2	194	157	*	147	133	133	120	143	126
3	174	164	143	148	147	137	129	150	133
4	186	168	144	147	149	141	150	126	125
5	185	157	151	146	146	140	147	136	140
Avg.	181	159	147	148	144	136	137	138	132

* Slow leak observed in flask during first hour; no readings taken.

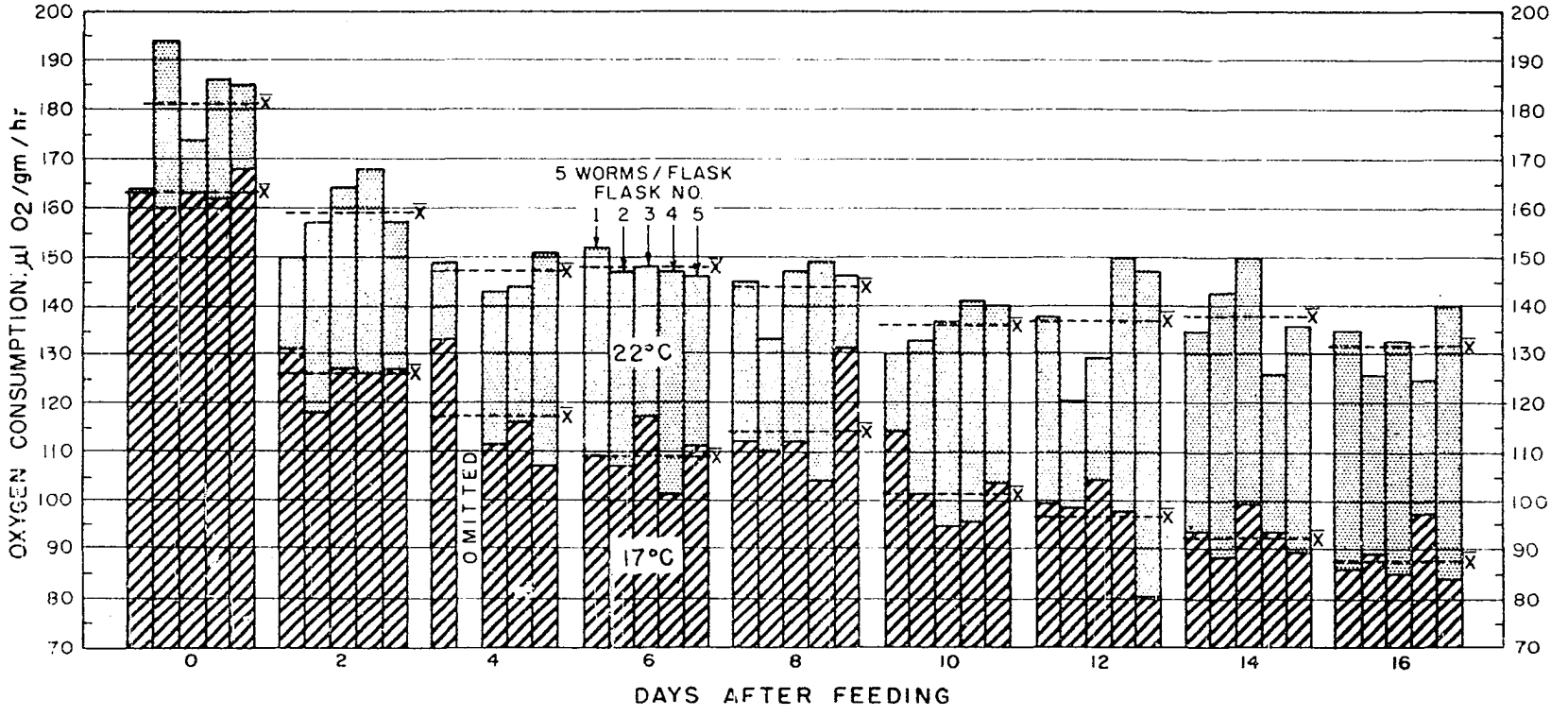

Fig. 1. Oxygen consumption of planarians in water at 17° and 22° C.

than those tested at 17° C., although the totals consumed during the three hours, and therefore the averages, were quite consistent (Table III.) The figures show the same trend from day to day that was found with the worms tested at 17° C., although the oxygen uptake at 22° C. was consistently higher, as shown in Fig. 1.

Since movement of animals will increase oxygen consumption, the planarians were observed regularly in order to note their responses to the conditions of the experiment. For the most part they remained attached to the floor of the flask. Occasionally one would be noted swinging to and fro in the water as the flask was shaken. Neither gliding nor crawling was noticed at any time, although it is presumed the variations in oxygen consumption may be due in part to such activity occurring during the time the apparatus was stopped for readings to be taken.

The Warburg manometric technique as employed was not perceptibly injurious to the worms. With one exception, the worms appeared normal in every way during and after the experiment. The one exception was a planarian which crawled off the weighing pan and fell to the oily floor of the balance. It was picked up, apparently unharmed, but one worm in the flask died later, presumably this one.

CHAPTER III

THE EFFECT OF GOITROGENS ON OXYGEN CONSUMPTION IN PLANARIANS

The second group of experiments was devoted to a study of the effects of goitrogens on oxygen uptake. Two sets of experiments were performed; one to test the effects of prolonged exposure and the other to determine immediate effects. All were conducted at 20° C. The worms were not fed during the course of the experiment.

Procedure

Three goitrogens were used for this study: thiourea, phenylthiourea and thiouracil. In order to determine the concentration of each to be used, cut posterior ends of planarians were allowed to regenerate in a graded series of molar concentrations of each of the chemicals as follows: thiourea: 0.1 M, 0.05 M, 0.01 M, 0.005 M, 0.001 M, 0.0005 M; phenylthiourea: 0.01 M, 0.005 M, 0.001 M, 0.0005 M, 0.0001 M, 0.00005 M; thiouracil: 0.005 M, 0.001 M, 0.0005 M, 0.0001 M.

In each case, the concentration finally chosen was the highest which allowed regeneration to occur without superficial evidence of any abnormality other than failure of eye-pigment formation. These concentrations were: 5×10^{-3} M thiourea, 5×10^{-4} M phenylthiourea and 5×10^{-3} M

thiouracil. Worms tested at these concentrations were not fed for seven days prior to the beginning of the experiment.

Since a more dilute solution of a drug may sometimes have a different effect from that of a more concentrated one, a duplicate series of experiments was performed using a solution of each of the three goitrogens of half the molarity of that given above. These concentrations were used for animals not fed for eight days before the beginning of the experiment.

Utilizing planarians starved for seven and eight days for the higher and lower concentrations respectively permitted the use of worms from the same stock pan for both concentrations of any one goitrogen, due to the fact that the series of oxygen consumption determinations were made on alternate days over a period of approximately two weeks. The choice of the seventh and eighth day after feeding was made on the basis of the earlier experiments which demonstrated that phase C, the period of most gradual decline of metabolic activity which occurs some time after feeding in planarians, was established in these animals after about the sixth day.

On the day that an experiment was begun, thirty of the largest specimens in one stock pan were examined with a binocular microscope. Animals exhibiting any abnormality were discarded. They were then divided randomly into six groups of five worms. Each group was thereafter treated as a unit.

The first determination of oxygen consumption, on Day 0, was made with the animals in water. At the close of the readings the worms were weighed, then placed in labeled fingerbowls, each containing 100 ml of

the higher concentration of one of the goitrogens. Readings on the second, fourth and sixth days were made with the animals in the same solution in which they had been living. On the sixth day, after the readings had been made, three of the six groups of animals were returned to water, the other three being kept in the chemical. The final two measurements were made on the eighth and tenth days.

The procedure for worms starved for eight days was identical, except that these animals were placed in the lower concentrations of the chemicals.

For determinations of oxygen consumption the technique described previously was used, with the exception that a thirty-minute equilibration period was employed. This was to allow time for the worms to come to rest after being placed in new surroundings. Readings were then taken every half-hour for three hours. At the termination of the day's readings, each group of worms was weighed.

Two series of oxygen consumption measurements on controls kept in water were made in a similar manner, one for worms starved seven days and a second for those without food for eight days.

In the second set of experiments, performed to test the immediate effect of the goitrogens on respiration, the procedure was similar to that outlined above, with the following exceptions: After the first measurement in water on Day 0, the worms were returned to water for twenty-four hours. For the second measurements, on Day 1, the worms were placed in reaction flasks prepared with the proper goitrogen, and measurements of oxygen consumption during the first three and one-half hours in the chemical were made. The worms were then placed in the drug overnight and

the third and final reading taken after twenty-four hours exposure to a goitrogen.

Results

The data obtained with these treatments were calculated according to standard methods (Umbreit et al., 1949) and the results expressed in microliters of oxygen consumed per gram of wet weight per hour. The results were found to be significant at $P < .001$ (Tables IV, V and VI).

TABLE IV

EFFECT OF HIGHER CONCENTRATIONS OF GOITROGENS ON OXYGEN CONSUMPTION OF PLANARIANS

	$\mu l\ O_2/gm/hr$					
	Days					
Treatments	0	2	4	6	8	10
Water	140	134	132	126	120	119
Thiourea, 5×10^{-3} M	145	126	117	126	117	119
Thiourea - water	146	132	123	122*	118	124
Phenylthiourea, 5×10^{-4} M	129	107	132	142	159	137
Phenylthiourea - water	143	127	135	143*	153	135
Thiouracil, 5×10^{-3} M	138	127	125	122	125	114
Thiouracil - water	132	124	131	124*	129	114

* Planarians returned to water after measurements made.

Both thiourea and thiouracil depressed oxygen consumption slightly, but not significantly below the usual gradual decline found in planarians subjected to prolonged starvation (Jenkins, 1960). A return to normal levels was established within four to six days in the animals exposed to

TABLE V

EFFECT OF LOWER CONCENTRATIONS OF GOITROGENS ON OXYGEN CONSUMPTION OF PLANARIANS

Treatments	µl O_2/gm/hr Days 0	2	4	6	8	10
Water	127	122	120	113	117	118
Thiourea, 2.5×10^{-3} M	125	123	119	106	117	119
Thiourea - water	130	120	112	112*	118	123
Phenylthiourea, 2.5×10^{-4} M	130	127	119	129	127	125
Phenylthiourea - water	124	134	128	126*	132	133
Thiouracil, 2.5×10^{-3} M	131	115	128	123	119	130
Thiouracil - water	133	120	125	125*	120	130

* Planarians returned to water after measurements made.

TABLE VI

EFFECT OF GOITROGENS ON OXYGEN CONSUMPTION OF PLANARIANS WITHIN 27½ HOURS AFTER INITIAL EXPOSURE

Treatments	µl O_2/gm/hr Days 0	1	2
Water	140	147	142
Thiourea, 5×10^{-3} M	121	122	135
Thiourea, 2.5×10^{-3} M	120	117	127
Phenylthiourea, 5×10^{-4} M	143	116	127
Phenylthiourea, 2.5×10^{-4} M	138	105	119
Thiouracil, 5×10^{-3} M	141	143	136
Thiouracil, 2.5×10^{-3} M	132	140	133

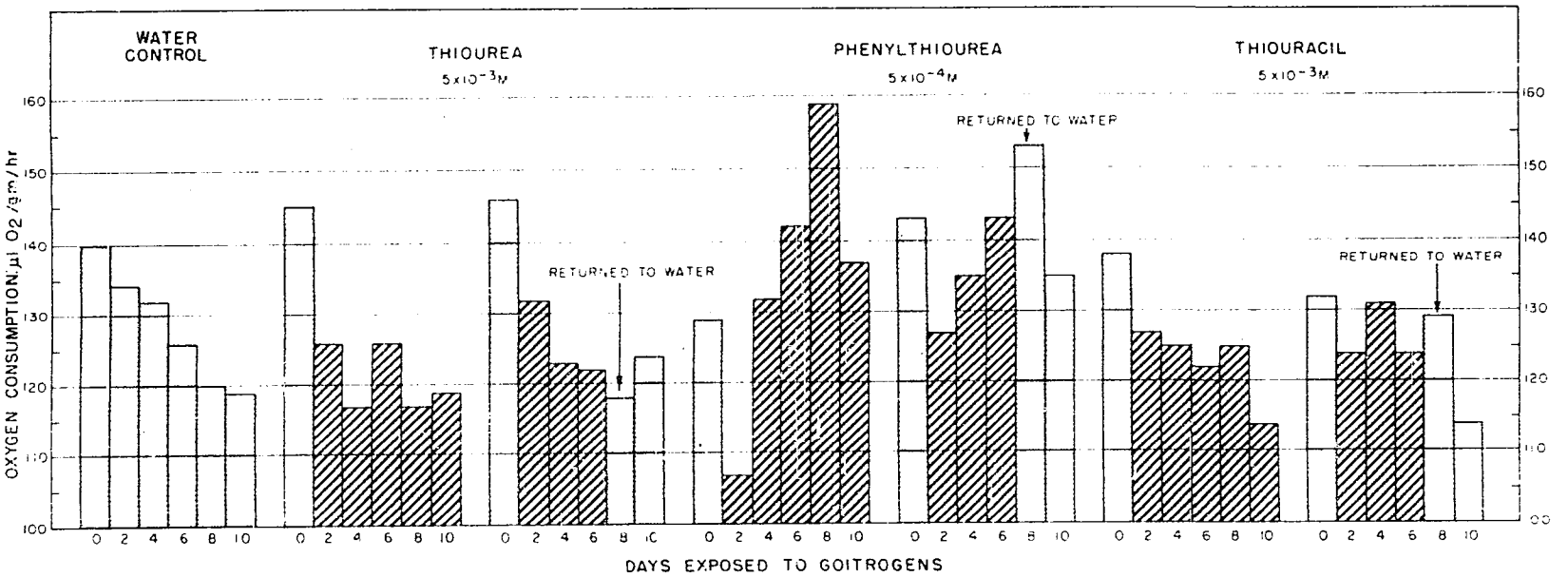

Fig. 2. Effect of higher concentrations of goitrogens on oxygen consumption of planarians.

the goitrogens for a prolonged period.

Planarians placed in 5×10^{-4} M phenylthiourea showed a significant fall in oxygen consumption within the first three and one-half hours after they were placed in the goitrogen. A return to normal levels occurred within two to four days, followed by a gradual rise which was significantly above normal by the sixth day, the maximum being reached on the eighth day. The effect was similar in the lower concentration, but less pronounced. A return to water slightly diminished the rise in oxygen uptake, but a return to normal levels was not established within four days.

The effect of the higher concentrations of goitrogens on the respiratory rate of the planarians as compared with the usual gradual decline exhibited by the water controls is illustrated in Fig. 2.

CHAPTER IV

THE EFFECT OF THYROID COMPOUNDS ON OXYGEN CONSUMPTION IN PLANARIANS

The third group of experiments dealt with the effects of thyroid compounds on oxygen uptake. All animals were maintained at a constant temperature of 20° C. Experimental animals were taken on the seventh day after feeding, and were not fed during the course of the experiment.

Procedure

Compounds used for this investigation were thyroxine, 3,5,3'-triiodothyronine and 3,5-diiodotyrosine. In order to determine the concentration to be used, groups of cut posterior ends of planarians were allowed to regenerate in a graded series of molar solutions of each of the compounds in lake water, and the regenerated animals examined for signs of any abnormalities. In worms in both the thyronines, eye spots were visible under a dissecting microscope by the third day, compared to the fifth day for the animals in water and in DIT. It was noted, however, that the worms in the 3×10^{-5} M dilution of T_3 appeared to have a slight thickening across the head behind the eyes. This was not apparent in the 2×10^{-5} M solution; the latter was therefore chosen as the higher concentration for the experiment.

All three chemicals were made up at this concentration so their effects could be compared. In addition, a solution of half the molarity given above was used for each in order to determine if a more dilute solution would have an appreciable physiological effect. The controls were cultured in lake water.

The procedure used was similar to that employed for observing the effect of the goitrogens on oxygen consumption, with the following exceptions: The Latin square method was used in order to provide maximum randomization. Seven replicate experiments were performed. Each replicate experiment employed seven groups of five planarians each; one group for each of the two concentrations of the three chemicals used, and one water control. On the day a replicate experiment was begun, thirty-five of the largest specimens in one stock pan were chosen in the usual manner and divided randomly into seven unit groups of five specimens.

For each replicate experiment, oxygen consumption determinations were made as follows:

Day 0: Oxygen consumption was measured over a period of three hours with all seven groups of animals in water. At the close of the day's readings, each group was placed in an individual fingerbowl of water until the following day.

Day 1: The Warburg flasks were prepared with the solutions of thyroid compounds (or of culture water) to be used. The planarians were placed in the flasks and manometer readings were made during the first three and one-half hours of exposure to the chemicals. At the termination of the day's readings, each group was placed in a fingerbowl containing the same concentration of thyroid compound in which oxygen consumption

was to be determined during the remainder of the experiment.

Further readings for periods of three hours were made on the second, fourth and sixth days. The worms were placed in fresh thyroid compound solutions every second day.

Results

The oxygen consumption of each group of planarians was calculated in the usual manner and the results are given in Table VII. Little variation is shown among the groups in response to treatment on any one day, the normally occurring slight downward trend shown by the controls being apparent in each of the experimental groups (Fig. 3). The data show significantly that, under the conditions of this experiment, there is no demonstrable effect on the oxygen consumption of the planarians.

TABLE VII

EFFECT OF T_4, T_3 AND DIT ON OXYGEN CONSUMPTION OF PLANARIANS

	$\mu l\ O_2/gm/hr$				
	Days				
Treatments	0	1	2	4	6
Water	130	128	131	120	121
Thyroxine, 2×10^{-5} M	133	130	130	121	120
Thyroxine, 1×10^{-5} M	132	129	131	120	124
Triiodothyronine, 2×10^{-5} M	129	133	128	124	124
Triiodothyronine, 1×10^{-5} M	131	130	131	121	120
Diiodotyrosine, 2×10^{-5} M	133	132	128	123	119
Diiodotyrosine, 1×10^{-5} M	126	130	130	124	125

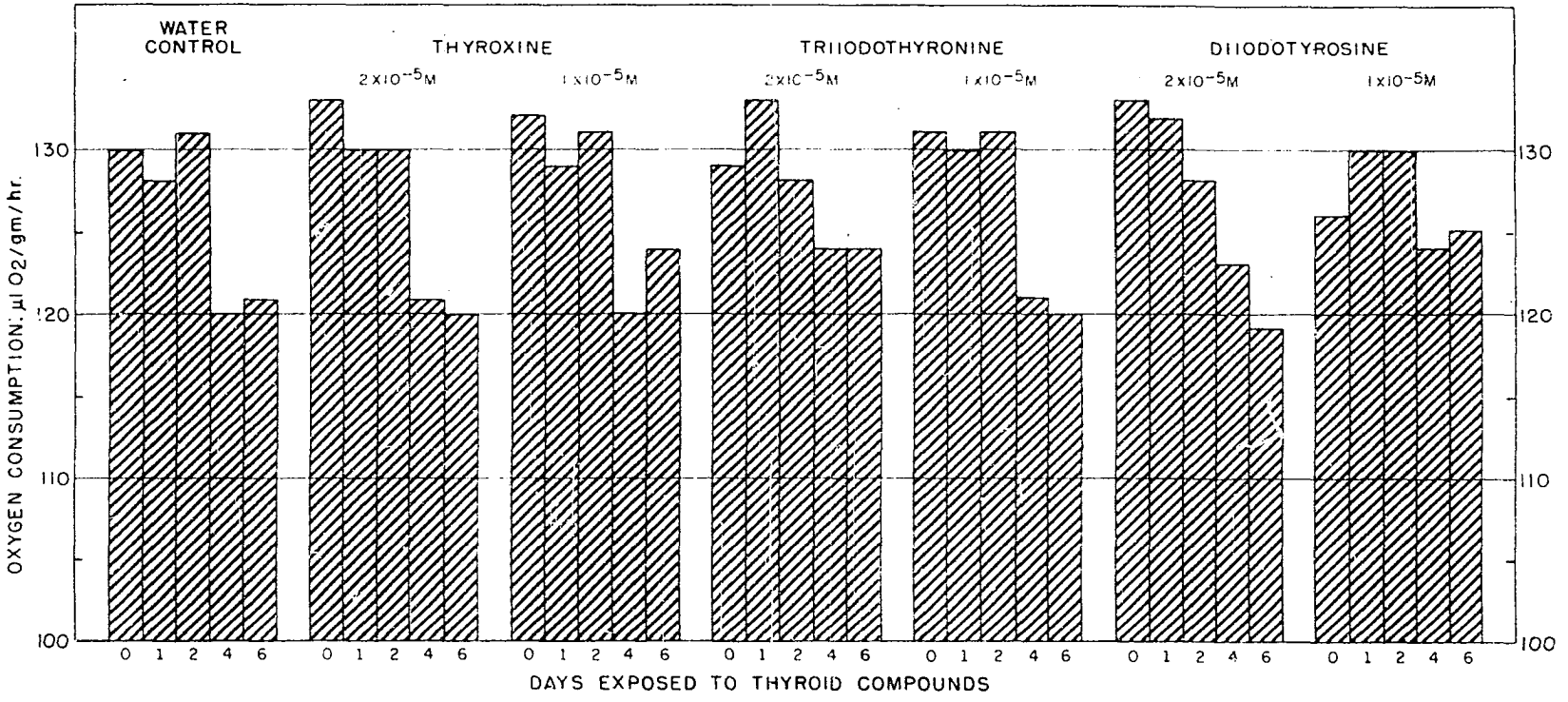

Fig. 3. Effect of thyroid compounds on oxygen consumption of planarians.

CHAPTER V

DISCUSSION

The data presented for the first experiment in this study show that the Warburg respirometer may be used successfully for measurement of the oxygen consumption of planarians. The worms were not injured by the techniques used, and the data indicate that the normal respiratory exchange of the planarians in the various flasks showed the same trends during the course of the experiment.

As noted above, one of the purposes of this phase of the study was to determine, in this species, how many days would elapse after feeding before a more or less constant level of metabolic activity was established, and whether or not it would continue for a period of time sufficient for other experimental work. For convenience in reference, these normal changes in metabolic rate have been divided into periods as follows:

Phase A. The initial rise in oxygen consumption which occurs soon after ingestion of food.

Phase B. The marked fall in respiratory rate which begins a few hours after feeding and continues for several days.

Phase C. The more or less constant level of metabolic activity which

succeeds phase B, and is characterized by a very slight and continued decline.

A final period, which might be termed phase D, has been reported to occur (Hyman, 1919a). It is marked by a noticeable and continuing rise in metabolic activity. This phase was not observed in this study because the experiment was ended during phase C.

The data show that the marked fall in respiratory rate (phase B) was not quite as pronounced in this experiment as the decline reported by Hyman (1919a), the oxygen uptake on Day 2 being 78 per cent and 87 per cent of that observed on Day 0 in the two groups of worms tested at 17° and 22° C. respectively. Hyman's figures indicate a range of 60 to 76 per cent under similar conditions. This was due, in part at least, to the fact that the measurements on Day 0 were based on the weight of the worms after feeding rather than before; the weights thus included the ingested food, which was more or less inert material. This was not considered important for the purposes of the experiment, since one object of the study was to find a sufficiently extended period during which the oxygen consumption of unfed planarians would show a minimum of either rise or decline. This period, phase C, was found to begin about the sixth day after feeding, and to last for at least ten days, at which time the measurements were terminated. This more or less constant level of metabolic activity has been reported to extend from one to three weeks in Dugesia dorotocephala (Hyman, 1919a, 1920) to as long as six weeks in the variety formerly known as Planaria agilis (Allen, 1919).

The data in Tables I and II show a much lower value than those Hyman (1920) reported for Dugesia dorctocephala, and a slightly lower value than

that given for the form previously called P. agilis. Aside from the difference in method, this variance is ascribed, at least in part, to the fact that the worms used in this experiment were much larger and heavier than the ones used by Hyman. The lowest weight obtained here was 140 mg for five worms after sixteen days starvation, or an average of 28 mg per worm. Hyman reported a weight of 472 mg for fifty worms after fourteen days starvation, or an average of less than 10 mg per worm. The values are in agreement with the conclusions of both Hyman (1919a, 1920) and Allen (1919), however, that large and old worms consume less oxygen per unit weight than do small, young ones.

Confirmation of the normal variations noted above in metabolic rate in the planarians used in this study was provided by the water controls used in the experiments with goitrogens and in the studies which made use of the thyroid compounds.

Goitrogens

The mechanism of goitrogenic action has not been completely elucidated, but current evidence favors the view that these compounds, by virtue of their reducing activity, produce their effect in the vertebrate thyroid by interfering with the conversion of iodide to iodine, presumably by inhibiting a peroxidase (Astwood, 1955), although the presence of the latter in the thyroid has not been demonstrated. Peroxidases are widespread in the plant world, but there is some question as to their occurrence and distribution in animals (Laidler, 1954).

It is possible that the action of the goitrogens in depressing oxygen consumption in planarians is mediated through a similar mechanism.

Although the fall in metabolic rate with thiourea and thiouracil was statistically insignificant, the depression in rate occasioned by the use of phenylthiourea was pronounced and continued for several hours. Jones and Wilson (1959) found that the phenylthiourea-induced fall in the rate of oxygen consumption in Cynthia silkworm larvae and pupae was accompanied by an inhibition of the blood phenolase (tyrosinase) activity, and suggested the possibility of this enzyme being implicated in some way with respiration. Its action is blocked by a number of inhibitors, including thiourea, phenylthiourea (Dubois and Erway, 1946) and thiouracil (Paschkis et al., 1944). Tyrosinase is considered to be a major component of the respiratory system of potato tubers and perhaps of many plants (Baker and Nelson, 1943).

Tyrosinase has not been demonstrated in planarians, but it is found in many invertebrates, the mealworm being the most commonly used source of invertebrate tyrosinase (Burris and Little, 1949). The presence of this enzyme in planarians is suggested, however, by reports of the effectiveness of thiourea compounds in causing eye-depigmentation in planarians. Kambara (1954) found that pigment granules in eye-spots disappeared as a result of thiourea treatment, but skin pigment was not affected. His results were confirmed by Kido and Kishida (1960). Jenkins (1959) noted that the formation of pigment was inhibited completely in the eyes of planarians allowed to regenerate in solutions of phenylthiourea at various concentrations. In the planarians returned to water, a steady development of pigment occurred. The colors appeared in the order commonly noted in melanin formation, namely tan, red to reddish brown, and finally black. In view of these facts, it appears quite possible that

the depressant action of phenylthiourea on oxygen uptake in planarians may be due to the inhibition of tyrosinase by the goitrogen. Further research to establish the validity of the suggestion is indicated.

The stimulatory effect of the phenylthiourea on planarian respiration is presumed to be due to its toxicity. The evidence for this view lies in the fact that upon continued exposure to the chemical the initial depression was reversed, then followed by a pronounced increase in respiratory rate accompanied by some degree of cytolysis. Although an effort had been made, as noted above, to use only physiologically tolerated concentrations, in the 5×10^{-4} M concentration of phenylthiourea, on the tenth day after the worms had been put in the chemical, one worm had several small lesions and five others had one or two small lesions each. None of the worms in the lower concentration of the drug showed any evidence of similar injury; nevertheless, the rise in oxygen consumption shows that they were affected and the cause, under the circumstances, is presumed to be the same in both instances. The toxicity of these chemicals, as evidenced by their ability to retard growth and differentiation, particularly when used in higher concentrations, has been demonstrated in Drosophila melanogaster (Harnley and Goldsmith, 1950) and in Eleutherodactylus martinicensis tadpoles (Lynn and Peadon, 1955).

It might be of interest to add that a possible effect of goitrogens on sexual activity in planarians was noted. During the time the experimental animals were kept in the solutions, both copulation and cocoon deposition occurred. Six pairs of copulants were seen in the thiourea and five in thiouracil, but none were observed in either water or phenylthiourea. Ten cocoons were deposited by the thiourea-treated animals, and

one by the water controls, but none were found in either of the other chemicals. Two of the ten cocoons were deposited in the Warburg reaction flasks while measurements were being made.

The ten cocoons were transferred to separate bowls of thiourea solution and kept for four weeks. By the fourth day they had begun to lose their original creamy whiteness, but complete darkening did not occur. No worms emerged from any one of the ten.

In view of the findings of Iwasawa (1959a, 1959b) that gonadal development was accelerated and sex reversal occurred in amphibian tadpoles following treatment with thiourea, it appears that further investigation is indicated.

Thyroid Compounds

Shortly after the discovery by Gudernatsch (1914) that differentiation in tadpoles was markedly hastened by thyroid feeding, M. Morse (1914) tested several iodine-containing compounds for their ability to accelerate metamorphosis in Rana pipiens larvae. He reported that experimental animals fed thyroid metamorphosed on the third or fourth day, and those given diiodotyrosine on the fifth day, in comparison with algae-fed control animals in which metamorphosis did not occur for two weeks. Since diiodotyrosine appeared to be almost as effective as thyroid in inducing metamorphosis, Morse concluded the specific effect of thyroid in shortening the period of differentiation in frog larvae was associated in some way with the amino acids which composed the complex iodinized globulin of the thyroid, and suggested that the metamorphosing effect was not a specific thyroid reaction. His work was soon confirmed by W. Morse (1918)

who also found diiodotyrosine to be effective in inducing early metamorphosis in frog larvae.

Swingle (1922) used this chemical to bring about differentiation in Rana sylvatica tadpoles which had been deprived of both thyroid and pituitary glands, but found tyrosine and dibromotyrosine to be ineffective in this respect. He also reported that large axolotls metamorphosed within a short time after injections of diiodotyrosine.

Later workers have largely ignored the use of diiodotyrosine as an effective agent in accelerating metabolic activity, due in part, no doubt, to the far greater effectiveness of thyroxine and its analogues. However, one of the more recent reports of the activity of diiodotyrosine (Vind et al., 1956) indicates the thyromimetic activity of a commercial preparation of the chemical can be reduced to one-third of the original level by one recrystallization. They suggest that a possible explanation for the reports of its activity may be due to thyroxine being slowly synthesized via the oxidative coupling mechanism discovered by Ludwig and von Mutzenbecher (1939).

The high biological activity of thyroxine and of triiodothyronine in mammals is too well known to require lengthy dircussion. With regard to the action of these chemicals in invertebrates, it is obvious that there is some homeostatic regulation in animals without a thyroid, and that metabolic processes do occur and are regulated within the animal, else life itself would cease to exist. The question is: what are the processes and how does regulation occur?

In a series of papers published in 1932 on the effect of iodine compounds on fertilization of eggs of Echinus esculentus and E. miliaris,

Carter reported that thyroxine had no effect on either fertilization or development of ripe eggs, but found that the addition of either fertilizin or thyroxine to both unripe and over-ripe eggs resulted in a high proportion of the eggs becoming fertilizable. He suggested the possibility that all healthy cells, regardless of origin, normally contained thyroxine, and were therefore not affected when more of the substance was added to the medium unless the concentration was too great, in which case the effect would be harmful. On the other hand, cells which were abnormal in any way, such as incompletely developed or overly mature eggs, might well be affected by concentrations within a physiological range.

If Carter's theory is correct, its confirmation would necessitate the demonstration of the presence of physiologically active thyroxine or thyroxine-like compounds in invertebrate cells. Although such compounds have been found in many invertebrate tissues, they have never been shown to have any effect on the metabolic activities of the invertebrate organism. However, the fact that there is no clear evidence that treatment of an invertebrate with thyroid hormone results in any kind of response would tend to support rather than to discredit Carter's view, since one of his assumptions is that normal invertebrate cells will not respond to thyroid compounds except when the concentration is great enough to have a harmful effect. The negative results obtained in the present experiment tend to support Carter's theory in that lack of response on the part of the planarians was correlated with concentrations of chemicals low enough that no evidence of any harmful effect was observed.

That physiologically active thyroxine-like compounds may be produced in tissues not subject to thyroid influence has been shown by a number

of workers. Chapman (1941) concluded, as a result of his experiments with thyroidectomized rats given extra iodine in the diet, that the iodine may play a role in body metabolism in the absence of thyroid tissue, possibly by the production of a thyroxine-like substance by the tissues themselves. Morton et al., (1943) demonstrated the presence of newly formed radioiodotyrosine and radicthyroxine in rats that had been deprived of their thyroid glands for several months. Pitt-Rivers and her co-workers (1955) have reported evidence that triiodothyronine also can be formed in the peripheral tissues of the thyroidectomized animal.

These observations, coupled with the demonstrations of von Mutzenbecher, Reineke and others, that thyroxine can be formed from iodinated proteins in vitro, point to the possibility that thyroxine and its precursors may play a role in the metabolism of invertebrates. That they have not been demonstrated unequivocally to do so may be due in part to the ability of the poikilotherms, particularly the smaller organisms, to carry on normal activities with only infinitesimal amounts of the regulatory materials available. It would appear that clarification of this controversial subject might well involve the use of more delicate techniques than those hitherto employed in the investigation of metabolic activities in small invertebrate organisms.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. The oxygen consumption of large planarians of the species Dugesia dorotocephala was studied during a period of sixteen days of starvation subsequent to feeding, at temperatures of 17° and 22° C. The Warburg respirometer was used and found to be adequate, under the conditions of the experiment, for measurement of oxygen consumption in planarians. Worms were not injured by the techniques employed.

2. Subsequent to feeding, the oxygen consumption of groups of planarians in water was found to follow a pattern divisible into typical periods or phases. The suggestion is made that these be indicated as follows: Phase A, initial rise; phase B, marked decline; phase C, minimum rate of decline; and phase D, final rise. In this experiment phase A continued for three to five hours after feeding, and phase B for five to six days. Phase C was found to begin about the sixth day after feeding and to extend for at least ten days until the termination of the experiment. Although oxygen consumption at 22° C. was consistently higher than at 17° C., the same phases and pattern of rise and decline were observed.

3. The worms were subjected to the influence of the goitrogens thiourea, phenylthiourea and thiouracil, and the effect of these chemicals

on their oxygen consumption was measured. Thiourea and thiouracil were found to have no statistically significant effect. With phenylthiourea there was a marked initial depression, then a gradual return to normal levels followed by a significant rise. In the animals returned to water, oxygen consumption was not reduced to normal levels within four days. It is suggested that the depressant action of the phenylthiourea might be ascribed to its ability to inhibit tyrosinase, while the stimulatory effect might well be due to the toxicity of the goitrogen.

4. Using the Latin square treatment, a study was made of the effect of the thyroid compounds diiodotyrosine, triiodothyronine and thyroxine on respiration in planarians. No statistically significant effect was found with any one of the three chemicals under the conditions of the experiment.

5. A possible effect of thiourea upon sexual activity and cocoon development in planarians was reported.

6. Regulation of metabolic processes in invertebrates was discussed briefly. The suggestion was made that the same fundamental pattern of metabolic regulation is to be found throughout the entire animal kingdom.

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