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THE EFFECT OF THYROID AND MO911 ON LOCOMOTOR ACTIVITY AND MONOAMINE OXIDASE ACTIVITY IN MICE

BY

VINCENT EUGENE REID

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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(Pharmacology)

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1963

MASTER OF SCIENCE THESIS

OF

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UNIVERSITY OF RHODE ISLAND

1963

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ABSTRACT

The relationship of thyroid and monoamine oxidase activity to locomotor responses was investigated.

Locomotor activity was measured by use of actophotometers. Four mice were placed in the actophotometer and counts were recorded at specific time intervals up to two hours. The administration of thyroid, MO911, and combined thyroid and MO911 did not alter spontaneous activity significantly.

Monoamine oxidase activity of brains and livers was measured in vitro using the Warburg apparatus. The elapsed time for tissue respiration was 28 minutes. Thyroid administration did not alter monoamine oxidase activity significantly in liver but brain activity was decreased. Therapy with MO911 decreased liver monoamine oxidase activity sharply and also depressed brain activity. Combined therapy with thyroid and MO911 decreased liver monoamine oxidase activity less effectively than MO911 alone. Brain monoamine oxidase activity was also depressed.

No correlation was found to exist between monoamine oxidase activity and locomotor responses following thyroid, MO911, and combined thyroid and MO911 therapy.

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I. INTRODUCTION

The advent of psychic energizers in 1952 represents a new frontier in the treatment of primary mental depression. Therapeutic doses in humans achieved excellent results by elevating the mood of the patient and improving psychic energy. Responses to these drugs were correlated with the decrease of monoamine oxidase (MAO) activity in the body thereby elevating the concentrations of brain and tissue catecholamines.

Diverse effects have been described following the use of thyroid. Among these is the decrease in MAO activity. There have been dissenters to this view but the evidence preponderantly favors the assenting viewpoint. The precise mechanism which is altered to produce the enzyme reduction has not been clarified.

Theoretically, combination therapy of a MAO inhibitor and thyroid should combat depression more effectively than either alone. If another metabolic effect of thyroid is considered, such as an elevated basal metabolic rate, a more potent therapeutic combination should be predicted. Inexplicably, this is not the result. Thyroid and MAO therapy produces retardation and apathy in depressed patients.

This investigation is an attempt to resolve the apparent paradox by studying the interrelationships of MAO activity in brain and liver. This will be approached by administering thyroid, MAO inhibitor and a combination of thyroid and MAO inhibitor to experimental animals.

II. SURVEY OF THE LITERATURE

At the turn of the century, Eppinger, Falta and Rudinger (cf. Burn & Marks, 1925) found that in thyroidectomized dogs an injection of adrenaline did not cause glycosuria. Burn and Marks (1925) studied the glycemic responses associated with adrenaline and insulin and found an inverse relationship resulting from the activity of adrenalin and insulin. Adrenaline promoted glycogenolysis and insulin produced a hypoglycemic state. These investigators also noted that thyroidectomy produces an increase in insulin hypoglycemia and a decrease in adrenalin hyperglycemia. Although Burn and Marks did not attribute this phenomena to MAO activity, the implication that this enzyme may have been responsible is evident from subsequent knowledge.

Interactions of thyroid and sympathomimetic amines were found on a gross tissue respone level. Sawyer (1935) explored the effects of thyroidectomy and thyroxine on the response of the denervated heart to injected and secreted adrenaline. Thyroidectomy decreased the response of the denervated heart of the cat to injected and secreted adrenaline. Their conclusion suggests that the "sensitization" of heart tissue to adrenaline is caused by thyroid. Oehme (1936) reported that the adrenal cortical hormone possesses an antithyrotropic action as it decreased the elevated basal metabolic rate (BMR) in guinea pigs. The action of adrenal cortical secretion was effective whether the hyperthyroid state was caused by parenteral thyroxine or by dietary feeding and furthermore, the activity of ascorbic acid paralleled the activity of the hormone of the adrenal cortex. Developing this theory Oehme (1937) experimented with thyroid, the adrenal cortex hormone, ascorbic acid and amino acids. The results indicated that the hormone of the adrenal cortex not only

lowers the increased metabolism of guinea pigs after thyroxine but also prolongs the life span of animals poisoned with thyroxine. Glycine exhibited essentially the same effect in experimental hyperthyroidism as the adrenal hormone, but in euthyroid guinea pigs it lowered metabolism after a prolonged period. Brewster et al. (1956) demonstrated the augmentation of the effects of epinephrine by thyroid. Hyperthyroid animals deprived of all sources of epinephrine returned to the euthyroid state. These experiments indicate that thyroid sensitizes heart tissue to adrenaline. Furthermore, thyroid's action is inhibited by the hormone of the adrenal cortex, ascorbic acid, and amino acids.

The hypothesis of a possible thyroid-adrenal interplay was demonstrated by the work of Rokhlina (1937). He has shown by implantation techniques in the chick embryo, that adrenal cortex tissue implanted in the chorio-allantoic envelope of the 8 day embryo inhibited the development of the thyroid gland. The inhibition was especially prominent 12 to 14 days after incubation. Lang (1938) noted that rats rapidly injected subcutaneously with adrenaline, demonstrated a slight increase in the colloid content of the thyroid gland with concomitant proliferation of the epithelium. Hoen (1939) determined hormonal relationships between the thyroid and adrenal glands. He found that desoxycorticosterone inhibited the metabolic effect of thyroxine but did not prevent the loss of weight orprolong the survival of rats. The hypertrophy of the adrenals in the hyperthyroid state was inhibited by the adrenal hormone and the same type of hypertrophy was observed in hypophysectomized rats. The inhibition of the thyrotropic stimulating hormone by thyroxine is accompanied by a release of corticotropic hormone which causes adrenal hypertrophy. Goetsch (1940) attempted to implant adrenaline pellets on

the thyroid of guinea pigs but was not successful. Using adrenaline in oil, rabbits were injected daily for 71 days and differences in thyroid mitochondria were observed in the experimental and controls. The change in the size of the mitochondria was deemed stimulation. Five years earlier Richter (1935) studied the effects of thyroid treatment on the production and discharge of adrenaline. This investigation demonstrated the increase in synthesis and discharge of adrenaline followed by adrenal exhaustion. Splanchnic sectioning did not alter the effect and thyroidectomy resulted in rapid formation of adrenaline but no discharge of the hormone. Insulin increased the secretion of adrenaline leading to exhaustion.

The scope of thyroid investigations has been kaleidoscopic. Attempts to correlate its activity on the tissue level have taken many tangents. There has been no formalization of approach and therefore the problem has been attacked from various directions. The results of the experiments previously cited may have been influenced by oral feeding (Barker, 1951). The marked weight loss following the feeding if diets containing 0.1 to 1.0 percent desiccated thyroid plus added vitamins disturbs the caloric balance. As the hyperthyroid state develops, the amount of thyroid ingested is increased leading to liver damage (Frazier, 1935; Haban, 1935 and Bartlett, 1938; cf. Barker, 1951).

Interest in enzyme interactions led to an investigation by Bacq (1936). This investigator found a prolongation of responses to sympathetic nerve stimulation after pyrogallol administration. The effect was attributed to the auto-oxidant properties of the catechol. Barker (1951) states that liver cytochrome oxidase, succinic oxidase, and d-amino acid oxidase systems were more active after the administra-

tion of thyroid. Dehydrogenase activity was reduced by thyroidectomy and had the opposite effects. Blaschko and Schlossman (1940) corroborated these findings with the specification that cytochrome oxidase oxidation of adrenaline is augmented by thyroxine, dried thyroid or the thyroid stimulating hormone (Tipton and Nixon, 1946).

Investigations into the effects of thyroid and MAO interactions were reported by Spinks and Burn (1952). They found that MAO activity was increased in thyroidectomized rats. A decrease in the activity of MAO activity followed thyroid feeding. Spinks and Burn also postulated that amine oxidase levels were responsible for the reduction of adrenaline hyperglycemia with thyroidectomy and the elevation of adrenaline hyperglycemia with thyroid feeding.

Zile and Lardy (1959) using rat liver mitochondria, demonstrated that thyroid feeding decreased MAO activity but no observable change was elicited after thyroidectomy. Using thyroid in vitro, no direct inhibiting effect could be shown manometrically. Further work by Zile (1960) and Dubnick et al. (1960) demonstrated the effect of thyroxine and other thyromimetic compounds on heart MAO activity. Zile contends that no measurable change of heart MAO activity was apparent in the rat. Changes in the brain were inconsistent. Upon doubling the dose, no further decline in MAO was demonstrated. Dubnick et al. (1960) demonstrated a decrease in brain MAO activity following thyroidectomy. The work of Novick (1961) substantiated the experiments of Zile and Lardy (1959) in one aspect. Using manometric methods, Novick reported that a 2 percent desicated thyroid diet reduced the MAO activity of the livers by 50 percent. This investigator's experimental results with heart MAO activity apparently conflicted with those of Zile (1960).

Thyroid feeding or administration of L-triiodothyronine did not decrease MAO levels in the heart but instead increased the content. Novick also observed oxygen stimulation of various substrates to different degrees. In 100 percent oxygen the uptake of oxygen was elevated by a factor of 2. Serotonin, the substrate used by Zile (1960), was shown to be stimulated in a total oxygen atmosphere by a factor of 1.4.

Skillen et al. (1962) made an important contribution by evaluating the effect of thyroid on MAO in male and female rats. Male rats possess greater heart MAO activity than females with no difference in 5-hydroxytryptophan decarboxylase or 5-hydroxytryptamine levels. Thyroid feeding did not lower heart MAO in males but did elevate MAO levels in females. Chemical thyroidectomy, by the administration of propylthiouracil, decreased male heart MAO activity but did not alter female activity. This work skillfully correlates the work of Novick, Zile, and Zile and Lardy. Novick used mixed sexes; Zile and Lardy used male rats.

Other paradoxical events have contributed to the argument. Five hours after the administration of an MAO inhibitor, trans-2-phenylcyclopropylamine, norepinephrine levels were shown to be falling at a time of complete MAO inhibition. If a feedback mechanism to arrest further synthesis becomes operable, there should be a leveling of norepinephrine levels but not a decline. The alternative would be another pathway of metabolism such as the catechol-O-methyl transferase (COMT) system as suggested by Axelrod (1957).

Ostensibly, MAO activity is modified by MAO inhibitors and the amount of inhibition of MAO should correlate with the effect, but there are dissenting views. Euler (1955), Corne and Graham (1957), Axelrod and

Larouche (1959), Axelrod et al. (1959), and Kopin (1960) suggest that deamination is a minor pathway of administered catecholamines. The fact that COMT is mainly responsible for the degradation of epinephrine is a view held by Axelrod (1957) and Axelrod and Tomchick (1958). Griesemer et al. (1953) and Brown and Gillespie (1957) believe that exogenous and endogenous catecholamines are not prolonged in their action by MAO inhibitors. They further state that more than one-half of the epinephrine and norepinephrine is converted to metanephrine or normetanephrine in two minutes. This implicates COMT as the enzyme which terminates most of the activity of epinephrine and norepinephrine.

Axelrod et al. (1959), with infusion techniques, found that the concentration of epinephrine localized in heart, spleen, adrenals, and pituitary exceeds plasma levels several fold. Muscle and brain epinephrine levels were lower than the plasma. Metanephrine concentration was comparable to epinephrine in all tissues, but higher in skeletal muscle. When epinephrine was injected rapidly, the concentration of metanephrine was much higher than epinephrine particularly in heart, lung, liver and muscle. In the pituitary, however, epinephrine levels were higher than metanephrine levels. Plasma also exhibited higher metanephrine levels with a trace of mandelic acid derivatives. Since epinephrine was found to be in lower concentrations after rapid injections, it was postulated that epinephrine was bound after fast injections and then released for metabolism. Epinephrine's biphasic degradation was also noted. The first phase, which was rapid, is explained by diffusion into the tissues and O-methylation. The second phase consists of the release of epinephrine from the binding sites and concomitant metabolism. This information suggests strongly that O-methylation is a major degradative pathway for

the catecholamines.

If MAO is considered a secondary path of metabolism of the catecholamines, MAO inhibitors would not be expected to elevate catecholamine levels significantly. Reports of increased levels of catecholamines and the potentiation of effects of catecholamines have been made by Pletscher (1957), Glylys et al. (1959), and Burford et al. (1960). Serotonin levels have been increased after 24 hours using an irreversible inhibitor, phenelzine. When these levels receded, a dose of a short acting inhibitor, harmaline, produced a booster effect (Dubnick et al., 1962). It may be questioned as to whether 100 percent inhibition had been achieved. Spector et al. (1960a) consider the rise in serotonin with phenylalkylhydrazines unusual when compared with iproniazid, since the amount of MAO in the brain is almost completely inactivated. The possibility of inacessibility of MAO to iproniazid is considered primary. The function of MAO is seen as an intracellular deactivation mechanism for the amines in neurons to prevent spillage onto receptor sites. If the MAO is located in the same neuron as norepinephrine, epinephrine, and serotonin, the amines are limited in action when storage sites are saturated. The delay in effect of MAO inhibitors after blockade is ostensibly caused by the lag in the filling of the storage sites and the eventual overflow onto the receptors. The pharmacological effect is seen only after the amines have reached a maximal concentration.

The suggestion of Dubnick et al. (1962) is somewhat contradicted by an earlier report of Weissbach et al. (1961), postulating the presence of variable low levels of MAO in the brain which are insensitive to MAO inhibitors. The enzyme can catalyse the formation of 5-hydroxyindole

acetic acid.

The fact that the physiological action of MAO inhibitors are not manifest unless the enzyme is inhibited 85 percent or more has been reported by Gey and Pletscher (1961) and Chessin et al. (1959). Since MAO is believed to be stored in granules (Giarman and Schanberg, 1958; Walaszek and Abood, 1959), it is possibly protected from enzymic transformation. Gey and Pletscher (1961) suggest that MAO is present in excess in rat brain and the function of the enzyme is to inactivate free amines rather than to regulate total amines.

Major actions which do not involve inhibition of deamination have been attributed to MAO inhibitors. The "true" action of MAO inhibitors was emphasized by Green and Erickson (1960), who observed the falling of norepinephrine levels after MAO had been completely blocked. Axelrod et al. (1961) using long and short acting MAO inhibitors and tritium-labeled epinephrine demonstrated that epinephrine has a diphasic degradation curve after treatment with reserpine. The MAO inhibitors did not vary the rapid phase of degradation but extended the slow degradation curve. Axelrod interpreted this as an inhibition of reserpine release of catecholamines from their binding sites. Spector et al. (1960), using a blood platelet system, refuted reserpine block as a mechanism of action of MAO inhibitors.

The fact that thyroid reinforces the effects of epinephrine (Thibault, 1948) and reduces the MAO levels in various tissues displays the importance of this interrelationship. Epinephrine has been shown to stimulate the release of triiodothyronine and tetraiodothyronine in dogs using the levels of protein bound iodine (PBI^{131}) in the thyroid vein as criteria (Ackerman and Arons, 1958). Increases in PBI^{131} levels

after injections of epinephrine ranged from 1.5 to 17.1 times the control values. The thyroid stimulating hormones increased PBI^{131} secretion 7.4 to 18.9 times that of the controls. Increases in PBI^{131} were comparable in the hypophysectomized dog indicating a direct effect on the thyroid. The fact that Axelrod et al. (1959) observed that the highest amount of brain epinephrine is found in the pituitary gives an indication of the complexity of the modifying influences on the pituitary hormonal picture.

With the advent of the psychic energizers, an enigmatic response was noted by Kline (1961). The combination of the psychic energizer with thyroid extract produced a retardation and apathy in depressed patients. The basis for this action is not known and generally unexplored.

III. INVESTIGATION

A. Objectives

The fact that thyroid extract and MAO inhibitors combined cause a retardation in the mental health of the depressed patient has been considered a paradox since each singly produces the opposite effect. There has not been an attempt to relate the cause of the retardation and apathy with locomotor activity and enzyme levels.

Specifically, the object of this investigation is to analyze the unpredictable responses observed when thyroid and MAO inhibitors are administered concurrently. Locomotor activity experiments were performed on mice after the administration of thyroid, MO911, and combined thyroid and MO911. Furthermore, MAO enzyme activity was determined at the conclusion of the locomotor studies. The results of these experiments showed the role of MAO in apathetic response associated with MAO inhibitors and thyroid combined therapy.

Locomotor relationships were evaluated by the use of actophotometers.[1] The actophotometer is suitad for locomotor studies since it records animal movements by a light beam interruption technique. A light beam with a red filter energizes photo-electric cells. The animals' movements will interrupt the beam indicating a pulsating voltage. The number of the pulsations is counted and is used as an index of activity. This technique offers an indirect quantitation of central nervous system activity.

B. Materials and Methods

1. Group Selection and Actophotometer Calibration:

---

1. Actophotometer, Metro Industries, Long Island City, N.Y.

Male mice weighing 19-25 grams were placed in each of four actophotometers, distributed 6, 5, 4, and 3 in each cage. Counts were taken at specific time intervals up to 2 hours. These counts were recorded and plotted on semilog paper to determine the ratio of change during each time interval.

The relationships showed that 3, 4, 5, or 6 animals could be used. Four animals were selected because of convenience in ease of handling.

The actophotometers were located in a soundproof room and counts were obtained in total darkness. All counts were taken at approximately the same time each evening. The temperature variance of the room from 80°F. was minimal.

Calibration of the actophotometers was accomplished by rotating an implement around the inner circumference in such a manner that 5 rotations produced a count of 60. Identical counts were obtained by the adjustment of the sensitivity.

2. Drugs:

Forty-eight male mice were divided into three sections of sixteen animals. Each section was subdivided into 4 groups of four animals designated I, II, III, IV. One group in each section, or a total of twelve animals, served as controls. Section I received 1 mg/kg desiccated thyroid intraperitoneally. Section II and III received 6 mg/kg N-benzyl-N-methyl-2-propynylamine hydrochloride (MO911) a non-hydrazine MAO inhibitor, and combined administration of desiccated thyroid and MO911 respectively.[1] Injections were administered daily.

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1. The structural formula for MO911 appears in the Appendix.

Desiccated thyroid was suspended in physiological saline and administered intraperitoneally. MD911, which is very soluble (1.6 g/ml) in water, was also administered intraperitoneally. Controls received sham injections of the vehicle. Upon introduction of combined therapy, the control mice were injected with an amount of water equivalent to the total fluid intake of the mice.

3. Locomotor Determinations:

Four male mice were placed in each of four actophotometers. After equilibrating for 15 minutes, the counters were activated and counts taken at 5, 10, 20, 30, 60, and 120 minutes. The temperature of the activity room was recorded throughout the experimental period by utilizing a Tempscribe[1] thermometer.

The basis of comparison of the two groups, the experimental and controls, was the student "T" test as modified by Snedecor (1956). The formula utilized is:

$$t = (\bar{X}_1 - \bar{X}_2)\sqrt{\frac{n(n-1)}{\Sigma x^2}} \quad \text{or}$$

$$t = (\bar{X}_1 - \bar{X}_2)\sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 - n_2)\ \Sigma x^2}}$$

$\bar{X}_1 - \bar{X}_2$ designates the difference between the two group means.

Σx^2 is the pooled sum of the squares.

Actophotometer experiments were initiated 24 hours after injection of a specific drug. Drug dosages were administered following the removal of mice from the actophotometers. Activities were tested daily 24 hours

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1. Tempscribe, Bacharach Industrial Institute, Pittsburgh, Pa.

subsequent to injections. Sham controls were used throughout the period of investigation.

4. MAO Analysis:

MAO activity was determined by the method of Creasey (1956) and the mitochondrial-microsomal preparation according to Hawkins (1952). Hawkins (1952) indicated that two thirds of the MAO activity is present in the mitochondria and the remainder is present in the microsomes.

Male mice were decapitated and the brains and livers were removed. The tissues were frozen by immersion into a freezing slush of dry ice and trichloroethanol. The tissues were kept in a freezer and MAO activity was analyzed within 30 days.

Crushed ice was used throughout the period of preparation of mitochondria and microsomes to obtain maximum yields of MAO activity. All implements utilized in preparing the tissues were placed in crushed ice.

The frozen tissues were weighed on a tissue balance[1] and minced with scissors while in isotonic sucrose (0.25M) and the resulting pulp was transferred to a homogenizing flask. An amount of isotonic sucrose was used in a ratio of 6:1 with the tissue. The homogenates were prepared with a teflon and pyrex homogenizer.[2] The homogenate was then transferred to a refrigerated centrifuge[3] and centrifuged at 755 x g for 10 minutes. This procedure sedimented the unbroken cells, the incompletely homogenized cell fragments, the nuclei, and red blood cells.

---

1. Vereenigde Draadfabrie Ken Nijmegen, Holland.
2. Tri R Homogenizer, Tri-R Instruments, Jamaica, N. Y.
3. Servall Refrigerated Centrifuge, Model RC-2, Ivan Servall Inc., Norwalk, Conn.

The supernatant containing the soluble cell constituents including the microsomes and mitochondria was decanted. This fraction was centrifuged at 22,000 x g for 45 minutes in order to separate the soluble proteins from the formed elements. The pellet formed consisted primarily of the mitochondria and microsomes. The supernatant was carefull discarded and cold sucrose added so that the mitochondria-microsome mixture was equivalent to 200 to 350 mg of fresh tissue.

MAO activity was determined by use of the Warburg apparatus.[1] The main compartment of the conical flask contained 1 ml of the protein, 0.2 ml of 0.24M sodium phosphate buffer (Dawson, 1959) pH 7.3, and 0.2 ml neutralized semicarbazide. Water was added to bring the final volume to 2.5 ml. The side arm contained 0.4 ml of 0.05M tyramine hydrochloride as the substrate or 0.4 ml water in the controls. Carbon dioxide absorption was facilitated by 2M KOH in the center well. Filter paper with accordian folds was placed in the center well extending approximately 1-2 mm above the lip to increase the surface area of the KOH. The analysis was performed at 38° Centigrade and the preparation was gassed with oxygen (2 liters/min.) for 2 minutes. Five flasks were used per assay consisting of three experimental, two controls and one thermo-barometer. Readings were taken at four minute intervals and total time was 28 minutes. Extrapolation of time to one hour was achieved by use of the derived equation.

MAO catalyzes the reaction:

$$R\text{-}CH_2NH_2 + H_2O + O_2 \longrightarrow R\text{-}CHO + H_2O_2 + NH_3$$

---

1. Bronwill Scientific Inc., Rochester, N.Y.

Theoretically one molecule of oxygen is absorbed for each mole of substrate deaminated. The work of Kohn (1937), Pugh and Quastel (1937), and Luschinsky and Singher (1945), demonstrated that crude MAO preparations contain peroxidases and aldehyde oxidase which elevate the theoretical values of MAO activity. Creasey (1956) demonstrated that semicarbazide reduces the spontaneous oxidation of the aldehyde (parahydroxyphenylacetaldehyde). Aldehyde oxidase was found not to affect this system. The schematic representation of the oxidation of tyramine substrate is depicted in Figure 1.

Total respiration was determined by the method of Umbreit (1957).

IV. RESULTS

All tables and figures are contained in this section.

TABLE 1

Oxygen Uptake of Livers Treated with Thyroid, MO911, and Combined MO911 and Thyroid

	µl O_2 Uptake		µl O_2 Uptake		µl O_2 Uptake	
Time	Thyroid Section	Control	MO911 Section	Control	MO911-Thyroid	Control
4 min.	11.3	12.4	0.22	12.8	3.9	17.0
8 min.	18.6	19.9	1.2	21.6	6.4	24.6
12 min.	25.7	26.5	2.9	28.3	9.2	29.9
16 min.	32.1	31.8	1.7	36.2	10.6	37.0
20 min.	37.7	36.4	1.4	42.9	12.4	41.8
24 min.	40.6	42.1	2.1	50.0	13.6	47.5
28 min.	45.6	45.4	3.0	55.6	20.1	54.8

TABLE 2

Brain Respiration

Thyroid	µl O_2/hr.	Percent Decrease	MO911 Section	µl O_2/hr.	Percent Decrease	Thyroid-MO911	µl O_2/hr.	Percent Decrease
Control	12.6	0	Control	49.6	0	Control	22.6	0
Group I	2.4	81	Group I	± 2.8	100	Group I	3.9	83
Group II	9.6	24	Group III	1.3	97	Group III	± 3.4	100
Group III	16.4	± 30	Group IV	12.2	75	Group IV	5.2	77

TABLE 3

Average Percentage Change of Actophotometer Counts of Mice Treated with Desiccated Thyroid

	15 min.*	SD	t	P	25 min.	SD	t	P
Control	101	16.6	-	-	83	25.6	-	-
Group I	108	29.9	0.638	>0.500	96	35.1	0.928	0.400 - 0.200
Group II	71	10.6	2.544	0.05 - 0.025	80	14.8	0.177	>0.500
Group III	89	30.7	1.087	0.400 - 0.200	102	43.5	1.186	0.400 - 0.200

	45 min.	SD	t	P	90 min.	SD	t	P
Control	119	22.6	-	-	89	18.3	-	-
Group I	97	30.5	0.851	0.500 - 0.400	83	19.8	0.684	>0.500
Group II	121	29.8	0.053	>0.500	93	26.3	0.150	>0.500
Group III	98	38.2	0.812	0.500 - 0.400	109	76.2	0.800	0.500 - 0.400

* represents mid point of time interval
"t" - t value

SD - Standard Deviation
P - P value

TABLE 4

Average Percentage Change of Actophotometer Counts of Mice Treated with MO911

	15 min.*	SD	t	P	25 min.	SD	t	P
Control	93	17.6	-	-	93	12.4	-	-
Group I	101	21.2	0.141	> 0.500	98	16.0	1.106	0.400 - 0.200
Group III	93	15.9	1.106	0.400 - 0.200	99	15.7	1.264	0.400 - 0.200
Group IV	100	23.6	1.050	0.400 - 0.200	91	23.7	0.340	> 0.500

	45 min.	SD	t	P	90 min.	SD	t	P
Control	86	20.6	-	-	104	49.7	-	-
Group I	92	21.0	0.705	0.500 - 0.400	103	15.1	0.360	> 0.500
Group III	96	14.6	1.665	0.200 - 0.100	98	9.1	0.186	> 0.500
Group IV	87	24.2	0.145	> 0.500	93	37.2	1.012	0.400 - 0.200

* represents mid point of time interval
"t" - t value

SD - Standard Deviation
P - P value

TABLE 5

Average Percentage Change of Actophotometer Counts of Mice Treated with MO911 and Desiccated Thyroid

	15 min.*	SD	t	P	25 min.	SD	t	P
Control	94	14.0	-	-	99	11.7	-	-
Group I	86	10.6	0.253	>0.500	106	47.2	0.556	>0.500
Group III	88	13.8	0.354	>0.500	103	15.6	0.768	0.500 - 0.400
Group IV	90	21.5	0.175	0.500	89	21.9	0.99	0.400 - 0.200

	45 min.	SD	t	P	90 min.	SD	t	P
Control	107	26.4	-	-	104	28.8	-	-
Group I	116	77.5	0.115	> 0.500	105	74.8	0.0979	> 0.500
Group III	95	9.1	0.415	> 0.500	93	8.5	0.347	>0.500
Group IV	84	30.7	1.93	0.100 - 0.050	79	35.7	1.89	0.100 - 0.050

* represents mid point of time interval
"t" - t value

SD - Standard Deviation
P - P value

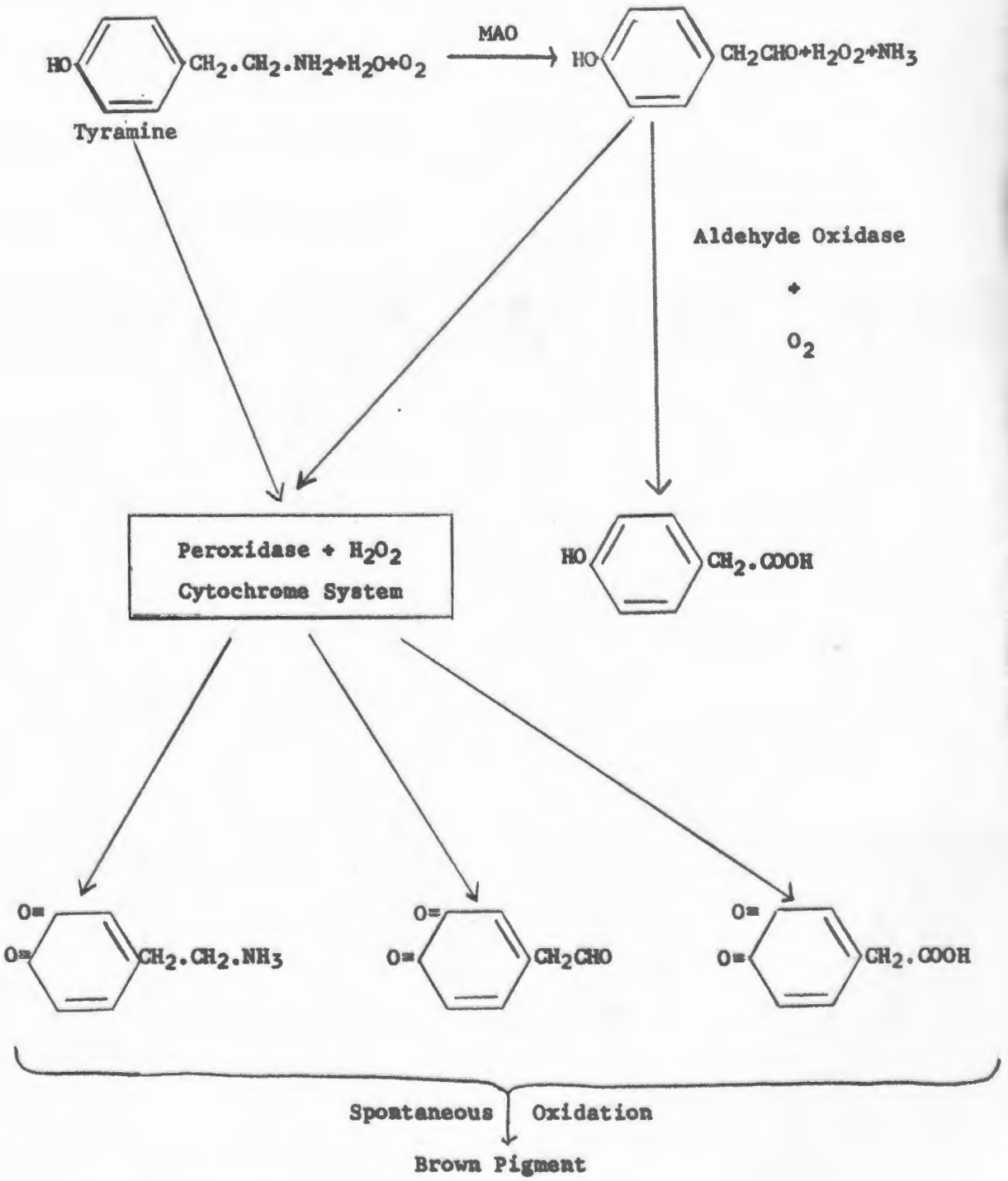

Fig. 1. Tyramine deamination by monoamine oxidase.

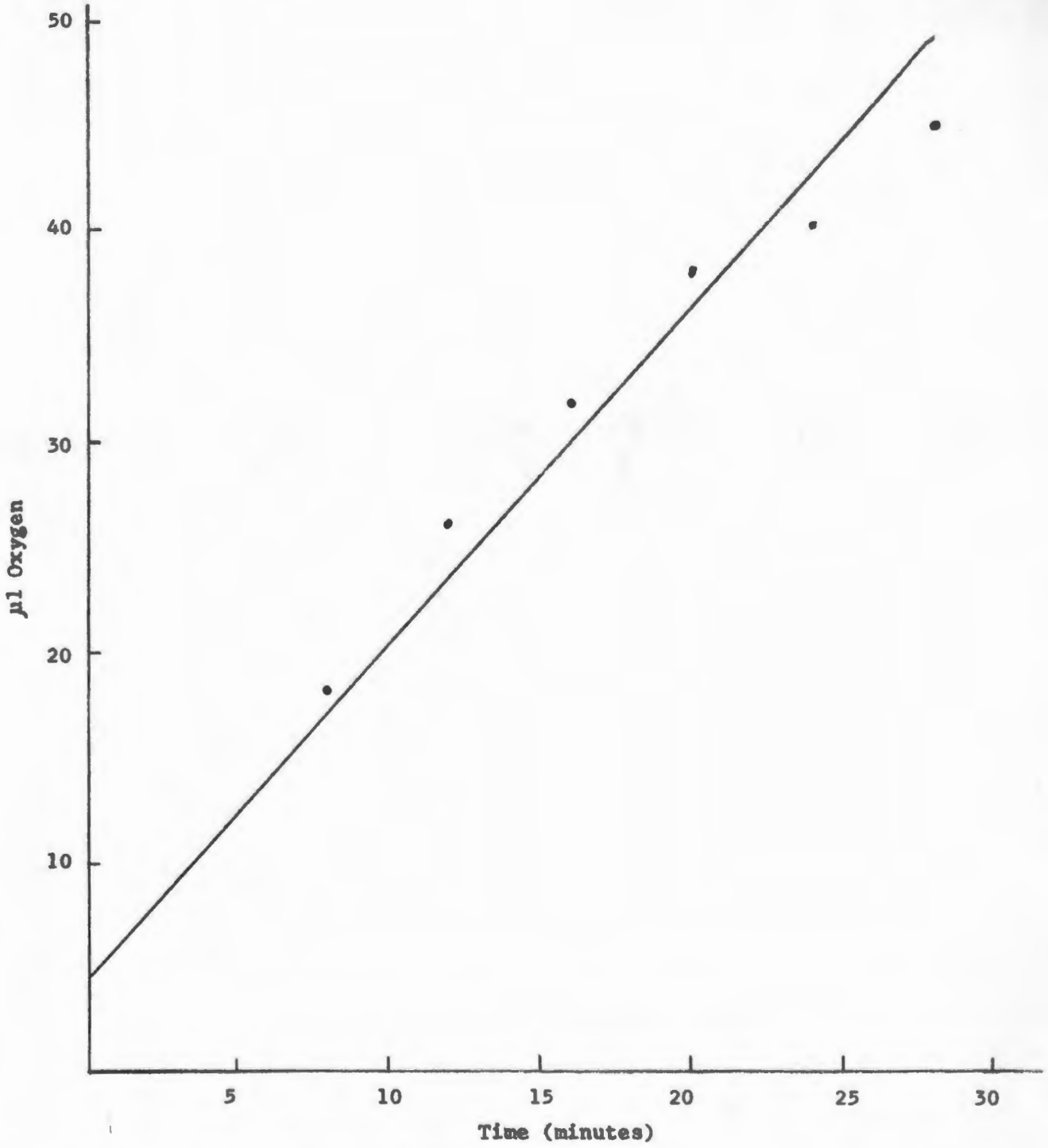

Fig. 2. Oxygen uptake of liver homogenates from mice treated with thyroid.

Each point represents the average oxygen uptake of 12 mouse liver homogenates analyzed manometrically. The calculated equation for the regression line is $Y_c = 4.4 + 1.6X$ by the method of least squares.

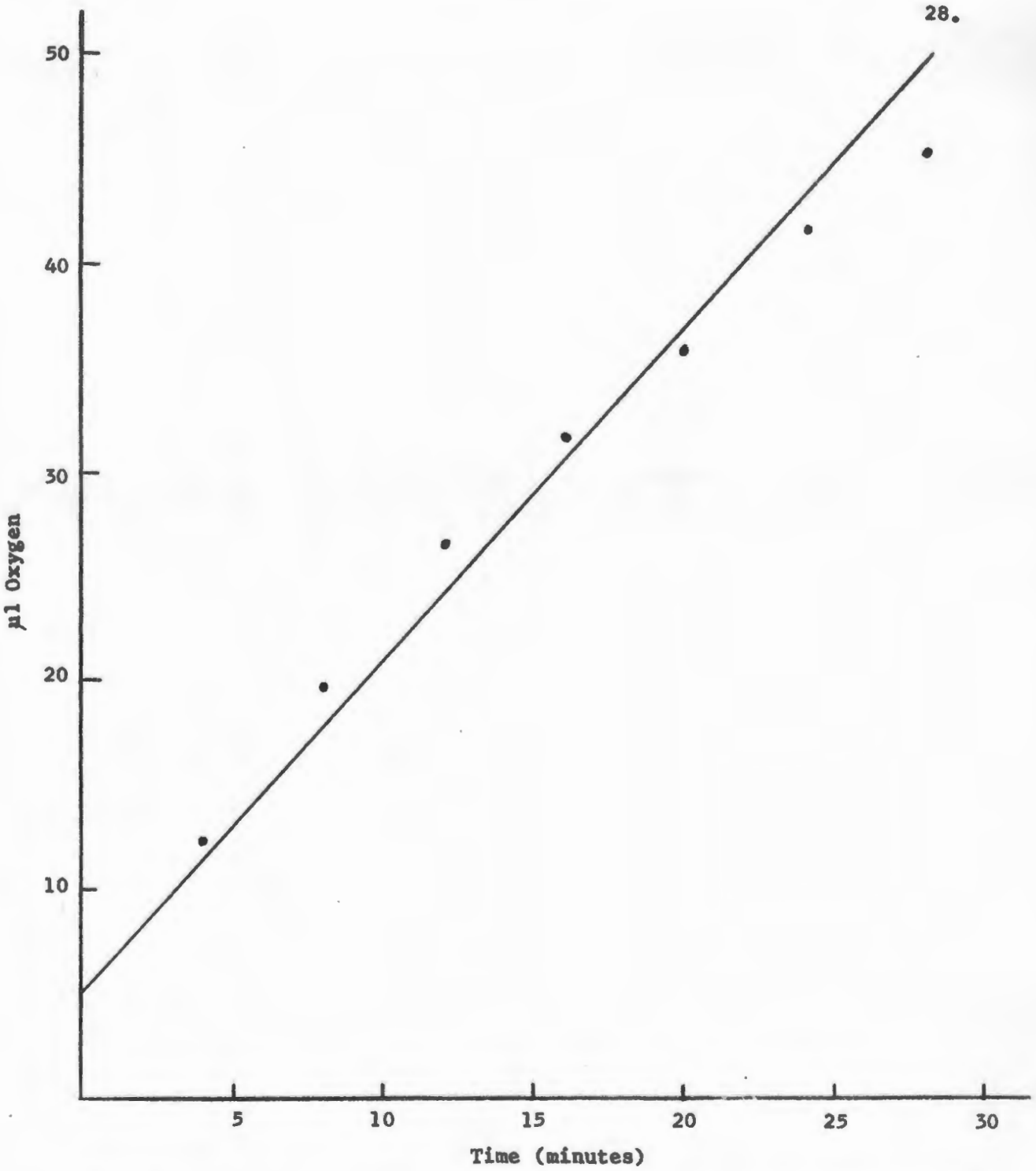

Fig. 3. Oxygen uptake of liver homogenates of the non-treated mice used in parallel studies with thyroid.

Each point represents the average oxygen uptake of 4 mouse liver homogenates analyzed manometrically.

The calculated equation for the regression line is $Y_c = 5.1 + 1.6X$ by the method of least squares.

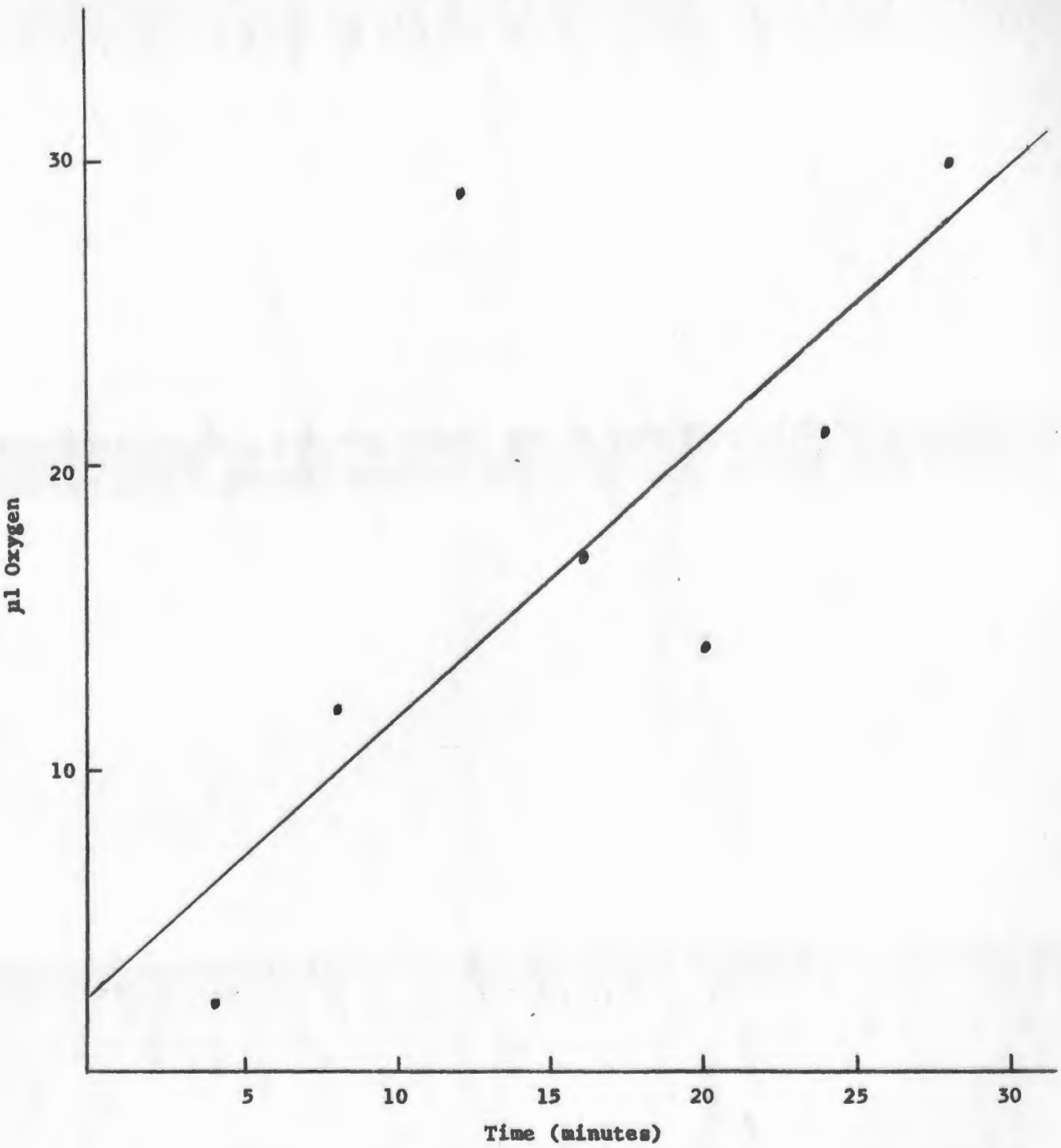

Fig. 4. Oxygen uptake of liver homogenates from mice treated with MD911.

Each point represents the average oxygen uptake of 12 liver homogenates analyzed manometrically.

The calculated equation of the regression line is $Y_c = 0.32 + 0.089X$ by the method of least squares.

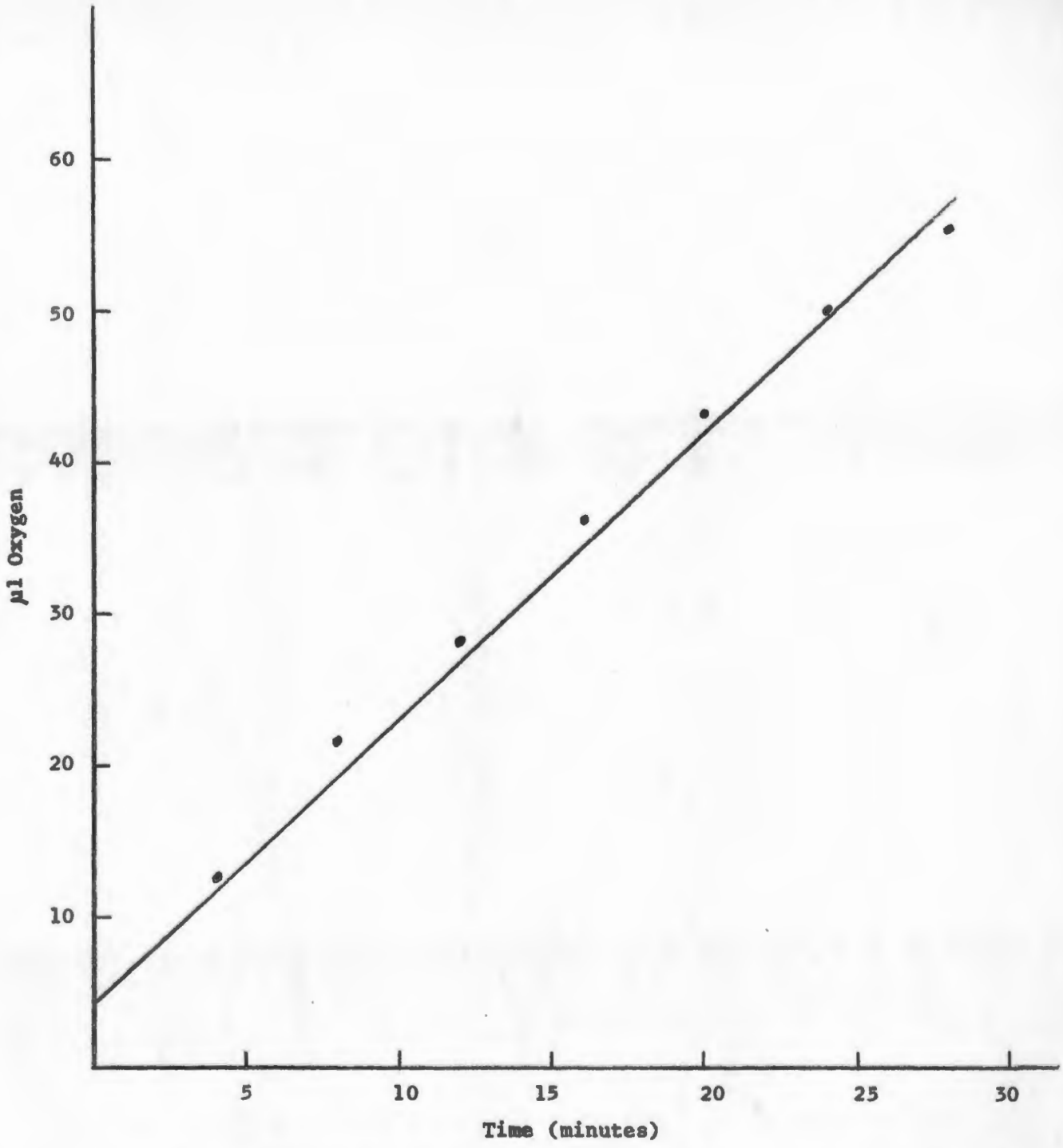

Fig. 5. Oxygen uptake of liver homogenates of the non-treated mice used in parallel studies with MO911.

Each point represents the average oxygen uptake of 4 mouse liver homogenates analyzed manometrically. The calculated equation for the regression line is $Y_c = 4.0 + 1.9X$ by the method of least squares.

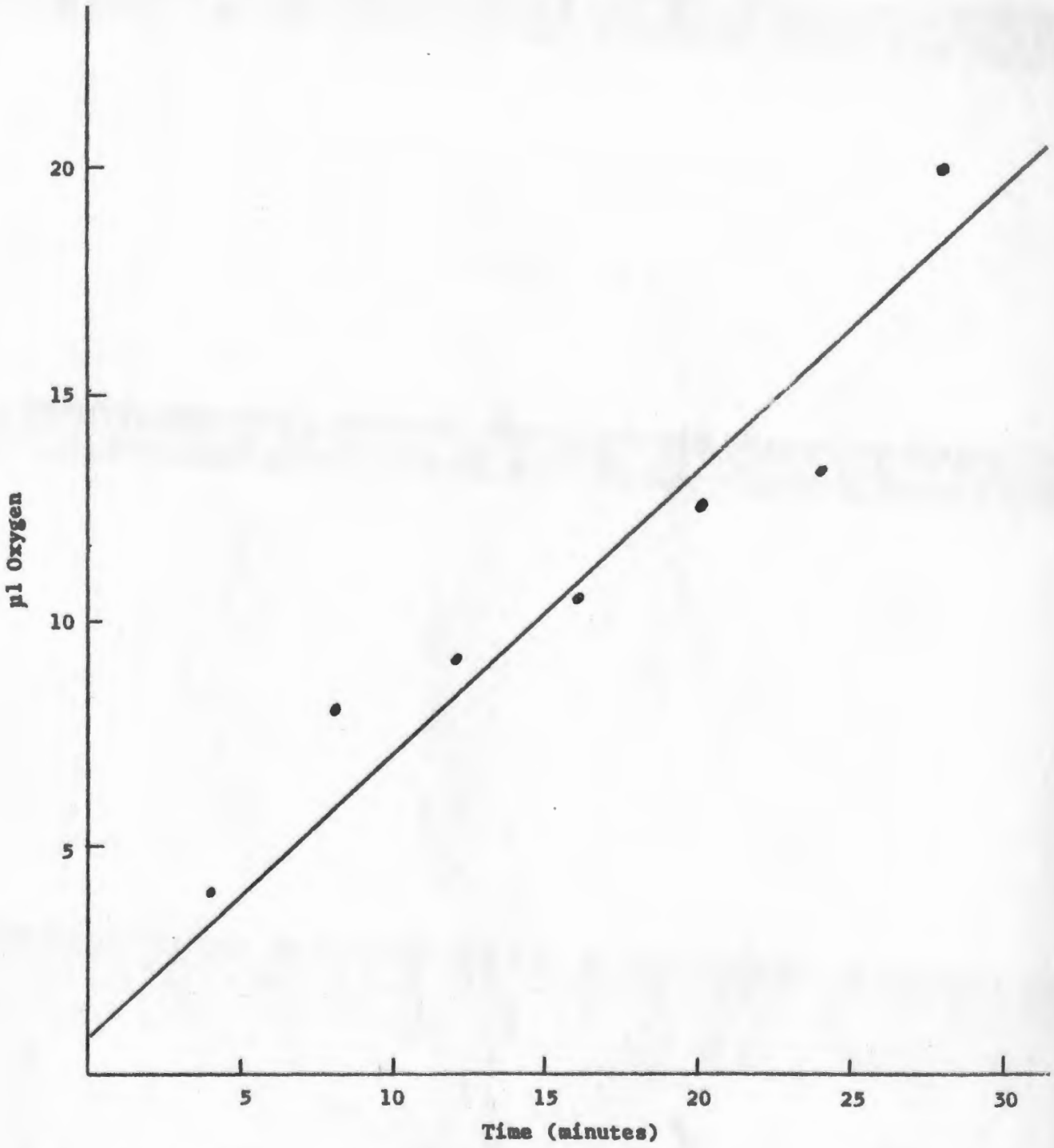

Fig. 6. Oxygen uptake of livers treated with MD911 and thyroid.

Each point represents the average uptake of 12 mouse liver homogenates analyzed manometrically.

The calculated equation for the regression line is $Y_c = 0.83 + 0.62X$ by the method of least squares.

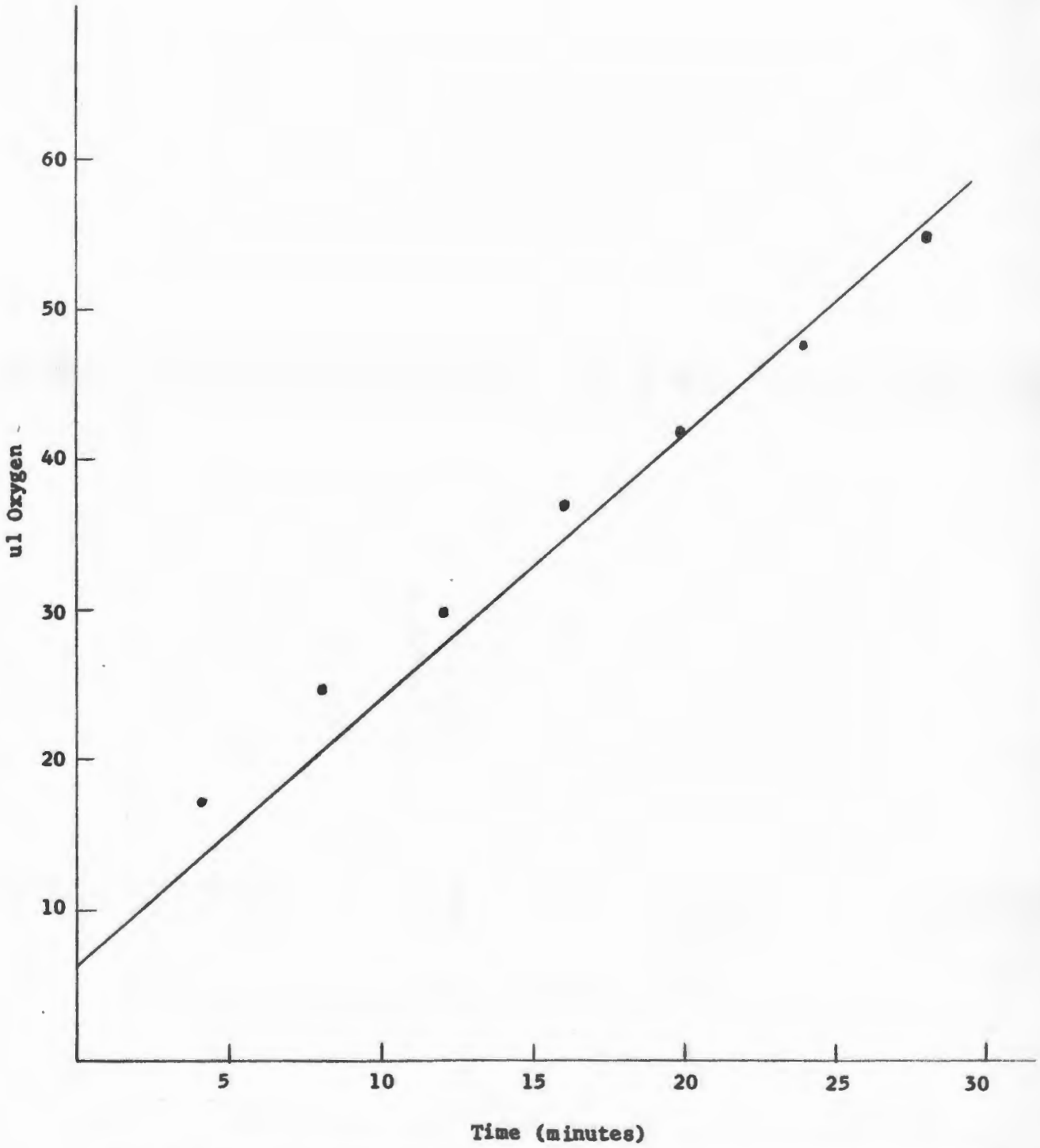

Fig. 7. Oxygen uptake of liver homogenates of the non-treated mice used in parallel studies with MO911 and thyroid.

Each point represents the average oxygen uptake of 4 mouse liver homogenates analyzed manometrically.

The calculated equation of the regression line is $Y_c = 6.8 + 1.77X$ by the method of least squares.

Section I

Group I

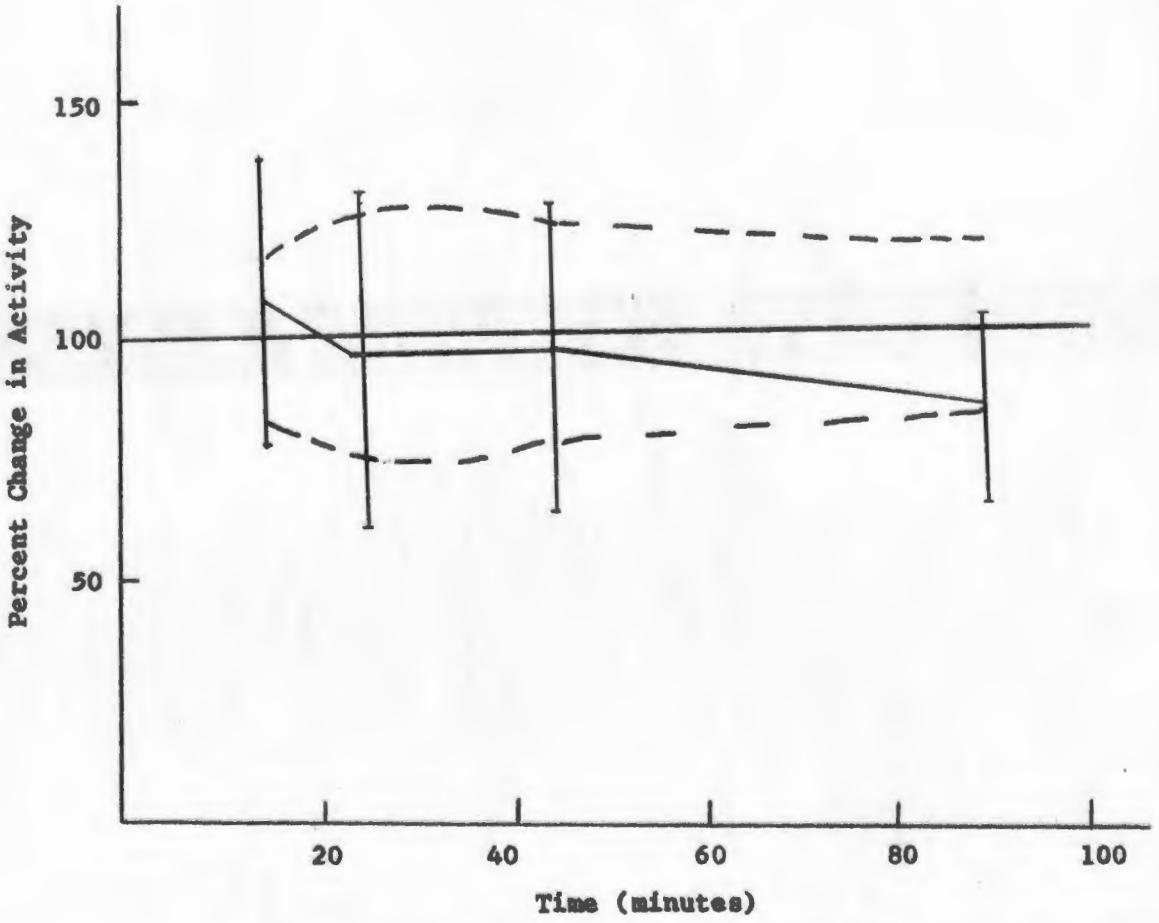

Fig. 8. Relationships of locomotor activity of normal mice and thyroid treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

Section I

Group II

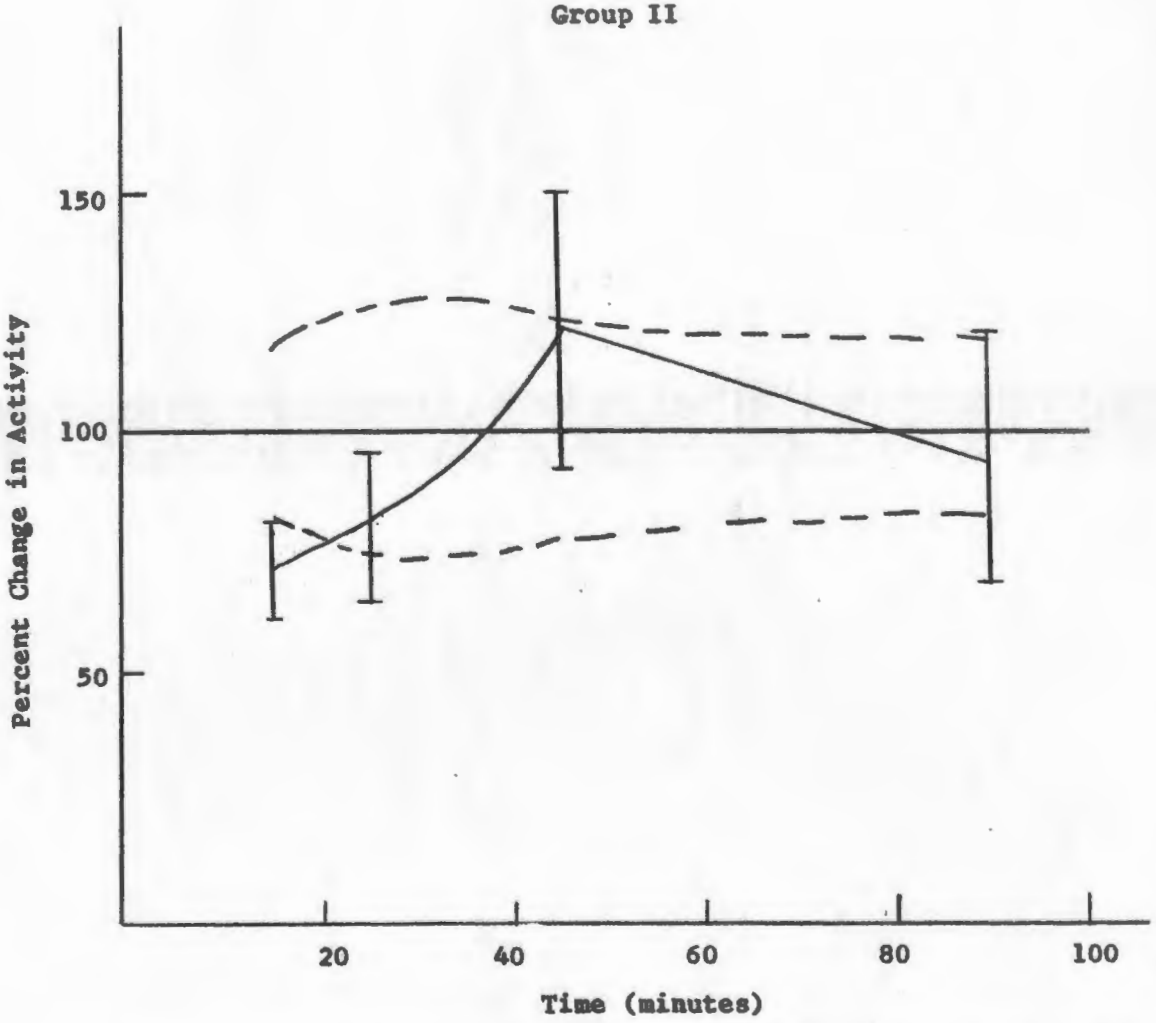

Fig. 9. Relationships of locomotor activity of normal mice and thyroid treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviation of the experimental.

Section I

Group III

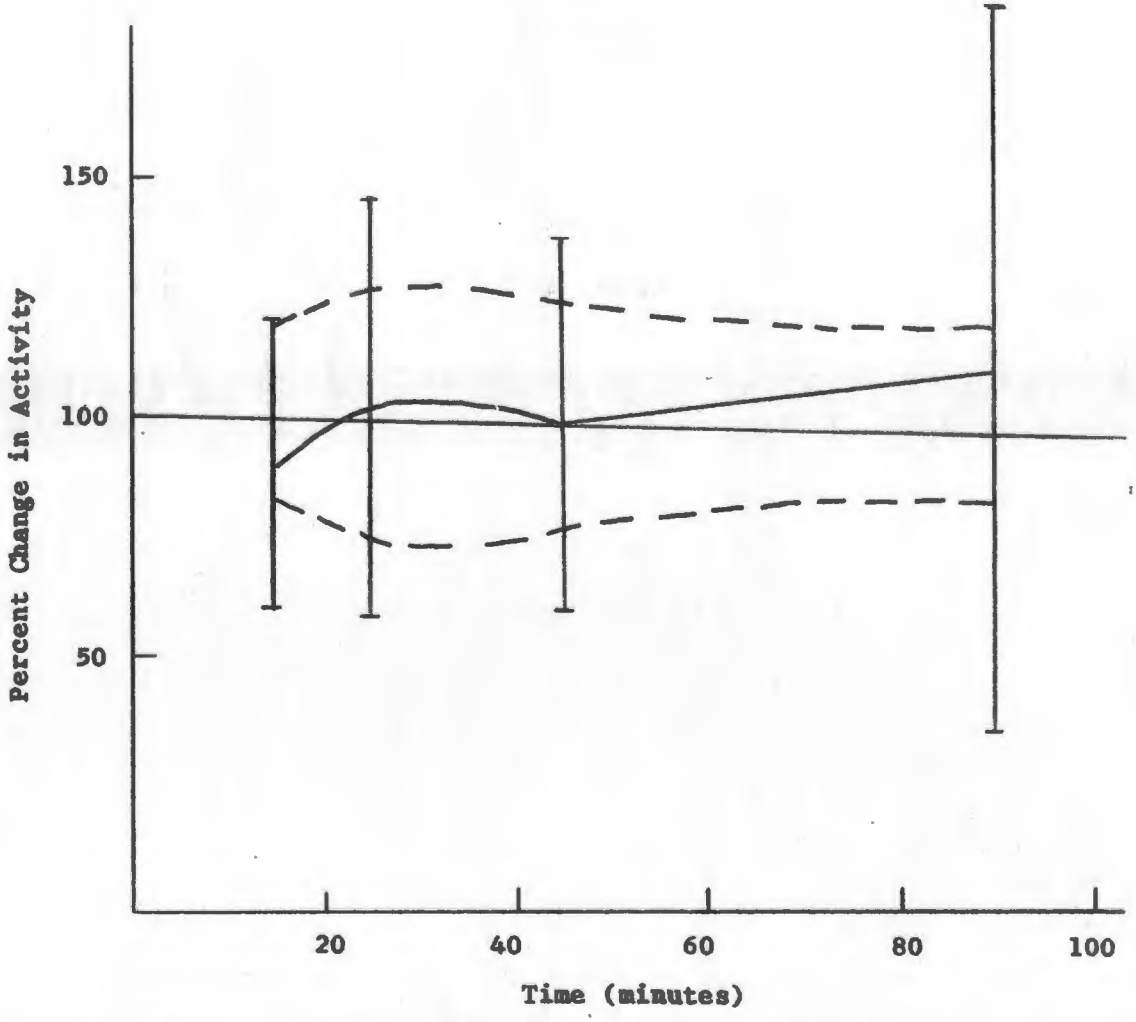

Fig. 10. Relationships of locomotor activity of normal mice and thyroid treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

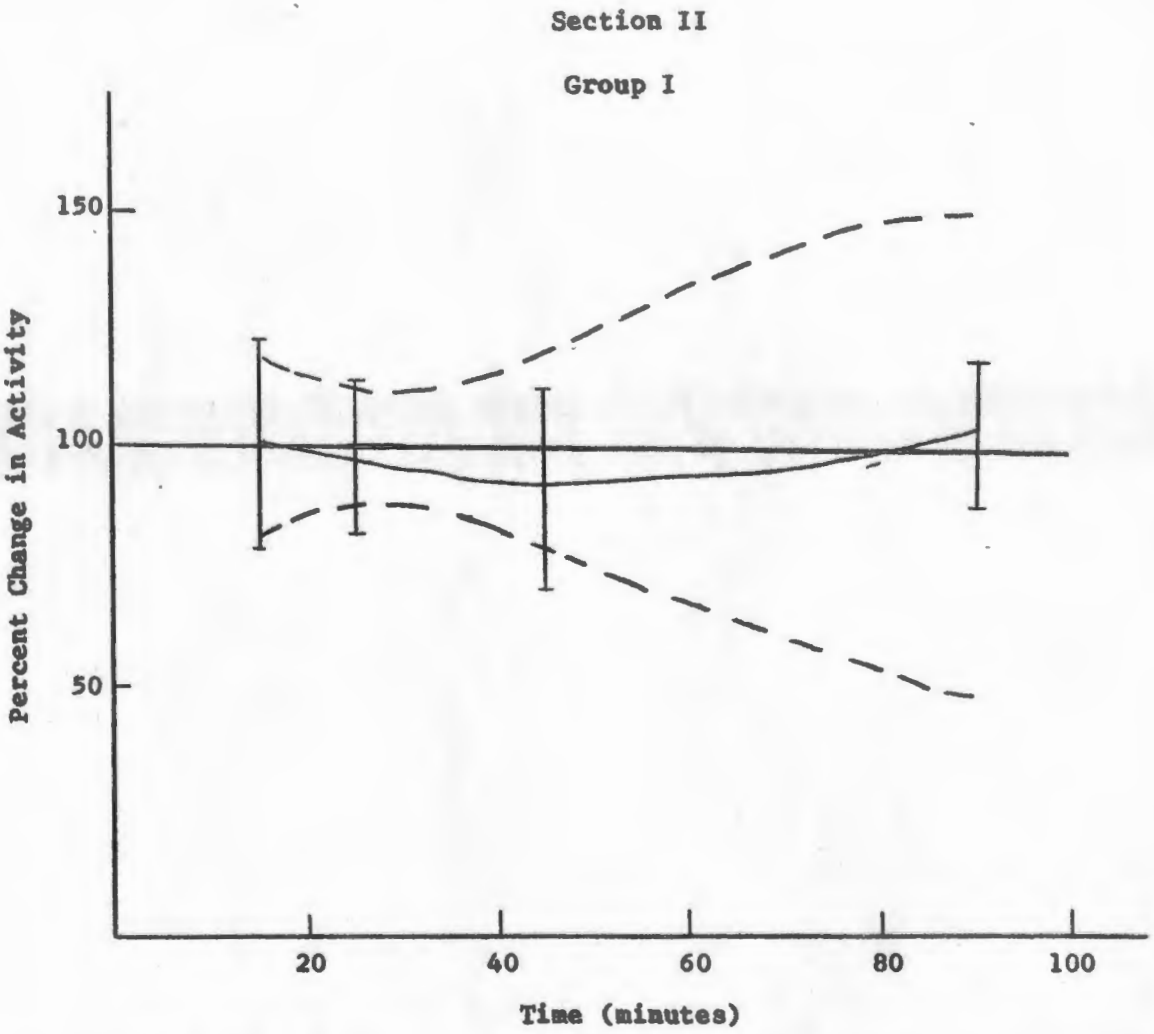

Fig. 11. Relationships of locomotor activity of normal mice and M0911 treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

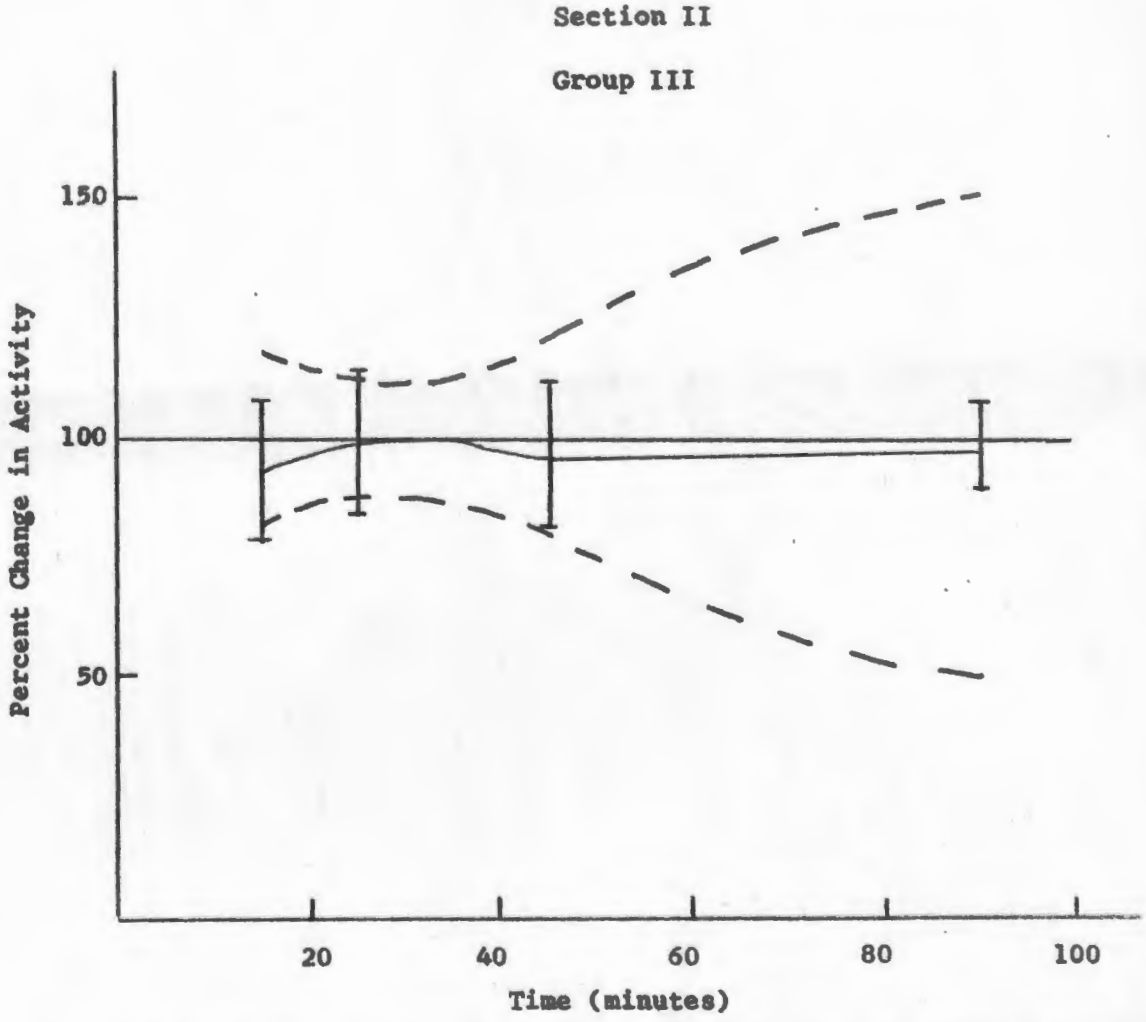

Fig. 12. Relationships of locomotor activity of normal mice and MO911 treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

Section II

Group IV

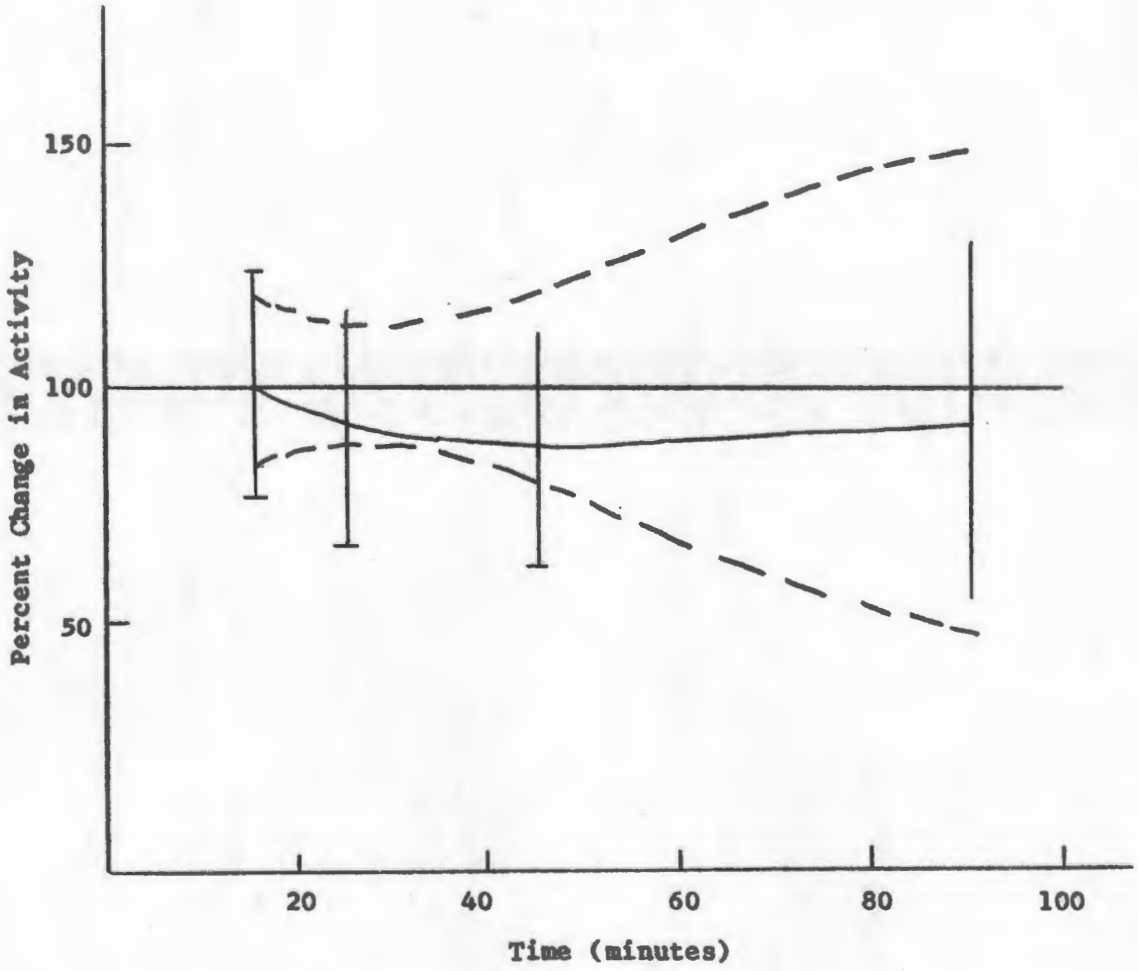

Fig. 13. Relationships of locomotor activity of normal mice and MO911 treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

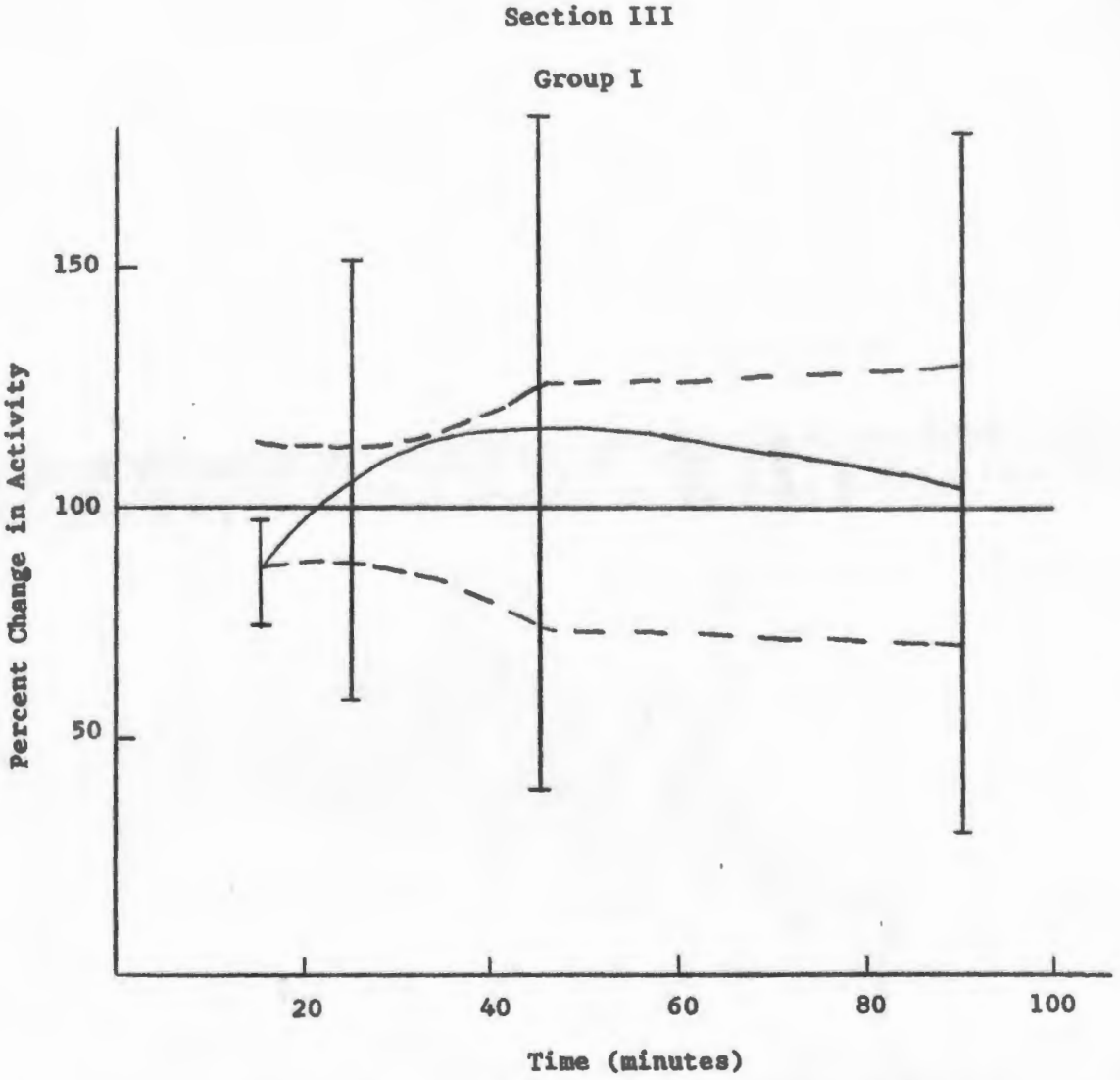

Fig. 14. Relationships of locomotor activity of normal mice and M0911-thyroid treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

Section III

Group III

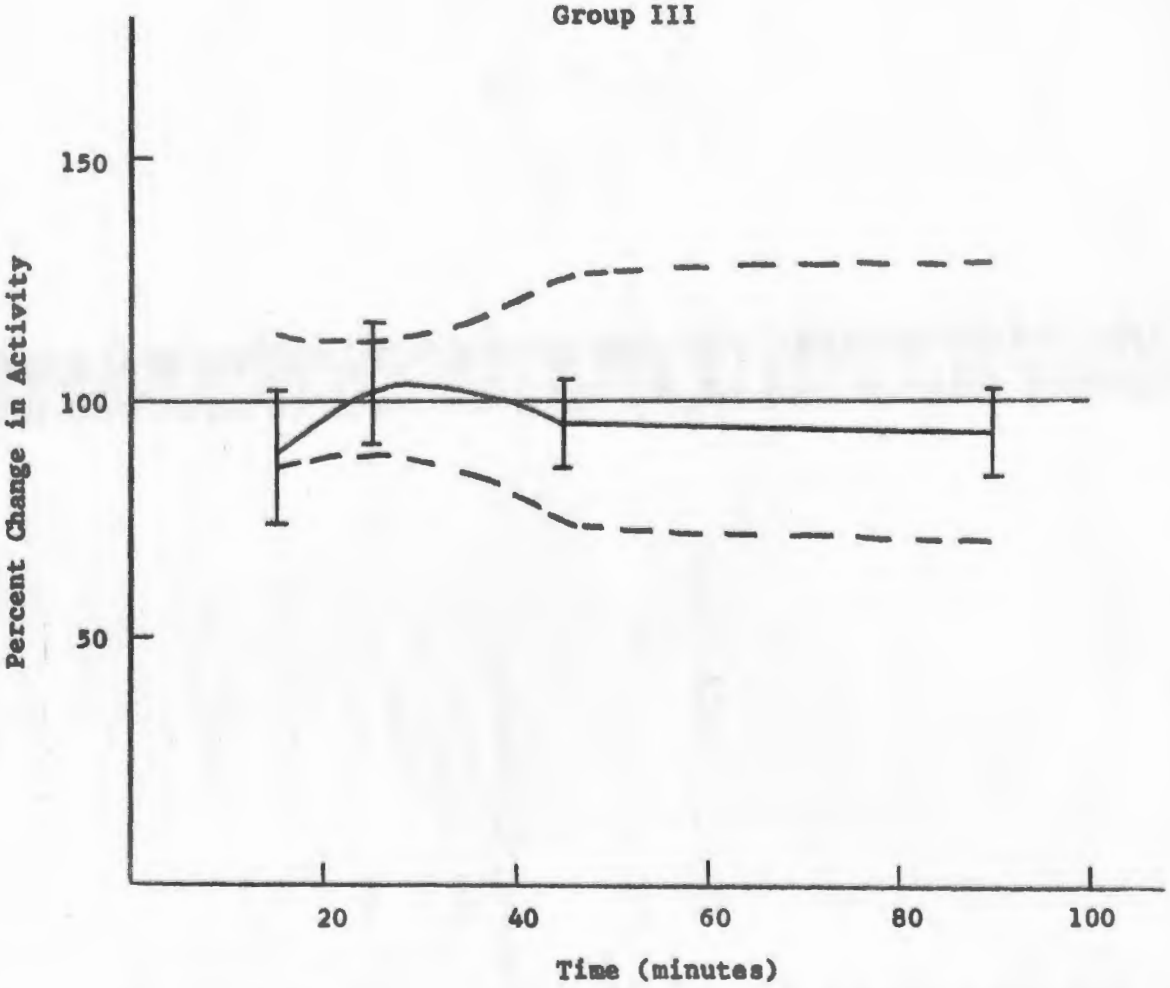

Fig. 15. Relationships of locomotor activity of normal mice and M0911-thyroid treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

Section III

Group IV

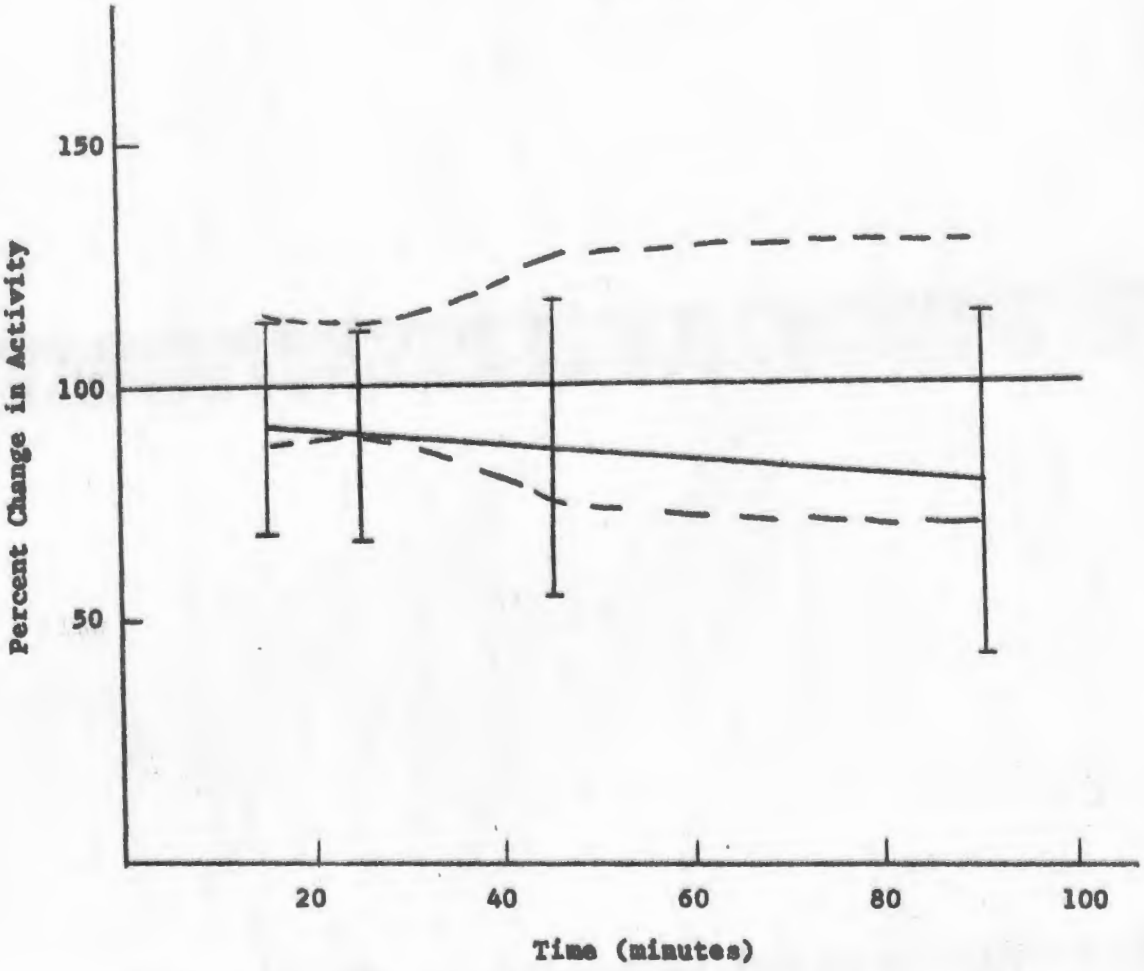

Fig. 16. Relationships of locomotor activity of normal mice and MO911-thyroid treated mice.

The values of the abscissa represent time. The ordinate values indicate the percentage change of activity variance from the control depicted as 100%.

The interrupted lines are the standard deviations of the controls. Solid bars are the standard deviation of the experimental.

V. DISCUSSION

Thyroid administration elicited changes in locomotor activity but not to any great extent. In group I (Figure 8), the 10-20 minute average was 7 percent above that of the control which was not statistically significant. This indicates that locomotor activity of the control and the experimental at this time interval were not significantly different. The actophotometer counts which are an indication of central nervous system activity did not deviate significantly from the controls throughout the various time intervals.

Comparisons of the two remaining experimental groups yielded the same information except in one instance. In the first time interval the activity in group II (Figure 9) was depressed. Following this depression, the activity returned to values within the control levels. Since the depression was not apparent in the other experimental group and did not persist for a reasonable time interval, the interpretation that thyroid administration was the causative factor is not justified. This transient effect is apparently caused by a lag in equilibration of this group of animals to the actophotometers or by the increased social activity of the mice.

Generally, thyroid at the dosage employed did not increase nor decrease locomotor activity as measured by actophotometers (see Table 3 and Figure 10). The thesis that thyroxine acts by uncoupling oxidative phosphorylation (Lipmann & Dutoit, 1951; Matuis & Hess, 1951; and Lardy & Maley, 1954) with concomitant production of a higher metabolic rate may suggest that thyroid's systemic effects should produce a measurable rate of decreased locomotor activity. Uncoupling oxidative phosphorylation releases the regulatory mechanism for maximum utilization of energy rich

bonds and prevents ADP rephosphorylation.

Administration of MO911 did not increase locomotor activity (Figures 11-13). During all time intervals the response of the experimental animals was not different than that of the controls (see Table 4). Wiegand and Perry (1961) stated that no behavioral changes were elicited by treatment with MO911 alone. Upon addition of DL-dihydroxyphenylalanine (DL DOPA) after pretreatment with MO911, an increase in activity and aggressiveness was noted. They suggest that the increased activity is due to increases in dopamine or norepinephrine. Carlson et al. (1959) (cf. Wiegand and Perry, 1961) agree with this hypothesis after measuring dopamine and norepinephrine levels in mice 6 hours after MAO inhibition.

The latter proposal is contradicted by the evidence of Everett and Wiegand (1961). These investigators demonstrated a gradual increase in central amines along with increasing motor activity and aggressiveness. Their effects were also augmented after giving DOPA. This may be the pivotal statement.

The postulated correlation of increased locomotor activity and aggressiveness with dopamine or norepinephrine appears reasonable, but the fact that precursors must be added to initiate increased activity indicates that the rate of synthesis of the catecholamines is not sufficient to elevate norepinephrine levels or that inhibition of MAO does not affect catecholamine metabolism to a significant degree in brain tissue. If this hypothesis is accepted then the lack of locomotor activity of the experimental animals versus the controls is not enigmatic.

Taylor and Krause (1962) have invalidated arguments concerning the possibility that MO911 does not cross the blood brain barrier.

C^{14}-labeled MO911 was found in adequate concentrations in the brain of rats and dogs, with detectable amounts up to 48 hours in rat brain. The extrapolation of this information to mice would be in order.

There are other effects of MO911 which could modify its total activity. These diverse effects are present in other MAO inhibitors of different structure. Horowitz and Sjoerdsma (1961) demonstrated a decrease in sympathetic nerve activity. Costa and Brodie (1961) also noted similar effects by pretreating with MO911 and then following with Ford (1961), and DeCosta et al. (1961). The mitigating influences of these physiological responses on gross locomotor activity are apparent. Any of these alone or in combination could reduce a gross effect to the limits of a measuring instrument. These factors were not controlled in this experiment. Efforts to control these factors would present serious problems in evaluating the results of in vivo locomotor studies.

Combined therapy of thyroid and MAO inhibitor did not significantly alter locomotor activity (see Table 5 and Figures 14-16). The response noted by Kline (1961) of retardation and apathy was not duplicated with mice. Since the retardation has been demonstrated only in depressed humans, a similar alteration of the psyche must be induced in experimental animals for duplication of this response. There has been no documentation of normal patients responding to thyroid and MAO inhibitor medication in this manner.

In all of the actophotometer studies, fluctuations from the control values were evident on particular days. None of the departures from the normal or control values were significant. Trends were not established and the fluctuations were random. Since all animals in a

particular section were handled under the same conditions, it is apparent that the drugs did not alter locomotor responses in a significant manner.

The method of Creasey (1956) and Hawkins (1952) was satisfactory in measuring MAO activity manometrically.

Oxygen uptake of mice liver mitochondria-microsome as a measure of MAO activity after thyroid treatment was linear with time (Figure 2). The coefficient of correlation was 0.99, indicating a high degree of linear association. The coefficient of determination indicates that 97 percent of the variations occurring in O_2 uptake can be explained on the basis of the linear regression of oxygen uptake with time. Total oxygen consumptions was 45.6 µl for 28 minutes (Table 1).

Respiration of control mice liver mitochondria for the thyroid experiment also indicates linearity with time (Figure 3). The coefficient of correlation was 0.98. Variation of the dependent variable could be explained by the variation of the independent as demonstrated by the calculated value of 0.96. Oxygen uptake was 45.4 µl for 28 minutes (Table 1).

With the aid of covariance analysis (Snedecor, 1956), the probability that the two regression lines are in fact the same line is 99 percent. Since these lines also possess the same slopes, this suggests that the regression lines are not different. The total oxygen uptake per hour for the thyroid treated animasl would be 100.4 µl O_2. The experimental consumption was not significantly different from the control value of 101.1 µl O_2.

These values do not agree with Spinks and Burn (1952), Zile and Lardy (1959). Skillen et al. (1962) found that thyroid decreases MAO activity in rat liver. Novick (1961) also found that thyroid decreases

MAO activity 50 percent below that of controls. Zile (1960) found insignificant changes in brain and heart MAO activity with thyroxine and related compounds and that increased dosages did not elicit a more profound effect.

One theory concerning the mechanism of action of thyroid on MAO is that thyroid decreases enzyme levels indirectly. Zile and Lardy (1959) suggest that thyroid regulates the biosynthesis of MAO or controls the enzymes that govern the activity of MAO in the liver.

Westermann (1956) found an increase in MAO following thyroid feeding in rat livers.

It is apparent that no definite statement can be made concerning the effects of thyroid on MAO activity in rodents. Barker's (1951) observation that thyroid feeding even with supportive vitamin therapy produces changes in the liver not attributable to thyroid alone may have valid application. If there are changes in the physiology of the liver, the differences in MAO activity may not accurately reflect changes produced by drug administration.

Mice liver mitochondria-microsomes, removed from MO911 treated animals, showed reduced respiration linear with time (Figure 4). Total oxygen uptake was 5.6 µl/hr. The coefficient of correlation was 0.79 and the coefficient of determination 0.62. The latter coefficient size is indicative of influences of factors other than the independent variable influencing the regression line.

The oxygen uptake by the control animals was also linear (see Figure 5). The coefficient of correlation was 0.93 constituting an excellent correlation. The unexplained variation was 13 percent which is relatively small.

Reduction in liver MAO activity by MO911 has been reported by Taylor et al. (1960). The results of this experiment corroborate Taylor's work.

The decrease in MAO activity of mice liver mitochondira was 18 times that of the controls (Table 1). It is apparent that MO911 is a powerful MAO inhibitor and that significant decrease in MAO levels can be achieved and maintained by daily injections.

Combined therapy of MO911 and thyroid reduced MAO activity of livers significantly over the controls (see Figure 6). Total activity of treated livers was determined to be 38 μl O_2 per hour as opposed to the control value of 115 μl O_2 per hour (see Figure 7). Comparing the results of combined therapy with MO911 therapy, a six fold increase in MAO activity is observed (see Table 1). This suggests that thyroid antagonizes the effects of MO911 in liver.

The fact that thyroid elicits no change in MAO activity but suppresses the total effect of MO911 may indicate a specific mechanism of action. Thyroid, a metabolic stimulator, apparently causes an increased turnover of constituents in the cell. The ability of an exogenous inhibitor of an enzyme system to exercise its usual effect may be reduced. This may be caused by increased synthesis of the enzyme or the inability of the inhibitor to reach the substrate in an effective concentration. The result would be increased activity of the enzyme when combined thyroid and MAO inhibitor therapy is utilized.

Brain mitochondrial MAO activity after thyroid administration was variable (Table 2). Enzyme levels were lower in two groups with percentage decreases of 81 percent and 24 percent. The third experimental group showed a 30 percent increase in MAO activity. The re-

fractory response of the latter group cannot be explained in terms of penetration of the "blood brain barrier" by thyroid since two groups did show definite decreases in MAO activity. These results agree with Zile (1960), who stated that the changes in MAO activity in brain after thyroid feeding were insignificant and inconsistent.

There is no reason to suggest that the increase in MAO activity is an artifact. Apparently, this is an individual response with obscure prerequisite cell constituents which would predetermine whether an increase or decrease in MAO activity is observed.

MO911 therapy decreased MAO activity in three experimental groups. Several investigators have observed a decrease in brain MAO activity after MO911 therapy, including Taylor et al. (1960; 1960a), Wiegand and Perry (1961), and Barondes (1962). In our investigation enzyme activity declined 100 percent, 97 percent and 75 percent of control values. This indicated that there is no impediment for access of MO911 to active sites of enzyme inhibition in the brain.

Thyroid and MO911 therapy did not differ from MO911 alone in its effects on MAO depletion in brain tissue. The experimental groups showed decreases of 100 percent, 83 percent and 77 percent in activity (see Table 2). It is apparent that no augmentation of effect or antagonistic response occurred at this level. Apparently, if any changes in the metabolism of the catecholamines are to be shown, another enzyme system, possibly the COMT system, may be involved.

Since the brain is such a complex system, other modifications in function cannot be overlooked. Changes in other systems could account for the retardation and apathy observed in patients.

VI. SUMMARY AND CONCLUSIONS

The interrelationships of thyroid, MAO, and locomotor responses have been investigated.

Locomotor activity of mice was evaluated by the use of actophotometers. The administration of thyroid, MO911, a MAO inhibitor, and combined thyroid and MO911 therapy, did not alter spontaneous activity significantly.

The apathetic response was not produced in mice with combined thyroid and MO911 administration. Reasons for the lack of response by the experimental animals were discussed.

MAO activity was measured manometrically using the Warburg apparatus. MAO activity, measured by uptake of oxygen, was shown to vary with the administration of thyroid, MO911, and combined thyroid and MO911. MAO activity could not be correlated with locomotor activity since spontaneous activity was not affected by the administration of the drugs employed.

Brain MAO activity did not show consistency after drug administration. MAO activity decreased in two groups but increased in one group after the administration of thyroid, MO911, and combined MO911 and thyroid.

MO911 treated animals exhibited no change in locomotor activity but significant decreases of MAO activity in brain and liver were observed. Combined therapy of MO911 and thyroid resulted in an increase in MAO activity in livers above that of MO911 and below thyroid administered separately. The increase in MAO activity with combined therapy indicates that thyroid antagonizes MAO inhibitors outside the central nervous system.

The central action of thyroid differs from its peripheral activity in that brain mitochondria MAO was lowered on the average. Apparently, thyroid action is not direct and the presence or absence of intermediates determine observed results.

Since combination therapy did not show a difference in MAO activity from MO911 therapy tissue, the response previously noted in humans, retardation and apathy, could not be ascribed to changes in MAO activity in the brain.

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VIII. APPENDIX

Constants for total oxygen uptake were calculated by the use of the formula:

$$k = \frac{V_g \frac{(273)}{T} + V_f}{P_o}$$

V_g = volume of gas phase in flask including connecting tubes.

T = temperature of bath in absolute degrees.

V_f = volume of fluid in vessel.

= solubility in reaction liquid of gas involved. Expressed as ml gas/ml liquid when the gas is at a pressure of 760 mm Hg at the temperature T.

P_o = standard pressure. (Brodie's solution is equivalen to 10,000 mm Hg.)

The method the least squares was used for regression lines for the manometric plots and the regression line relationships were calculated according to Lewis (1953).

$$\Sigma Y = Na + b\Sigma X$$
$$\Sigma XY = a\Sigma X + b\Sigma X^2$$

H_o the null hypothesis stating that the regression line is not a sample from the universe from which it is drawn.

$$r = \frac{\sqrt{1-r^2}}{\sqrt{N-2}}$$ standard error of the regression

$$t = \frac{r^2-u}{r}$$ critical ratio

$$S_y = \sqrt{\frac{(Y-Y_c)^2}{N}}$$ scatter

$$r = 1 - \frac{Sy^2}{y^2}$$ coefficient of correlation

$$r^2 = 1 - \frac{S_y^2}{y^2}$$ coefficient of determination