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The Effects of Chronic Thyroxine and Pargyline Treatment on Locomotor Activity, Blood Pressure, Urinary Electrolytes, Body Weight and Liver and Brain Monoamine Oxidase Activity in Male Albino Rats Maintained on Fixed Caloric Input

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THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY, BLOOD PRESSURE, URINARY ELECTROLYTES, BODY WEIGHT AND LIVER AND BRAIN MONOAMINE OXIDASE ACTIVITY IN MALE ALBINO RATS MAINTAINED ON FIXED CALORIC INPUT

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ABSTRACT

The effects of chronic treatment with thyroxine, pargyline, and combined thyroxine and pargyline were simultaneously determined on locomotor activity, systolic blood pressure, urinary sodium and potassium excretion, body weight gains and terminal monoamine oxidase activity in brain and liver of male albino rats.

Chronic locomotor exploratory activity was shown to be best measured by comparing only the first 30-minute counts of groups of rats run on alternate days in the actophotometer. Significant ($P \leq 0.05$) depression of exploratory activity occurred during 11 days of daily intraperitoneal injection of thyroxine, pargyline or both these drugs concurrently. Rats receiving thyroxine or both thyroxine and pargyline lost weight beginning on day 2 or 1, respectively, but rats receiving pargyline alone lost no weight. Treatment with thyroxine elicited continuous hypertension and apparently increased heart rate and stroke volume; these first appeared on day 2 and were present 17 treatment days later. After 4 days of treatment with both drugs there was no hypertension, but heart rate and stroke volume appeared increased. Treatment with pargyline alone appeared to reduce these latter effects, and induced continuous hypotension beginning between days 2 and 7.

Urinary excretion of sodium and potassium was continually increased after 7 days of treatment with thyroxine, but not with pargyline or with both drugs. Terminal monoamine oxidase activity in brain and liver was not affected by thyroxine treatment but was completely suppressed in rats receiving pargyline or both drugs.

MASTER OF SCIENCE THESIS

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ANGELO BENEDICT MENDILLO III

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I. INTRODUCTION

In 1961 Kline reported that concurrent administration of thyroid extract and monoamine oxidase inhibitors to psychotic patients resulted in increased depression and apathy. Kline felt that this response was paradoxical since he had observed a measure of reduction in psychotic depression after administration of either thyroid alone or a monoamine oxidase inhibitor alone.

It was decided to examine this problem in the laboratory using normal rats. Thyroxine and pargyline were selected as representative of thyroid hormones and the monoamine oxidase inhibitors, respectively, and a method was developed that permitted the efficient measuring of locomotor activity in rats. A study was then designed in which a simultaneous evaluation could be made of the effects, in rats, of chronic treatment with thyroxine, pargyline or both thyroxine and pargyline on locomotor activity, systolic blood pressure, body weight gains, urinary sodium and potassium excretion and terminal monoamine oxidase activity in brain and liver. It was felt that any drug-induced changes in locomotor activity might parallel changes induced by the drugs in the other parameters being considered. These parameters were chosen because they were perhaps the most likely to be affected by thyroxine and pargyline, and the whole study was developed in order to obtain a correlated profile of the effects of these drugs on the parameters studied.

II. SURVEY OF THE LITERATURE

On Christmas Day 1914, E. C. Kendall isolated from an iodine-containing protein fraction of the thyroid gland a physiologically active crystalline product which he called "thyroxin" (Trotter, 1964). Half a century of subsequent research has specified many effects of thyroid hormones in the body, but Wolff and Wolff (1964) have stated flatly that the mechanism of action of these hormones is still unknown.

From the discussion of Morgans (1964) the pertinent metabolic and clinical phenomena observed in hyperthyroidism can be summarized as follows:

1. Increased caloric production.
2. Increased plasma volume and increased rate of water filtration through capillary walls.
3. Increased cardiac output and decreased circulation time.
4. Increased heart rate.
5. Increased renal blood flow and glomerular filtration rate.
6. Loss of weight and increased appetite.
7. Excessive thirst.
8. Increased nervousness, irritability and tremors.
9. Excessive sweating and heat intolerance.

A. The Hemodynamic Effects of Thyroid Hormone

Barker et al. (1965) showed that thyroxine and many structural analogs increased the heart rate in rats. Bray (1964) demonstrated that tri-iodothyronine (T-3) administered subcutaneously (s.q.) to rats every other day for a week and then daily for a second week also significantly increased the heart rate. He showed that the tachycardia was accompanied

by an increased sensitivity to catecholamines and was abolished by reserpine or guanethidine. He suggested that the tachycardia of the hyperthyroid state is due to adrenergic stimulation. Wurtman et al. (1964) showed in addition that the epinephrine-induced increase in cardiac output was potentiated in hyperthyroidism. The mean blood pressure of the hyperthyroid rats in this study did not, however, appear to be significantly elevated over the controls. On the other hand Zsoter et al. (1964) showed that daily oral administration of l-thyroxine, thyroid powder or T-3 for 2 weeks did significantly increase systolic blood pressure in dogs and cats. Danowski et al. (1964) reported similar findings in healthy adult volunteers: large doses of desiccated thyroid induced thyrotoxicosis accompanied by increased sensitivity to injected epinephrine. Danowski also measured the pressor response of guinea pig aortic strips to the serum and urine collected during these studies. It was found that the tachycardia and increases in systolic blood pressure induced by thyroid excesses were accompanied by increases in the aortic-strip pressor activity of urine but not of serum. Exogenous epinephrine produced a greater rise in systolic blood pressure and tachycardia in the hyperthyroid state, but these responses were not accompanied by increased titers of pressor activity in serum. It was postulated, then, that the excess thyroid hormone was potentiating the effect of exogenous epinephrine in the heart. A peripheral effect was ruled out because epinephrine in the doses used in this study produced increased cardiac output and systolic pressure, accompanied by net peripheral vasodilation with decreased diastolic pressure. Danowski referred to the work of Wurtman et al. (1964) who showed that "in thyrotoxicosis the heart receives the same fraction of circulating labeled epinephrine per gram of cardiac tissue but its capacity to inactivate the epinephrine by binding is diminished. On

the basis of these findings it was suggested that the consequent maintenance of elevated levels of free epinephrine in the heart during hyperthyroidism may be a major factor in the enhanced hemodynamic responses to epinephrine."

Thus it appears likely that the hemodynamic responses to thyroid hormones are closely related to levels of epinephrine in the heart and probably not to peripheral effects of epinephrine.

B. The Hemodynamic Effects of Pargyline Hydrochloride

Pargyline hydrochloride (Eutonyl[1], MO 911), (N-benzyl-N-methyl-2-propynylamine hydrochloride) is a potent, irreversible nonhydrazine monoamine oxidase (MAO) inhibitor with striking ability to effect reductions in elevated blood pressure. In preliminary studies Maronde et al. (1963) reported that pargyline produced a significant drop in the standing blood pressure of 29 hypertensive patients, but showed little effect on pressures of supine patients. Horwitz and Sjoerdsma (1963) found that pargyline produced orthostatic hypotension and was effective in reducing a case of hypertension that could not be controlled even after administration of guanethidine in doses of 450 mg daily. In addition, they found that pargyline elevated mood and showed no parasympatholytic properties.

The findings of Brest et al. (1963) agreed with those of Maronde et al. (1963) that pargyline had less effect in the supine position than in the erect position. Brest et al. attributed the overall reduction in blood pressure primarily to a reduction in peripheral vascular resistance. They noted that in most of the cases studied pargyline elicited no consistent change in pulse rate or cardiac output but did decrease total peripheral resistance in all cases. They also reported that because renal vascular resistance was

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similarly reduced, renal blood flow and glomerular filtration rate are affected only to a moderate extent.

The sites and mechanisms of action of pargyline in lowering blood pressure have been the subjects of much controversy. Wolf et al. (1963) ruled out adrenergic blockade and depression of central sympathetic centers as being of any major importance and suggested that MAO inhibitors decrease blood pressure by sympathetic ganglionic blockade. This mechanism is probably not a likely one, however. Horwitz and Sjoerdsma (1963) had noted that pargyline produced no parasympatholytic effects, implying perhaps that there was no blockade of autonomic ganglia. More recently Winsor (1964) stated categorically: "Pargyline hydrochloride does not behave like a ganglionic blocking agent." He noted that although the antihypertensive effects are primarily orthostatic and that occasional reports of parasympatholytic side-effects such as dryness of mouth and constipation are seen, "there is no clear-cut clinical evidence to justify its classification as a ganglionic blocker". His data showed that although in pargyline therapy the antihypertensive effect was not always accompanied by increased urinary excretion of tryptamine, it was always accompanied by an abolishment of the digital reflex. This latter phenomenon indicates a pargyline-induced reduction in peripheral resistance.

Thus it appears likely from these data and from the work of Brest et al. (1963) that pargyline exerts its antihypertensive effect primarily by decreasing peripheral resistance. Winsor suggested a blockade of postganglionic sympathetic fibers as a possible mechanism. Although this appears likely since other sites in the autonomic nervous system have been ruled out, it obviously tells us little about a specific site of action. Indeed, Winsor made it clear that his work failed to show any consistent positive correlation

between MAO inhibition as reflected in the increased urinary excretion of tryptamine, and the antihypertensive effects of pargyline as mediated by reduced sympathetic activity.

In searching for an explanation of the hypotensive effects of MAO inhibitors, Goldberg (1964) also agreed that pargyline-induced orthostatic hypotension appears to be produced by decreased peripheral resistance mediated by peripheral depression of sympathetic activity. He points out that since the action of injected norepinephrine is not blocked in conditions of MAO inhibition it is certain that the MAO inhibitors are not adrenergic blockers. But he emphasizes that evidence for the postulated blocking effect of these drugs on postganglionic release of norepinephrine is not unequivocal. Pressor agents such as tyramine that act indirectly by releasing bound norepinephrine act with greater effect in MAO-inhibited animals than in normal animals. Goldberg suggests that this happens because the usual continuous destruction of norepinephrine by MAO in nerve endings is blocked; consequently, bound norepinephrine accumulates, and the relatively larger amounts subsequently released by agents such as tyramine cause greater pressor response. But the pressor response of <u>injected</u> norepinephrine is <u>not</u> potentiated in MAO-inhibited animals. Goldberg suggests that catechol-O-methyltransferase (COMT) in the liver inactivates injected norepinephrine so that exaggerated responses are not seen. But the increased levels of endogenous norepinephrine bound in nerve endings as a result of MAO inhibition are released by agents such as tyramine and amphetamine directly onto the receptor sites and consequently an exaggerated pressor response is seen with these drugs.

It is clear from the foregoing that the hypotensive effects of MAO inhibitors are probably caused by peripheral sympathetic blockade at some

site in the postganglionic apparatus rather than by central effects or by peripheral adrenergic blockade. The specific role of MAO inhibition in this sympathetic blockade has not been clarified, but recent work by Kopin et al. (1964, 1965) forms the basis for a new approach to this question.

Kopin et al. (1964) reported that increased tissue and urinary levels of amines were seen after MAO inhibition. In the rabbit, elevated tissue levels of octopamine, a relatively inactive amine (the beta-hydroxylated derivative of tyramine) were observed after MAO inhibition, and it was shown that chronic treatment with MAO inhibitors resulted in diminished release of norepinephrine from the isolated, perfused cat spleen upon stimulation of its sympathetic nerve supply. From these and other data it appeared likely, then, that an impaired release of norepinephrine from sympathetic nerve endings resulted from an accumulation of amines in the tissues following treatment with MAO inhibitors.

When MAO is inhibited, tyramine excretion increases; moreover, C^{14}-tyramine is rapidly converted to C^{14}-octopamine and selectively retained in the tissues after all the tyramine is destroyed. Since very little octopamine is formed after sympathectomy it is likely that these processes occur in the sympathetic nerves. It was the object of Kopin et al. (1964), therefore, to determine whether octopamine accumulates in the sympathetic nerves after inhibition of MAO, and whether this amine can be released as a "false neurochemical transmitter": that is, "a substance normally not present in significant amounts in the sympathetic nerves, which can be made to accumulate in the nerve endings and which can then be discharged by sympathetic nerve stimulation".

The following results were reported in this study:

1. After 2 days of treatment with pheniprazine, an inhibitor of MAO, octopamine levels in the cat salivary glands were greatly increased. This

effect was not seen if the gland was sympathetically denervated.

2. After administration of C^{14}-tyramine, C^{14}-octopamine was found in much higher concentrations in intact salivary glands than in denervated glands. When the cats were first given pheniprazine there was no increase in C^{14}-octopamine levels in denervated glands, but a tenfold increase was found in intact glands.

3. Isolated, perfused spleens from untreated cats were infused with C^{14}-tyramine; 30 minutes later the sympathetic nerves were stimulated and there resulted a marked increased in the rate of appearance of C^{14}-octopamine in the effluent. After administration of epinephrine the spleen contracted as it did on nerve stimulation, but no increase in C^{14}-octopamine release was seen.

From these data Kopin et al. formulated the following hypothesis:

> Following inhibition of monoamine oxidase, endogenously formed amines, such as tyramine, usually destroyed by this enzyme, are taken up by these vesicles and converted to the beta-hydroxylated derivatives. These beta-hydroxylated amines take the place of a portion of the norepinephrine normally contained in the vesicles and are released with the catecholamine. If each impulse releases a limited number of transmitter molecules, less norepinephrine would be released, a portion being replaced by less active molecules, even if tissue levels of norepinephrine are elevated. This would result in apparent sympathetic blockade, especially at low rates of nerve stimulation. At high, unphysiological rates of nerve stimulation, a sufficient number of vesicles could be made to release their contents so that the apparent block would be overcome.

Thus the most current views of the mechanism whereby MAO inhibitors effect a sympathetic blockade, resulting in decreased blood pressure, rest on the concept of the formation and release of a false neurochemical transmitter.

C. Thyroxine, Pargyline and Urinary Sodium and Potassium

Gaunt and Birnie (1951) reported that administration of thyroid

hormones to hypothyroid patients resulted in diuresis and increased sodium (salt) excretion. Euthyroid subjects, however, showed increased potassium excretion after thyroid administration. In thyrotoxicosis there is an increased urinary excretion of both sodium and potassium, and increased amounts of urinary nitrogen, phosphorus, calcium, chloride and creatine are also seen (Williams and Bakke, 1962).

Ganog (1963) suggested that the increased urinary excretion of potassium was due to the protein catabolism accompanying hyperthyroidism. If food intake is not increased, catabolism of body protein and fat stores occurs; weight is lost and urinary nitrogen levels increase. The latter is an index of amino acid degradation and hence of protein degradation. It is probable that the excess potassium is of intracellular origin. As protein is degraded the integrity of body cells is lost, and the potassium normally kept inside the intact cells by a process of active transport is liberated and lost into the urine.

It is somewhat difficult to reconcile the increased urinary sodium and potassium seen in hyperthyroidism with the findings of two recent studies concerning the effects of thyroid on serum constituents. Although administration of desiccated thyroid to healthy adult prisoners did result in increases in serum potassium levels one week after institution of daily therapy, the serum sodium was decreased (Moses et al., 1964). The investigators indicated that these alterations had not previously been recognized, and speculated that the hyponatremic effect might reflect a lowering of intracellular solutes as a result of tissue wasting. On the other hand Liu and Overman (1964) found no change in serum sodium and potassium levels in rats after administering toxic doses of thyroxine (1.4-2.0 mg Na thyroxine/kg) daily for 25 days. Some difficulty remains, then, in understanding how tissue

potassium presumably released in hyperthyroidism and appearing in the urine is nevertheless not always reflected in increased serum levels.

Following a clinical trial of pargyline hydrochloride in 25 outpatients Sutnick et al. (1964) reported that no significant changes in urinary or blood constituents had occurred. The glomerular filtration rate is moderately reduced in pargyline therapy (Brest et al., 1964; Onesti et al., 1964), but apparently no changes in urinary or blood electrolytes have been noted. Progressive renal failure is an indication that pargyline therapy should be stopped (Abbott Laboratories, 1964).

D. Thyroxine, Pargyline and Behavior

The behavioral effects observed in hyperthyroidism have been known for many years. Clinical observations noted below have been summarized from the discussion of Williams and Bakke (1962).

Muscle weakness and fatigue are very common in hyperthyroidism. Skeletal muscle apparently degenerates in some cases; generally, muscle weakness may be accounted for by the reduced capacity of the body to phosphorylate creatine and by reduced levels of nutrients. These observations are, of course, the manifestations of thyroid action at the subcellular level. An enormous amount of work has been done in an attempt to explain the increased oxygen consumption and the generally depressed P:O ratios seen as a result of thyroxine administration or in vitro action. It is not possible here to discuss this work. Wolff and Wolff (1964) stated that depending on the dosage, the particular hormone used and the experimental conditions, uncoupling of oxidative phosphorylation, "loosened coupling" or even stimulation of oxidative phosphorylation have been reported.

The effects of thyroid hormones on the nervous system are unexplained, but symptoms almost always include nervousness, difficulty in concentrating,

and continual, often purposeless movement of parts of the body. Tremor is common, as are hyperactive reflexes and emotional displays. Development of psychosis is sometimes seen.

Pargyline has been reported to produce a feeling of "well-being" in hypertensive patients (Horwitz and Sjoerdsma, 1963). Its effects in depressed or schizophrenic patients are variable, however. Sharpley et al. (1964) reported that in a double-blind study on 80 chronic psychiatric patients, mostly schizophrenic, neither pargyline nor tranylcypromine (a MAO inhibitor) effectively improved or worsened the psychiatric condition. Barsa and Saunders (1964) administered pargyline or tranylcypromine in combination with phenothiazine tranquillizers to 28 female schizophrenic patients. In three studies 82 comparisons were made: in 9 instances pargyline was superior to tranylcypromine; the reverse was true in 42 instances, and the drugs were judged equally effective in 31 instances. Drug effectiveness was evaluated by observing whether the patients became more alert and interested in the environment. The investigators concluded that pargyline combined with an antipsychotic tranquillizer is at times effective in schizophrenia, but not so effective as tranylcypromine.

Turner and Merlis (1964) administered pargyline and dihydroxyphenylaline (DOPA) to psychotic patients on the assumption that increases in brain norepinephrine might lead to increased confidence and awareness. There resulted some improvement in mood, resembling the effects of amphetamine but of longer duration. Other investigators also subscribe to this "catecholamine theory of affective disorders" which proposes, according to Klerman et al.(1964) "that depressed patients have an absolute or relative deficiency of central nervous system (CNS) catecholamines and that conversely, patients with elation have an excess of CNS catecholamines". The discovery that

reserpine depressed CNS levels of serotonin and norepinephrine and at the same time exhibited a tranquillizing effect apparently prompted the development of this concept. Unlike Turner and Merlis, however, Klerman et al. could not demonstrate that administration of phenelzine, a MAO inhibitor, plus DOPA elicited any reversal of psychotic depression. They suggested that perhaps orally-administered DOPA does not enter the brain as rapidly as is supposed.

Felstein et al. (1964) administered C^{14}-serotonin orally to 22 depressed patients and to 22 normal volunteers; urinary levels of C^{14}-5-hydroxyindoleacetic acid in these two groups were not significantly different, suggesting that MAO activity in unmedicated depressed patients is not reduced. Serotonin, however, does not enter the brain appreciably (Brodie, 1958); thus, no conclusions concerning MAO levels in the brains of depressed patients can be drawn from this work.

It thus appears that therapy with MAO inhibitors alone exerts variable and inconsistent effects in depressed patients. Indeed, Barsa and Saunders (1964) pointed out that the MAO inhibitors frequently cause an increase in psychotic symptoms. But when used in conjunction with other drugs, especially antipsychotic tranquillizers or DOPA, the MAO inhibitors are apparently capable of relieving depression in some patients.

Several recent studies relating MAO inhibitors to animal behavior are of interest. Poschel and Ninteman (1964) showed that MAO inhibitors, including pargyline, produced marked increases in self-stimulation in rats trained to press a lever for rewarding electrical stimulation of the lateral hypothalamus. Mantegazza and Riva (1964) reported that treatment of mice with iproniazid plus beta-phenylethylamine produced increases in spontaneous gross activity as measured in photoelectric recorder-activity cages. This

effect, however, was not seen in rats. van Rossum and Hurkmans (1963) reported that pretreatment of mice with pargyline reverses the sedative effect of alpha-methyldopa. Moreover, a few hours after injection of the alpha-methyldopa a strong central excitation occurs, as measured in photoelectric activity cages equipped with pulse shapers.

Everett and Wiegand (1962) found increasing degrees of general motor activity in mice after administration of pargyline plus DOPA. Single-beam photoelectric recorder-activity cages were employed for the study, using 4 mice per cage. Using an arbitrary program for rating activity by gross observation Everett and Wiegand were also able to demonstrate that mice responded to increasing acute doses of pargyline and DOPA by becoming more aggressive. Intraperitoneal (i.p.) administration of 100 mg/kg of pargyline HCl followed four hours later by administration of 100 mg/kg of DOPA produced aggressive attacking and fighting in mice. The dose-response curve of the combined drugs correlated both with increasing levels of brain dopamine and with increasing inhibition of MAO. Pargyline alone produced no change in activity in mice even at the same acute dose as above; brain dopamine was only slightly elevated. These results and earlier work by Everett (1961) suggest that increases in alertness and aggressiveness may be related to increases in brain amines and also to decreases in brain MAO activity.

Daily i.p. injection of rats with pargyline HCl at a dose of 25 mg/kg for 25 days was reported to result in reduced gross activity (Carrier and Buday, 1963). More recently Guarino *et al.* (1964), using the same daily dose, found general muscular tenseness in 13-25% of the rats while being handled or injected; this effect was first noted after 23 days of daily treatment and continued to the end of the 32-day study.

The work of Carrier and Buday (1963) showed that rats receiving daily doses of 25 mg/kg of pargyline HCl and fed a 2% thyroid diet *ad lib.* appeared

more depressed than animals receiving either drug alone. This work is interesting in view of the reported increases in apathy seen when both thyroid and MAO inhibitors were administered to depressed patients (Kline, 1961). Working with mice, however, Reid (1963) found no changes in locomotor activity after treatment with pargyline, thyroid or both of these agents.

E. The Effects of Thyroid Hormones on Monoamine Oxidase

Monoamine oxidase, IUB 1.4.3.4; Monoamine: oxygen oxidoreductase (deaminating), is a cuproprotein that acts on primary, secondary and tertiary amines, in combination with H_2O and O_2, to yield an aldehyde + NH_3 + H_2O_2. Oxygen acts as the acceptor. (Enzyme Nomenclature, 1965). Although MAO is secondary to COMT in catabolizing circulating adrenergic amines it is apparently the main enzyme for the inactivation of these monoamines in the central nervous system (Sice, 1962). About 70% of MAO activity is found in the mitochondria (Baudhuin, et al., 1964).

"One can hardly imagine a more conflicting series of reports than those existing on the effects of thyroid feeding on monoamine oxidase activity" (Harrison, 1964). Since the appearance of the now-classic paper of Spinks and Burn (1952) several investigators have reported on the effects of administered thyroid hormones on MAO activity in liver, heart and brain. In these studies there were considerable differences in types and sex of animals used, thyroid doses, enzyme preparations and assay methods. These differences have undoubtedly contributed to the confusion that follows when comparisons are made of the results of different workers. Much of this confusion may be eliminated, however, if one groups together all the available data for each organ studied and subdivides according to animal, sex and treatment. An attempt to do this is presented below.

Unless otherwise noted below, the method of MAO assay used in the studies to be described was the Warburg manometric technique with tyramine

as substrate. Oxygen utilization was measured.

MAO activity in the tissues of thyroid-treated animals is expressed as per cent change from control values. These percentages were taken from the reports or, if not given, calculated from the data presented.

1. The Effect of Thyroid on Liver MAO

a) Burn and Spinks (1952). Spinks and Burn (1952). Rabbits were fed 200 mg dried thyroid daily for 14 days. Tissue was frozen at -15°C until use. The assay for MAO activity was carried out on a 5% homogenate in 0.067 M phosphate buffer (pH7.4), and disclosed a 16% ($P = 0.015$) decrease from control values.

b) Trendelenburg (1953). Rabbits were treated and fresh homogenates were prepared as above (a). The assay for MAO activity disclosed a 10% ($P = 0.05$) decrease from control values.

c) Zile and Lardy (1959). Male rats were sustained for 3-4 weeks on a diet containing 2% desiccated thyroid. The assay for MAO activity was carried out on mitochondria isolated in 0.25 M sucrose. The mitochondria were either fresh, or frozen and thawed for assay. The assay disclosed a 51% ($P = 0.015$) decrease from control values.

d) Zile, (1960). Male rats were injected (s.q.) with Na thyroxine · $5H_2O$, 2.5 mg/kg daily for 10 days. The assay for MAO activity was carried out on mitochondria isolated in 0.25 M sucrose, and disclosed a 52% decrease from control values.

e) Novick (1961). Rats of mixed sex were sustained for 12 days on a diet containing 2% desiccated thyroid. The assay for MAO activity was carried out on mitochondria suspended in 0.067 M phosphate buffer (pH 7.4), and disclosed a 50% depression from control values.

f) Wurtman et al.(1963). Male rats were injected (i.p.) with Na thyroxine, 1 mg/kg, daily for 7 days. The assay for MAO activity was carried

out on fresh homogenates in isotonic KCl. The production of C^{14}-indoleacetic acid from C^{14}-tryptophan was measured. No change from control values was seen.

g) Wurtman et al.(1963). Female rats were injected (i.p.) with Na thyroxine, 1 mg/kg daily for 10 days. The assay for MAO activity was carried out as above (f), and disclosed a 30% increase ($P = 0.05$) over control values.

h) Wurtman et al.(1963). Female rats were injected (i.p.) with Na thyroxine, 5 mg/kg daily for 5 days. The assay for MAO activity was carried out as above (f), and disclosed a 16% decrease ($P < 0.05$) from control values.

i) Utley (1964). Female rats were injected (s.q.) with l-triiodothyronine (l-T-3), 0.3 mg/kg daily for 12-14 days. The assay for MAO activity was carried out on fresh homogenates in 0.067 M phosphate buffer (pH = 7.4), and disclosed a 15% decrease ($P = 0.005$) from control values.

j) Reid (1963). Male mice were injected (i.p.) with suspended thyroid powder, 2 mg/kg daily for 14 days. The assay for MAO activity was carried out on a mitochondria-microsome mixture suspended in 0.25 M sucrose and prepared from frozen tissue. No change from control values was noted.

2. The Effects of Thyroid on Heart MAO

a) Zile (1960). Male rats were injected (s.q.) with Na thyroxine $\cdot$ 5 H_2O, 2.5 mg/kg daily for 10 days. The assay for MAO activity was carried out on mitochondria isolated in 0.25 M sucrose. Some of the studies were done using a frozen, thawed preparation. No change from control values was noted.

b) Novick (1961). Rats of mixed sex were sustained for 12 days on a diet containing 2% desiccated thyroid. The assay for MAO activity was carried out on mitochondria suspended in 0.067 M phosphate buffer (pH 7.4), and disclosed a 75% increase over the controls.

c) Novick (1961). Rats of mixed sex were injected (s.q.) with 1-T-3, 0.15 mg/kg daily for 8 days. The assay for MAO activity was carried out as above (b), and disclosed a 70% increase over the control values.

d) Skillen et al. (1962). Male rats were sustained for 21 days on a diet containing 0.15% desiccated thyroid. The assay for MAO activity was carried out by measuring fluorometrically the disappearance of serotonin. Homogenates prepared in water and frozen at -15°C for 24 hours before assay were used. No change from control values was noted.

e) Skillen et al. (1962). Female rats were treated and MAO assays were carried out as above (d). The assays disclosed a 173% increase ($P = 0.03$) over control values.

f) Utley (1964). Female rats were injected (s.q.) with 1-T-3, 0.3 mg/kg kaily for 12-14 days. The assay for MAO activity was carried out on fresh homogenates in 0.067 M phosphate buffer (pH 7.4) and disclosed a 28% increase ($P = 0.005$) over control values.

3. The Effect of Thyroid on Brain MAO

a) Zile (1960). Male rats were injected (s.q.) with Na thyroxine, 2.5 mg/kg daily for 10 days. The assay for MAO activity was carried out on fresh homogenates in 0.25 M sucrose. No change from control values was noted.

b) Reid (1963). Male mice were injected (i.p.) with suspended thyroid powder, 2 mg/kg daily for 14 days. The assay for MAO activity was carried out on a mitochondria-microsomes mixture suspended in 0.25 M sucrose and prepared from frozen tissue. No consistent changes from control values were noted.

4. Summary

From the foregoing tabulation several interesting trends may be seen.

Liver. In studies (a),(b),(c),(d),(e) and (i), a decreased MAO

activity was seen in thyroid-treated animals. In these six studies the Warburg technique was employed to measure O_2 utilization with tyramine as substrate, and in all six enzyme preparations the mitochondria were subjected to treatment resulting in swelling, aging or lysing. Type or sex of animal employed appeared not to influence the results of these six studies.

In studies (g) and (h) anomalous effects were seen in female rats Very high doses of thyroxine (5 mg/kg) depressed MAO activity, but the same dose (1 mg/kg) that had no effect in male rats in study (f) effected a 30% increase in MAO activity in female rats. Studies (f), (g) and (h) were carried out as part of one project (Wurtman et al., 1963) and the same assay method was employed in all three sections.

Heart. In male rat hearts, studies (a) and (d) showed no change in MAO activity after thyroid treatment. Different assay methods were used, but both employed preparations in which mitochondria are generally swollen, aged or lysed.

In female rat hearts, studies (e) and (f) increases in MAO activity were seen after thyroid treatment. Different assay methods were used, but bcth employed enzyme preparations in which the medium was hypotonic to mitochondria. Since both sexes of rats were used in studies (b) and (c), the increased MAO activity was probably due to the presence of female rats with higher MAO activity.

Brain. No changes in brain MAO were induced by thyroid treatment.

It appears, in summary, that thyroid-induced depression of MAO in liver was seen only when manometric techniques were employed on enzyme preparations in which mitochondria were not completely intact. Thyroid-induced increases of heart MAO are apparently seen only in female rats.

III. INVESTIGATION

A. Objectives

The point of departure for this study was the reported retardation and apathy resulting from concurrent thyroid and monoamine oxidase (MAO) inhibitor therapy in depressed patients (Kline, 1961). An experiment of broad design was consequently evolved in order to simultaneously evaluate, in rats, certain parameters in which drug-induced changes might in turn affect behavior.

No real correlation necessarily exists between the effects of drugs in depressed patients and in normal laboratory rats. Consequently it was necessary to first establish a reliable method for measuring the behavioral responses of normal rats to chronic treatment with thyroxine and pargyline hydrochloride, a potent non-hydrazine MAO inhibitor. Use of a reliable method for measuring activity would permit one to carry out the main objective of this work: to determine if, under conditions of chronic thyroxine and pargyline treatment in rats, a correlation exists between changes in behavior and changes in certain physiological or biochemical parameters thought most likely to be affected by these drugs.

In this study the effects of chronic treatment with thyroxine, pargyline, and combined thyroxine and pargyline were determined on systolic blood pressure, body weight gains, locomotor activity, urinary sodium and potassium excretion and terminal MAO activity in brain and liver.

B. Materials and Methods

1. General Considerations and Daily Protocol

In each study rats were divided into four groups for daily intraperitoneal injection with thyroxine, pargyline, combined throxine and pargyline, and vehicle, respectively.

Male, adult albino rats of the Sprague-Dawley strain[1] were distributed three to a cage in standard metabolism cage banks[2] and housed in a soundproof room maintained at about 50% relative humidity. Room temperature, measured by a Tempscribe recording thermometer[3] was maintained at 22.2 ± 0.5°C. The room was dimly lighted from 7 am to 7 pm daily. All studies except blood pressure determinations were carried out in this room, and all animals used in these studies were housed here both before and during experimentation.

The animals were each offered 20 g of Purina[4] rat chow daily at 6 pm. Unlimited access to water was provided by wiring two jars of 255 ml total capacity into each cage. All animals were weighed to the nearest gram and injected daily from 9 am - 12 noon. Water jars were refilled and the urine collecting apparatus was cleaned and reset at this time.

Drugs were prepared for intraperitoneal injection as follows: Na l-thyroxine[5] was suspended daily before use in glass-distilled water in a concentration of 1 mg/ml or 0.5 mg/ml. Pargyline HCl[6] was dissolved in a concentration of 25 mg/ml glass-distilled water. There was no mixing of drugs: animals receiving both thyroxine and pargyline had a separate injection of each drug. Animals treated with one of the drugs or with the vehicle, glass-distilled water, were given a second, sham injection.

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1. Charles River Breeding Farms, North Wilmington, Massachusetts.
2. Wahmann Mfg. Co., Baltimore, Maryland.
3. Bacharach, Pittsburgh, Pennsylvania.
4. Ralston Purina, St. Louis, Missouri.
5. Calbiochem, Los Angeles, California.
6. Kindly supplied by Dr. R. B. Hasbrouck and Mr. Dale Giddings, Abbott Laboratories, North Chicago, Illinois.

Drug doses were as follows:

Pargyline HCl	25 mg/kg body weight, i.p. daily.
Na l-thyroxine	1 mg or 0.5 mg/kg body weight, i.p. daily.
Vehicle, glass-distilled water	1 ml/kg body weight, i.p. daily.

This investigation was carried out in two separate phases. In both phases, however, animal housing and daily protocols were as described above.

In phase 1, involving 30 rats, the effects of chronic administration of thyroxine, pargyline, combined thyroxine and pargyline, and vehicle on blood pressure were evaluated. In addition, weight gains were analyzed and terminal MAO activity was determined in brain and liver.

In phase 2, involving 142 rats, the effects of chronic administration of thyroxine, pargyline, combined thyroxine and pargyline, and vehicle on gross activity, urinary sodium and potassium and weight gains were evaluated. Terminal MAO activity in liver and brain was also determined.

A preliminary study, involving 24 untreated rats, was first carried out in order to evaluate the method of analyzing gross activity.

Each phase of this study is divided into a principal and a supplementary group of animals. The principal animals were placed in the cages so that the initial weight range of the 3 rats per cage would not exceed 5 g. Moreover, the overall initial weight range in the 24 and 96 animals in each respective principal group was 34 and 57 g, respectively. The supplementary groups were composed of heavier rats with somewhat wider weight ranges. For this reason weight gain studies were carried out only on the principal groups in each phase of this study.

These studies are summarized in the following chart.

SUMMARY OF EXPERIMENTAL DESIGN

	PHASE 1	PHASE 2
1. Number of Rats:	30	142
2. Initial Weight Ranges (g):		
Principal Group	(24) 161-195	(96) 145-202
Supplementary Group	(6) 214-297	(46) 202-327
Overall Ranges	(30) 161-297	(142) 145-327
3. Blood Pressure Study:	Yes	No
4. Gross Activity Study:	No	Yes
5. Urinary Na^+ and K^+:	No	Yes
6. Weight Gains:	Principal Group (24)	Principal Group (96)
7. Terminal MAO:	Yes	Yes
8. Length of Study:		
Pretreatment Period	11 days	None
Drug Treatment Period	27 days (days 0-26 incl.)	12 days (days 0-11 incl.)
9. Daily i.p. Treatment:		
Na Thyroxine, 1 mg/kg	Days 0-19 incl.	Days 0-4 incl.
Na Thyroxine, 0.5 mg/kg	Days 20-26 incl.	Days 5-11 incl.
Pargyline HCl, 25 mg/kg	Days 0-26 incl.	Days 0-11 incl.
Vehicle, 1 ml/kg	Days 0-26 incl.	Days 0-11 incl.

PRELIMINARY STUDY

1. Number of Rats:	24
2. Initial Weight Range (g):	133-178
3. Purpose:	Evaluation of Gross Activity Method
4. Length of Study:	22 days
5. Drug Treatment:	None

2. Blood Pressure Study

The effects of daily treatment with thyroxine, pargyline, combined thyroxine and pargyline, and vehicle on the systolic blood pressure of rats were determined. This study, a part of phase 1[1], was carried out in an Environtrol constant-temperature chamber maintained at 28°C, in which low humidity was maintained by means of rapid turnover of air in the chamber. Rats were fed and housed as described previously; only during the actual determinations were they removed to the Environtrol chamber.

The tail cuff method of indirect systolic blood pressure determination was used. Rats were removed from their cages and placed in an incubator box at 40°C for 15-20 minutes. After incubation each rat was allowed to enter a clear plastic chamber[2] with dimensions 23 cm x 7 cm x 5 cm. This chamber is closed at the far end but provided with a movable piston in order to regulate the internal length. The sliding partition at the open end of the chamber was then put into position, leaving only the rat's tail protruding. After a few minutes in the chamber the rat generally became calm.

An inflatable tail-cuff[3], connected to a Physiograph manometer system[4], was placed about 1 cm from the base of the tail. Distal to the cuff the tail was passed through a small plastic holder designed so that when the screw clamp was slightly tightened the tail came into contact with a Beckman microphone transducer.[5] The transducer lead was fed into a Beckman Infraton[5]

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1. See Summary of Experimental Design, Page 28.
2. Constructed by Mr. David Coates, Dept. of Pharmacology, University of Rhode Island.
3. Harvard Instruments, Cambridge, Massachusetts.
4. E & M Instruments, Houston, Texas.
5. Beckman Co., Palo Alto, California.

signal divider set for maximum pulse, and the Infraton output lead was plugged into the direct current input of an HP oscilloscope[1] set at a sweep time of 20 msec/cm.

This system converts systolic pressure pulses in the rat's tail to peaks on the oscilloscope screen. Squeezing the rubber bulb of the manometer system increases pressure in the system; the cuff becomes inflated, and consequently the peaks disappear as tail circulation distal to the cuff is cut off. By opening the pressure escape valve slightly, pressure in the manometer system can be reduced at will. As tail circulation is restored peaks begin to appear again on the screen. At this point the pressure reading on the manometer dial is considered to be the systolic blood pressure, because it is assumed that for distal circulation to be restored the pressure in the tail arteries must be slightly greater than the pressure in the manometer system.

The recorded blood pressure of a rat on a given day was the average of at least 3 successive determinations. It was found that once the animal was quiet, successive determinations fell into a range not exceeding 4-5 mm Hg.

In the principal blood pressure study 24 rats caged and fed as described above were divided into four groups of 6. Blood pressures were determined on four separate days before drug treatment was begun: days -11, -9, -7, and -2. The first day of drug treatment was designated day 0, and blood pressures were determined on days 2, 7, 10, 12, 16 and 19 of daily drug treatment. Daily drug doses (i.p.) from day 0 were as follows: vehicle, 1 ml/kg; pargyline HCl, 25 mg/kg; Na thyroxine, 1 mg/kg. Animals receiving both drugs received the full dose of each.

For each day that blood pressure determinations were made, the data

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1. Model 130 B., Hewlett-Packard Co., Palo Alto, California.

from each of the three drug-treated groups were separately compared to the vehicle-treated group by means of Student's "t" test (Snedecor, 1956).

The data from the pre-treatment period (days -11, -9, -7 and -2) were similarly analyzed. But it was found that in this pre-drug period no difference ($P \leq 0.05$) existed between the group designated as the future vehicle-treated group and each of the three groups destined to receive drugs. These data were consequently pooled so that for each of the four days in the pre-treatment period one mean $\pm$ S. D. was obtained.

Because of the high mortality in the combined-drug group a second, supplementary blood pressure study was undertaken using heavier animals (214-297 g). Two groups, vehicle-treated and combined-drug treated, were used. Blood pressure determinations were made on days -6 and -4 before drug treatment was begun, and again on day 3 of drug treatment.

3. Activity Studies

Four multibeam "Actophotometer" units[1] were employed in determining the effects of daily treatment with thyroxine, pargyline, combined thyroxine and pargyline, and vehicle on the locomotor activity of rats.

The actophotometer consists of a circular cage 31 cm deep and 33 cm in diameter. Two centimeters up from the grid floor are 12 holes spaced equally apart around the inside diameter of the cage; from these holes six light beams pass through red filters and cross the cage to activate six respective photoelectric cells. When any one of these light beams is interrupted a digital counter advances one unit. Animals moving about in the cage interrupt the light beams, causing the counter to advance; in this way, counts per unit time can be used as a measure of locomotor activity.

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1. Metro Scientific, Inc., Carle Place, Long Island City, New York.

The light beams and photocells were adjusted for maximum sensitivity by varying the angle of incidence of light to the photocell and by adjusting voltage to the counter relay and to the photocells. It was found that each cage has its own sensitivity characteristics, resulting in varying total counts per unit time from one cage to another. Watzman (1964) found similar, inherent sensitivity differences among actophotometer units. It is not practical to attempt a calibration of four cages; therefore, comparisons from one cage to another cannot be made. Rather, the experiment is designed so that data from both vehicle-treated and drug-treated animals are obtained from the same cage. Thus for each cage one must have control values.

The four actophotometer cages were located in the same soundproof room described above. The counters were located in an adjacent room. Because animals were kept in the actophotometers for six-hour periods, these units were fitted with standard water bottles in order to insure a constant supply of water.

All animals used in the activity studies were placed on the same housing and feeding schedule as described above.

The same design was employed for all activity studies. Three animals were removed from their cage and immediately placed in the actophotometer; grids were then placed on top of each unit to prevent animals from escaping. The room was quickly secured and the counters in the adjacent room were turned on. A maximum time of three minutes elapsed between placing of animals in the four cages and turning on the four counters to begin recording counts. A clock was simultaneously set.

This procedure was always started between 11:30 am and 12 noon. Total cumulative counts appearing on the counters from time 0 were recorded every 30 minutes for six hours (i.e., once counting was begun the counters were never reset).

After the six hour period had elapsed the animals were returned to their respective cages.

Two separate activity studies were carried out. The preliminary study[1] employed 24 untreated, uninjected rats divided into 2 groups of 12 each. Each group was subdivided into 4 units of 3 animals each, and each unit of 3 animals was assigned to be run every other day in one of the four actophotometers. On day 1 of the study the 12 animals in group 1 were put into their respective actophotometers and counts were taken as described above. On day 2 the 12 animals in group 2 were similarly run, and on day 3 the animals in group 1 were again placed in the actophotometers. This alternation continued for a total of 22 days, resulting in 12 daily sets of data per cage per group (days 1,3,5,7,...21 for group 1 and days 2,4,6,8,...22 for group 2).

The second study was carried out using the principal group of phase 2[1]. The 3 animals in each unit of group 1 were injected daily at 9:45 am with vehicle, thyroxine, pargyline, and combined thyroxine and pargyline, respectively. The 3 animals in each of the 4 units of group 2 were injected with vehicle. This study was run for 11 days, resulting in 6 daily sets of data per cage for group 1 (days 0, 2, 4, 6, 8 and 10) and 5 daily sets of data per cage for group 2 (days 1, 3, 5, 7 and 9).

In the drug-treated groups deaths occasionally occurred. Only one rat died during an actophotometer run; data for that cage was discarded for that day. If animals being used in the activity study died during the night or early moring the entire unit of 3 was replaced with another unit of animals identically treated.

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1. See Summary of Experimental Design, Page 28 .

Recording of cumulative counts every 30 minutes for six hours resulted in 12 figures per cage per day. From these figures the counts that had accumulated during each of the twelve 30-minute increments were determined simply by subtracting the 30-minute total count from the 60-minute total count, the 60-minute count from the 90-minute count, and continuing successively. There resulted, then, 12 total counts, and 12 incremental counts of 30 minute periods per cage per day.

The data from each of the two studies were analyzed in two ways:

a) Incremental Counts per 30 Minutes

For a given actophotometer, two groups of animals were run on alternate days, resulting in two sets of data as indicated above. Using Student's "t" test (Snedecor, 1956) the data from each 30-minute interval of group 1 was compared to the data from the corresponding time-interval of group 2.

b) Total Counts per Hour

Total counts were recorded from each unit every 30 minutes, as indicated above. But only the total elapsed counts per hour were analyzed. The method of analysis was identical to that used for the 30-minute incremental data.

4. Urinary Sodium and Potassium Studies

This work, a part of phase 2[1], evaluated the effects of daily treatment with thyroxine, pargyline, combined thyroxine and pargyline, and vehicle on the daily output of sodium and potassium in the spontaneously-voided urine of rats.

From 9-11 am daily, animals were injected and drinking-water vessels were filled. The urine-collecting funnels were fitted with cotton plugs at the apex; the screens designed to keep food fragments and droppings out of the

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1. See Summary of Experimental Design, Page 28.

funnels were put into place, and finally the funnels were secured under the cages. Bottles were placed under the funnel spouts at 11:30-11:45 am, and urine collection commenced. The rats were fed as usual at 6 pm, without disturbing the funnels or bottles. At 9 am the next day each bottle was removed from the collection site; the contents were measured to the nearest 0.5 ml and a sample was stored in a stoppered glass tube in the refrigerator until assay. The funnels, bottles and screens were thoroughly washed and allowed to dry. At 11:30 am, urine collection began again.

A record was kept of daily water consumption in each cage. These values are not reported, however, because they do not reflect water actually consumed. The rats carried on a great deal of splashing and washing in the drinking water, especially in the thyroxine-treated groups; consequently it was impossible to determine how much water was consumed. Much of the spilled water appeared in the urine-collecting bottles. For this reason no conclusions with respect to the effects of the drugs on urine volume could be drawn.

This splashing had no effect on sodium and potassium determinations, since these are reported simply as total mEq/cage/day and not as a concentration of ions in urine.

Urine samples were analyzed for sodium and potassium on the Advanced flame photometer[1] using the lithium internal standard method.

Stock solutions of NaCl (1000 mEq/l) and KCl (100 mEq/l) were prepared using glass-distilled water. From these, standard concentrations of Na^+ (10-160 mEq/l) and K^+ (1-10 mEq/l) were prepared by suitable dilution with glass-distilled water. A 1:2000 solution of Li_2SO_4 (internal standard solution) in glass-distilled water was prepared. Standard curves were made by using

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1. Model 11B, Advanced Instruments, Inc., Newton Highlands, Massachusetts.

the standard concentrations of the ions, diluted 1:100 with the lithium sulfate solution. Urine was similarly diluted with this solution: 1:100 for the sodium assay and 1:2000 for the potassium assay. Each urine sample was read against the standard curve.

Total output of Na^+ or K^+ in terms of mEq/3 rats/day (i.e., mEq/cage/day) was determined by multiplying the Na^+ or K^+ concentration in the urine aliquot by the total volume collected for that cage.

Thus, for each day of urine collection there resulted data from several cages in each of the four treatment groups. For each day the data from control (vehicle-treated rats) cages were compared to the data from each of the three drug-treated groups by means of Student's "t" test (Snedecor, 1956).

5. Weight Gain Studies

The effects of daily treatment with thyroxine, pargyline, combined thyroxine and pargyline, and vehicle on the individual body weights of rats were determined.

All animals used in this work were routinely weighed daily, to the nearest gram, on an Ohaus small-animal balance[1]. Each rat was marked for identification and individual records were kept of the daily weights. Because of the small range of their initial weights the animals from the principal groups of phase 1 and phase 2[2] were used for weight gain analysis.

For each animal, then, there was compiled a record of daily weight from day 0, the beginning of the study. Three methods of data analysis were employed.

a) Daily Weights. For each day of the study, the individual daily

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1. Ohaus Scale Corp., Union, New Jersey.

2. See Summary of Experimental Design, Page 28.

weights of all animals in each treatment group were compared to the individual daily weights of the control group.

b) Cumulative Daily Weight Gains from Day 0. These were determined by subtracting the weight of each animal on day 0 from its weight on each of the remaining days of the study. Daily comparisons were made, as above, between the control group and each treatment group.

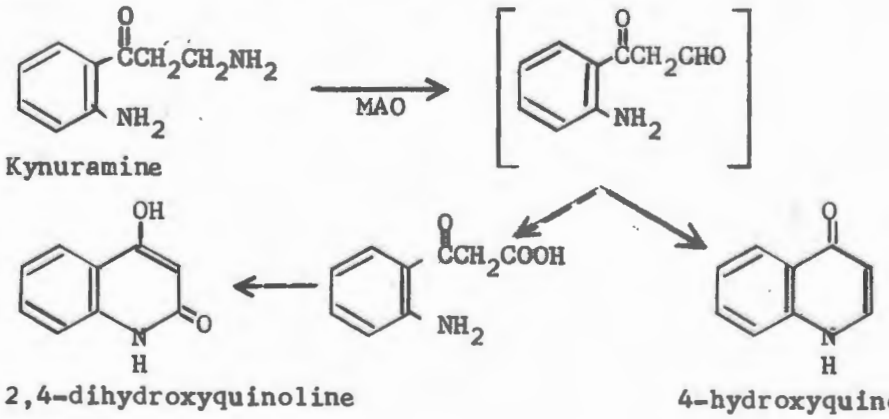
c) Daily Weight Gains. These were determined by subtracting the weight of each animal on one day from its weight on the next day. This was done for each day of the study. Daily comparisons were made, as above, between the control group and each treatment group.

Student's "t" test (Snedecor, 1956) was used to compare these data.

6. MAO Assay

The spectrophotometric MAO assay method of Weissbach et al. (1960) was modified for the determination of MAO activity in brains and livers of rats treated daily with thyroxine, pargyline, combined thyroxine and pargyline, and vehicle, respectively.

In this assay the disappearance of kynuramine[1] at 360 mμ is followed in the Beckman DU spectrophotometer. The action of MAO on kynuramine is postulated to be the following:



1. Kynuramine dihydrobromide, Regis Chemical Co., Chicago, Illinois.

As kynuramine absorbance at 360 mμ decreases, an absorbance peak at 310-335 mμ develops, and the final spectrum of an incubation of kynuramine with rabbit liver homogenate closely resembles the spectrum of 4-dydroxyquinoline, the postulated product (Weissbach et al., 1960).

Preliminary experiments with rat brain and liver homogenates satisfactorily reproduced Weissbach's data, showing that kynuramine disappearance at 360 mμ is linear with time. But the overall experimental design involved the sacrificing, on the last day of the study, of many animals on whom MAO assays were then to be carried out. Thus it was important to develop an assay method that would permit storing, for subsequent analysis, of MAO or MAO-containing tissues from these animals.

The majority of MAO activity is found in the mitochondria (Baudhuin et al., 1964; Weissbach et al., 1960; Zile and Lardy, 1959; Hawkins, 1952). Seiden and Westley (1962) reported a method for isolating and lysing brain mitochondria for subsequent ultrasound treatment to release soluble MAO. The soluble MAO was then assayed by the Weissbach method. Here, however, the Weissbach method was adapted for use with lysed, rehomogenized mitochondria prepared by a slight modification of Seiden and Westley's procedure. Furthermore, it was found that storage of a suspension of lysed mitochondria at -40°C for as long as three months did not affect their turnover of kynuramine. Finally, the assay was run at 30°C instead of at room temperature. The temperature inside the DU cuvette chamber was maintained at 30°C ± 0.5° by attaching to the instrument two Beckman Thermospacer[1] units connected to a Haake[2] water heater and circulator.

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1. Beckman Instruments, Inc., Fullerton, California.

2. Haake, Gerruder, Berlin.

a) Preparation of Mitochondria. Animals treated daily with thyroxine, pargyline, combined thyroxine and pargyline, or vehicle[1] were sacrificed by decapitation.[2] All subsequent procedures were carried out at 0°C. Each brain was quickly removed, placed in 10 ml of 0.25 M sucrose and homogenized in a pyrex homogenizing tube using a teflon pestle.[3] The homogenate was transferred to a 50 ml polypropylene tube and centrifuged[4] at 1500 x g for 6 minutes. The supernatant fluid, containing mitochondria and microsomes, was layered over 15 ml of 0.88 M sucrose in a 50 ml polypropylene tube and centrifuged at 18000 x g for 20 minutes. The resulting supernatant was aspirated out, leaving the mitochondrial pellet. The mitochondria were lysed when resuspended in 6.0 ml of 0.05 M phosphate buffer (pH 7.4) and dispersed in the buffer by rehomogenization. The lysed, resuspended mitochondria were transferred to a 12 ml polypropylene tube which was stoppered and stored at -40°C until assay.

MAO Assay. The tube of frozen, suspended lysed mitochondria was allowed to thaw and immediately placed in crushed ice. The temperature in the cuvette chamber of the DU spectophotometer was allowed to reach 30°C and maintained at this temperature throughout the assay. All constituents of the assay mixture were kept at 30°C in a water bath[5]; only the mitochondria were kept at 0°C.

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1. See phase 1 and phase 2, Summary of Experimental Design, Page 28.
2. Guillotine, Harvard Instruments, Cambridge, Massachusetts.
3. Tri-R Homogenizer, Tri-R Instruments, Jamaica, New York.
4. Servall Refrigerated Centrifuge, Model RC-2, Sorvall, Inc., Norwalk, Connecticut.
5. Scientific Glass Apparatus, Inc., Bloomfield, New Jersey.

Incubation mixtures were prepared in Beckman standard silica cuvettes by adding reagents in the following sequence:

Reagent	Blank	Experimental
0.5 M phosphate buffer, (pH 7.4)	0.3 ml	0.3 ml
0.3 μM kynuramine	--	0.1 ml
Water, glass-distilled	1.2 ml	1.1 ml
Mitochondrial suspension	1.5 ml	1.5 ml
	3.0 ml	3.0 ml

Immediately after the mitochondria were added, a clock was set and the cuvettes were mixed by inversion and placed in the DU cuvette chamber. The instrument was set at 360 mμ with a slit width setting of 0.1. Absorbance of the experimental cuvette was read against the blank approximately every 3 minutes for 30 minutes. Initial absorbance was about 0.3. A curve was constructed from the data by plotting absorbance readings on the ordinate against time (in seconds) on the abcissa. Because of settling of particles in the cuvette, initial readings were sometimes erratic. For this reason these curves were constructed using the last 20 minutes of the 30-minute assay period. In this time interval the curves were linear in each case.

A standard curve of kynuramine concentration against absorbance was constructed and found to be linear in the 0.05-0.71 absorbance range.

Protein Determination. The standard colorimetric biuret method of Gornall et al.(1949) was used to determine protein content of an aliquot of each mitochondrial suspension. These determinations were carried out on the DU spectophotometer. 1.0 ml of the mitochondrial suspension was added to 4.0 ml of biuret reagent, mixed and allowed to stand for 30 minutes. Readings were taken at 540 mμ against a blank containing 1.0 ml of water instead of mitochondria. Standard curves were prepared using bovine albumin protein

standard solution[1], and the protein content of the mitochondrial suspensions was determined by reading against the standard curve.

Method of Calculating MAO Activity. Decrease in absorbancy during the last 20 minutes of each 30-minute assay was determined from the slope of the assay plot. This value was extrapolated to one hour and divided by the slope of the kynuramine standard curve (change in absorbancy/µmol of kynuramine free base). This value was divided in turn by the amount of mitochondrial protein present in the cuvette to yield a rate of substrate turnover in terms of µmol kynuramine/hr/mg protein.

Determination of Mitochondrial Stability for MAO Assay. This preliminary study was carried out on a suspension of mitochondria prepared as described above from 8 rat brains. An aliquot of the fresh preparation was assayed and the remaining aliquots were stored at -40°C for assay at various intervals up to 3 months.

A comparison was also made between the rate of kynuramine turnover by crude homogenates and by the mitochondrial preparation.

b) Liver MAO Study. Slight changes were made in the brain MAO assay procedure described above in order to adapt it for use with liver mitochondria. Only the specific changes are noted here.

Preparation of Liver Mitochondria. A modification of the method of Myers and Slater (1957) was used. Rats were sacrificed by decapitation; each liver was removed, placed in 0.25 M sucrose at 0°C and minced with scissors. The liver particles were washed with 0.25 M sucrose, suspended in a total volume of 35 ml in 0.25 M sucrose, and homogenized. The homogenate was centrifuged at 300 x g for 10 minutes and the pellet discarded. The supernatant was centrifuged for 10 minutes at 2000 x g and the resulting supernatant was

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1. Armour Pharmaceutical Co., Kankakee, Illinois.

discarded. The remaining pellet was resuspended in 25 ml of 0.25 M sucrose and centrifuged at 4600 x g for 10 minutes; the resulting supernatant was discarded. The pellet was resuspended in 6.0 ml of 0.05 M phosphate buffer (pH 7.4) and rehomogenized. This final suspension was stored in a stoppered 12 ml polypropylene tube at -40°C until assay.

MAO Assay. The assay for MAO in liver mitochondria was carried out as described above for brain mitochondria. Because a smaller volume of suspended liver mitochondria was used, cuvettes were prepared as follows:

Reagent	Blank	Experimental
0.5 M phosphate buffer (pH 7.4)	0.3 ml	0.3 ml
0.3 uM kynuramine	--	0.1 ml
Water, glass-distilled	2.6 ml	2.5 ml
Mitochondrial suspension	0.1 ml	0.1 ml
	3.0 ml	3.0 ml

All other procedures used in the assay of liver MAO did not differ from the methods used for brain MAO.

IV. RESULTS

All tables and figures are contained in this section.

TABLE 1

PRINCIPAL BLOOD PRESSURE STUDY: THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON SYSTOLIC BLOOD PRESSURE IN MALE ALBINO RATS

DAY	I	II	III	IV	POOLED[c]
-11	112 ± 9[a] (6)[b]	114 ± 10 (6), N.S.[d]	120 ± 3 (5), N.S.	120 ± 12 (6), N.S.	116 ± 10 (23)
-9	118 ± 10 (6)	123 ± 7 (6), N.S.	118 ± 7 (6), N.S.	112 ± 3 (6), N.S.	119 ± 8 (24)
-7	115 ± 6 (6)	115 ± 10 (6), N.S.	119 ± 11 (6), N.S.	114 ± 9 (6), N.S.	116 ± 9 (24)
-2	109 ± 6 (6)	111 ± 15 (6), N.S.	116 ± 10 (6), N.S.	109 ± 6 (6), N.S.	114 ± 18 (24)

Daily Drug Injection Began on Day 0.

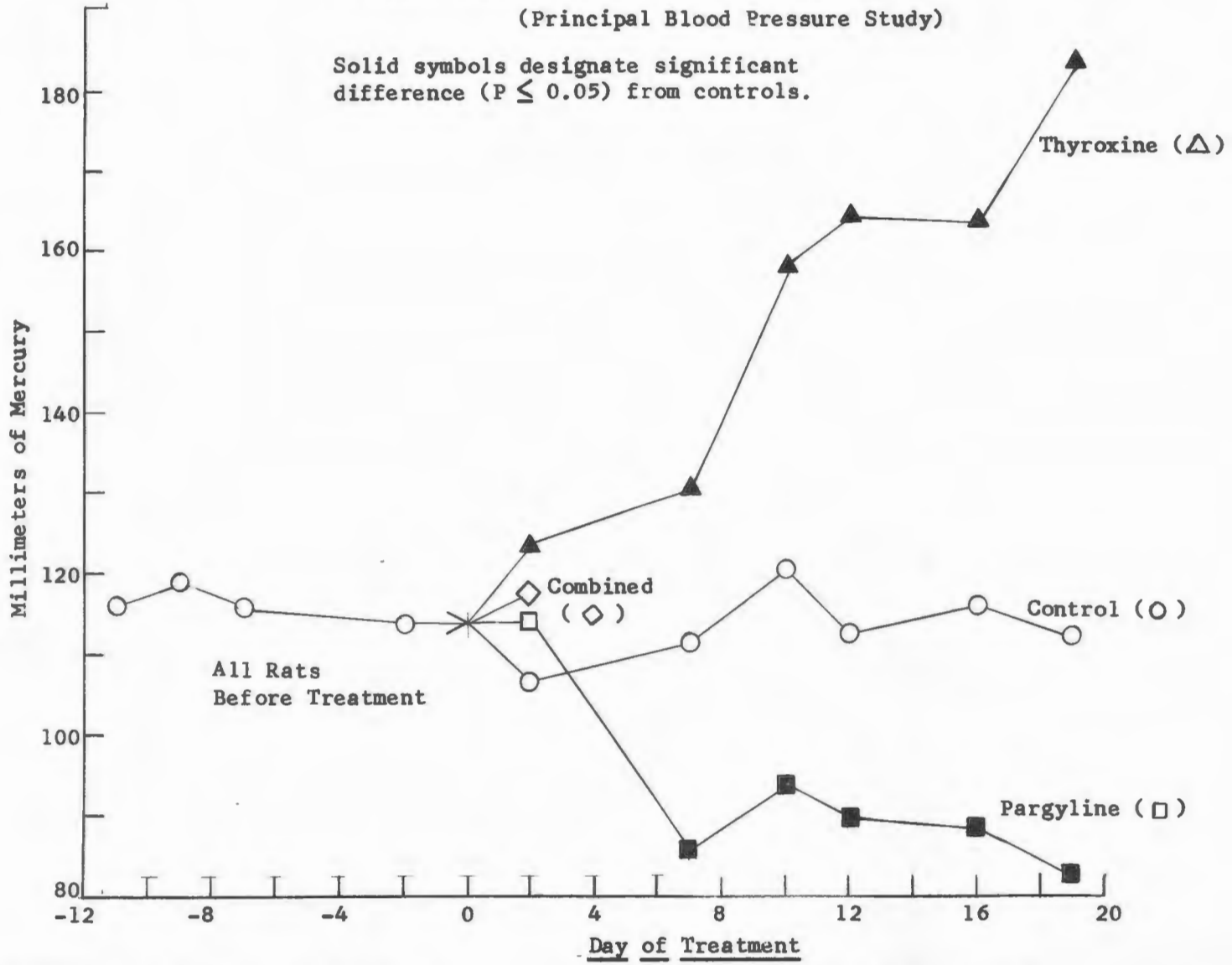
	VEHICLE	THYROXINE	PARGYLINE	COMBINED
2	107 ± 3 (6)	124 ± 12 (5), INC.[d]	115 ± 8 (5), N.S.	118 ± 15 (4), N.S.
7	112 ± 9 (5)	131 ± 3 (6), INC.	86 ± 19 (6), DEC.[d]	--
10	121 ± 13 (6)	159 ± 15 (6), INC.	94 ± 13 (6), DEC.	--
12	113 ± 7 (6)	165 ± 21 (4), INC.	90 ± 8 (6), DEC.	--
16	117 ± 11 (6)	164 ± 19 (4), INC.	89 ± 9 (6), DEC.	--
19	113 ± 6 (6)	184 ± 2 (2), INC.	83 ± 10 (6), DEC.	--

a: mean systolic blood pressure ± S.D.
b: number of rats.
c: mean systolic blood pressure ± S.D. of all four groups
d: at $P \leq 0.05$:

N.S. = not significantly different from group I (or vehicle).
INC. = significantly elevated over vehicle-treated group.
DEC. = significantly decreased from vehicle-treated group.

Drug Doses, Daily From Day 0: Na Thyroxine, 1 mg/kg; Pargyline HCl, 25 mg/kg; Combined, Na thyroxine, 1 mg/kg + pargyline HCl, 25 mg/kg; Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

FIG. 1. THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON MEAN SYSTOLIC BLOOD PRESSURE IN MALE ALBINO RATS



Doses (i.p.) daily from day 0: Na Thyroxine, 1 mg/kg. Pargyline HCl, 25 mg/kg. Combined, Na thyroxine and pargyline HCl as above. Control, glass-distilled water, 1 mg/kg.

TABLE 2

SUPPLEMENTARY BLOOD PRESSURE STUDY: THE EFFECTS OF CHRONIC COMBINED THYROXINE AND PARGYLINE TREATMENT ON SYSTOLIC BLOOD PRESSURE IN MALE ALBINO RATS

DAY	I	II	POOLED[c]
-6	121 ± 13[a] (6)[b]	112 ± 10 (6), N.S.[d]	116 ± 2 (12)
-4	113 ± 7 (6)	108 ± 6 (6), N.S.	111 ± 2 (12)
	Daily Drug Injection Began on Day 0.		
	VEHICLE	COMBINED	
3	113 ± 6 (6)	100 ± 3 (3), N.S.	

a: mean systolic blood pressure ± S.D.
b: number of rats.
c: mean systolic blood pressure ± S.D. of both groups.
d: at $P \leq 0.05$:

N.S. = not significantly different from group I (or vehicle).

Drug Doses, Daily From Day 0: Na Thyroxine, 1 mg/kg, + Pargyline HCl, 25 mg/kg; Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

TABLE 3

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON URINARY SODIUM EXCRETION IN MALE ALBINO RATS

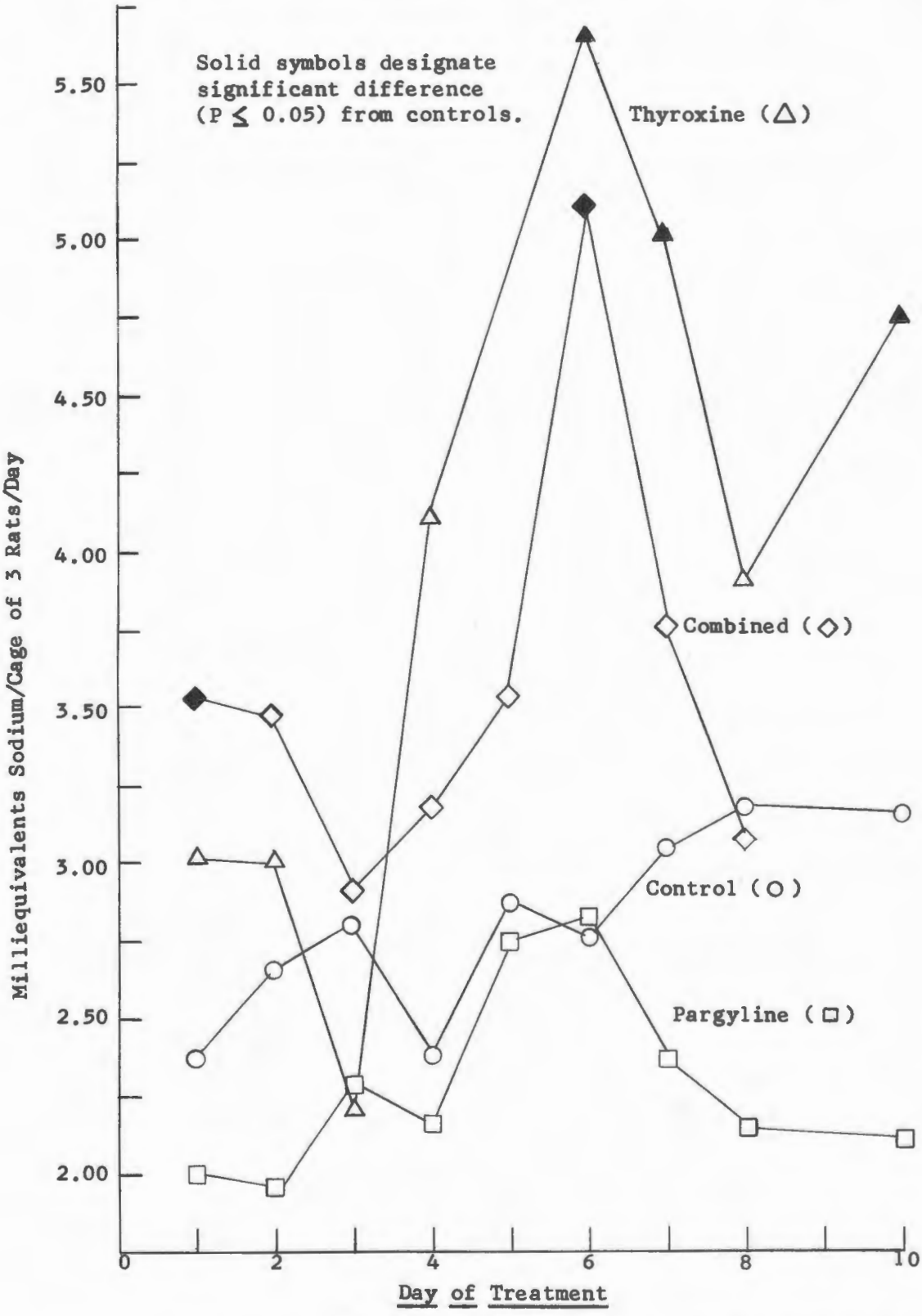
DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
1	2.38 ± 0.50[a] (4)[b]	3.02 ± 0.61 (4), N.S.	2.01 ± 0.44 (4), N.S.	3.53 ± 0.77 (12), INC.[c]
2	2.66 ± 0.53 (4)	3.00 ± 0.72 (4), N.S.	1.95 ± 0.58 (4), N.S.	3.48 ± 0.97 (8), N.S.
3	2.80 ± 0.66 (4)	2.20 ± 0.91 (3), N.S.	2.29 ± 0.99 (3), N.S.	2.92 ± 0.66 (9), N.S.
4	2.38 ± 0.60 (3)	4.11 ± 1.45 (2), N.S.	2.16 ± 0.91 (3), N.S.	3.18 ± 0.28 (5), N.S.
5	2.88 ± 1.26 (3)	--	2.75 ± 0.70 (4), N.S.	3.54 ± 0.67 (7), N.S.
6	2.76 ± 1.48 (7)	5.67 ± 1.28 (5), INC.	2.82 ± 1.12 (3), N.S.	5.10 ± 2.21 (7), N.S.
7	3.05 ± 0.84 (4)	5.00 ± 0.95 (6), INC.	2.37 ± 0.59 (4), N.S.	3.75 ± 0.45 (5), N.S.
8	3.18 ± 0.68 (5)	3.90 ± 0.77 (5), N.S.	2.14 ± 0.57 (3), N.S.	3.07 ± 1.48 (3), N.S.
10	3.15 ± 1.06 (5)	4.74 ± 0.74 (4), INC.	2.12 ± 0.05 (3), N.S.	--

a: mean, ± S.D., mEq Na^+/cage of 3 rats/day.
b: number of cages.
c: at $P \leq 0.05$:

N.S. = not significantly different from vehicle-treated group.
INC. = significantly elevated over vehicle-treated group.

Drug Doses, Daily, From Day 0: Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Pargyline HCl, 25 mg/kg. Combined, thyroxine as above + pargyline, as above. Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

FIG. 2. THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON MEAN URINARY SODIUM EXCRETION IN MALE ALBINO RATS



Doses (i.p.) daily from day 0: Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Pargyline HCl, 25 mg/kg. Combined, Na thyroxine and pargyline HCl as above. Control, glass-distilled water, 1 ml/kg.

TABLE 4

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON URINARY POTASSIUM EXCRETION IN MALE ALBINO RATS

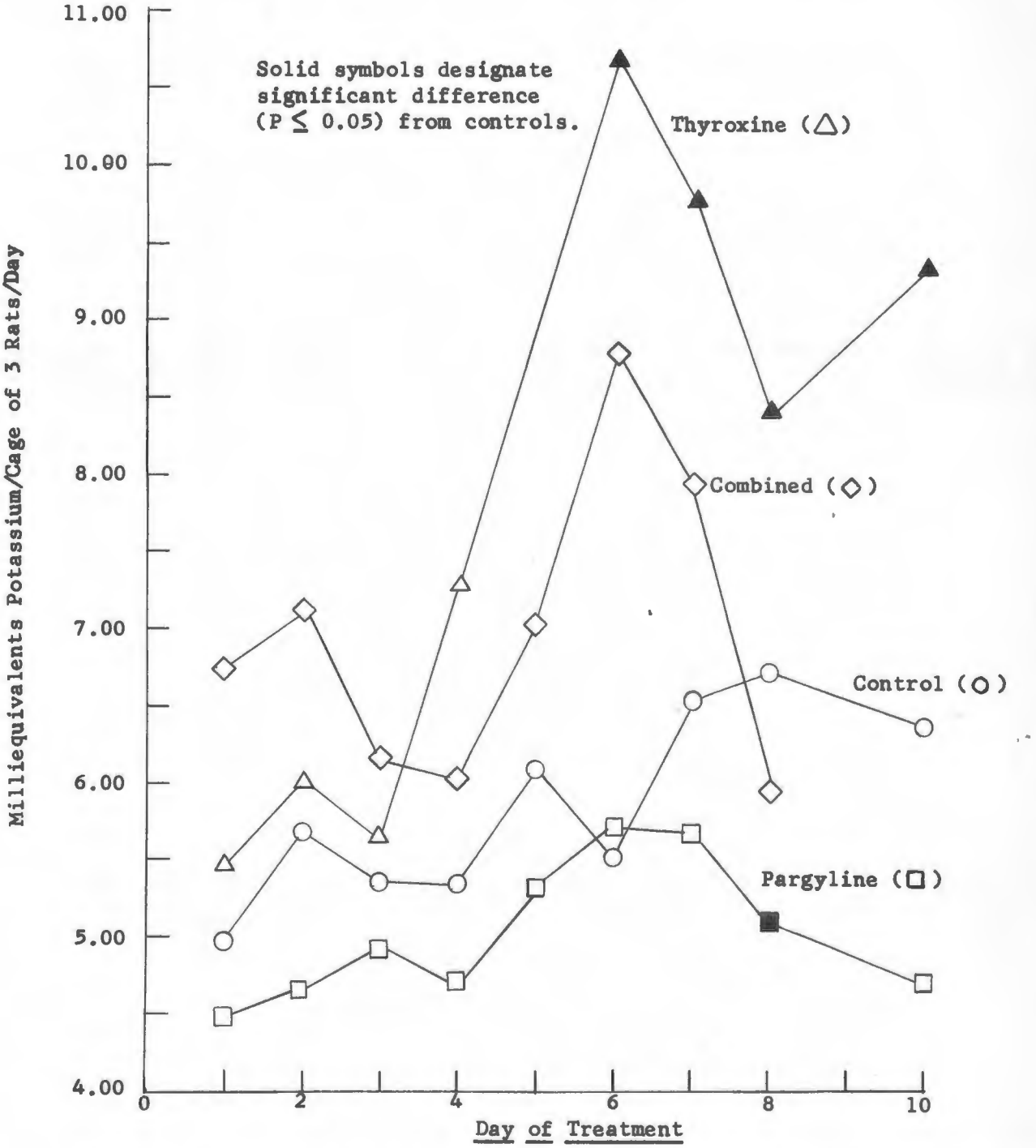
DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
1	4.98 ± 0.85[a] (4)[b]	5.47 ± 1.05 (4), N.S.[c]	4.50 ± 0.99 (4), N.S.	6.73 ± 1.32 (12), INC.
2	5.70 ± 0.91 (4)	6.04 ± 1.66 (4), N.S.	4.67 ± 0.97 (4), N.S.	7.13 ± 0.96 (8), INC.
3	5.36 ± 0.70 (4)	5.66 ± 2.49 (3), N.S.	4.94 ± 1.97 (3), N.S.	6.17 ± 0.94 (9), N.S.
4	5.34 ± 1.34 (3)	7.32 ± 1.75 (2), N.S.	4.74 ± 1.96 (3), N.S.	6.03 ± 0.19 (5), N.S.
5	6.12 ± 2.70 (3)	--	5.35 ± 1.16 (4), N.S.	7.07 ± 0.84 (7), N.S.
6	5.53 ± 2.70 (7)	10.75 ± 2.54 (5), INC.	5.73 ± 1.80 (3), N.S.	8.83 ± 3.19 (7), N.S.
7	6.58 ± 1.01 (4)	9.81 ± 1.15 (6), INC.	5.70 ± 0.86 (4), N.S.	7.95 ± 0.63 (5), N.S.
8	6.74 ± 0.95 (5)	8.42 ± 0.89 (5), INC.	5.13 ± 0.81 (3), DEC.	5.97 ± 2.64 (3), N.S.
10	6.41 ± 1.45 (5)	9.39 ± 1.21 (4), INC.	4.74 ± 0.32 (3), N.S.	--

a: means ± S.D., mEq K^+/cage of 3 rats/day.
b: number of cages.
c: at $P \leq 0.05$:

N.S. = not significantly different from vehicle-treated group.
INC. = significantly elevated over vehicle-treated group.
DEC. = significantly decreased from vehicle-treated group.

Drug Doses, Daily, From Day 0: Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Pargyline HCl, 25 mg/kg. Combined, thyroxine as above + pargyline, as above. Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

FIG. 3. THE EFFECTS OF CHRONIC THYROXINE AND PARGYLING TREATMENT ON MEAN URINARY POTASSIUM EXCRETION IN MALE ALBINO RATS



Doses (i.p.) daily from day 0: Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Pargyline HCl, 25 mg/kg. Combined, Na thyroxine and pargyline HCl as above. Control, glass-distilled water, 1 ml/kg.

TABLE 5

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS: I. DAILY WEIGHTS

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
0	209 ± 21[a] (6)[b]	213 ± 17 (6), N.S.[c]	230 ± 21 (6), N.S.	231 ± 13 (6), N.S.
1	212 ± (6)	211 ± 13 (6), N.S.	233 ± 18 (6), N.S.	227 ± 13 (6), N.S.
2	213 ± 21 (6)	210 ± 16 (6), N.S.	230 ± 19 (6), N.S.	227 ± 13 (6), N.S.
3	215 ± 24 (6)	206 ± 20 (6), N.S.	231 ± 20 (6), N.S.	233 ± 16 (5), N.S.
4	218 ± 24 (6)	202 ± 18 (6), N.S.	230 ± 19 (6), N.S.	225 ± 12 (5), N.S.
5	222 ± 26 (6)	201 ± 18 (6), N.S.	230 ± 20 (6), N.S.	220 ± 14 (5), N.S.
6	228 ± 26 (6)	201 ± 19 (6), N.S.	237 ± 22 (6), N.S.	224 ± 14 (5), N.S.
7	227 ± 27 (6)	190 ± 19 (6), DEC.	234 ± 20 (6), N.S.	220 ± 19 (4), N.S.
8	232 ± 31 (6)	192 ± 19 (6), DEC.	239 ± 22 (6), N.S.	229 ± 16 (2), N.S.
9	237 ± 30 (6)	188 ± 23 (6), DEC.	245 ± 21 (6), N.S.	228 ± 14 (2), N.S.
10	235 ± 29 (6)	179 ± 23 (6), DEC.	240 ± 21 (6), N.S.	216 ± 14 (2), N.S.
11	241 ± 32 (6)	193 ± 18 (4), DEC.	248 ± 24 (6), N.S.	--
12	241 ± 30 (6)	183 ± 25 (4), DEC.	248 ± 24 (6), N.S.	--
13	244 ± 32 (6)	182 ± 21 (4), DEC.	250 ± 27 (6), N.S.	--
14	255 ± 33 (6)	182 ± 22 (4), DEC.	258 ± 27 (6), N.S.	--

Table 5 (Continued)

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
15	260 ± 37 (6)	184 ± 20 (4), DEC.	265 ± 29 (6), N.S.	--
16	256 ± 36 (6)	175 ± 22 (4), DEC.	267 ± 32 (6), N.S.	--
17	257 ± 38 (6)	168 ± 15 (3), DEC.	268 ± 34 (6), N.S.	--
18	272 ± 37 (6)	179 ± 26 (3), DEC.	274 ± 36 (6), N.S.	--
19	270 ± 37 (6)	169 ± 25 (3), DEC.	276 ± 35 (6), N.S.	--
20	261 ± 34 (6)	171 ± 31 (2), DEC.	274 ± 33 (6), N.S.	--
21	266 ± 18 (5)	167 ± 38 (2), DEC.	279 ± 37 (6), N.S.	--
22	270 ± 26 (5)	--	282 ± 35 (6), N.S.	--
23	262 ± 25 (4)	--	285 ± 38 (6), N.S.	--
24	274 ± 36 (4)	--	286 ± 36 (6), N.S.	--
25	271 ± 34 (4)	--	290 ± 40 (6), N.S.	--
26	278 ± 35 (4)	--	302 ± 39 (6), N.S.	--

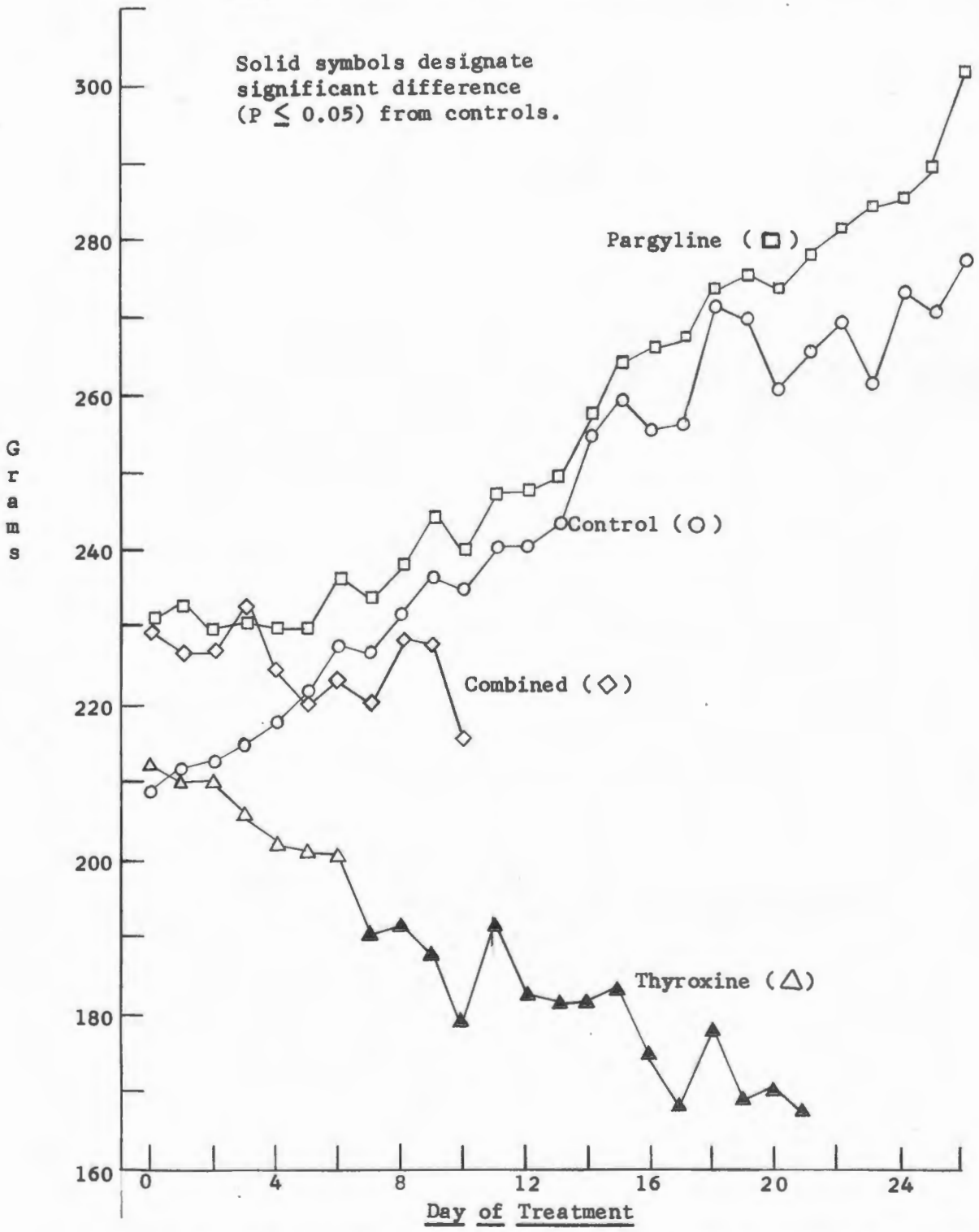
a: mean, ± S.D., daily weights.
b: number of rats.
c: at $P \leq 0.05$:

N.S. = not significantly different from vehicle-treated group.
DEC. = significantly decreased from vehicle-treated group.

Drug Doses, Daily, From Day 0: Na Thyroxine, 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-21. Pargyline HCl, 25 mg/kg. Combined, thyroxine as above + pargyline as above. Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

These animals form the principal group of phase 1 (see Page 28, Summary of Experimental Design).

FIG. 4. THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS. I. MEAN DAILY WEIGHTS.



Doses (i.p.) daily from day 0: Na Thyroxine, 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-21. Pargyline HCl, 25 mg/kg. Combined, Na thyroxine and pargyline HCl as above. Control, glass-distilled water, 1 ml/kg.

TABLE 6

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS: II. CUMULATIVE DAILY WEIGHT GAINS FROM DAY 0

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
1	3 ± 6[a] (6)[b]	-2 ± 7 (6), N.S.[c]	3 ± 7 (6), N.S.	-4 ± 5 (6), DEC.[c]
2	4 ± 2 (6)	-3 ± 6 (6), DEC.	0 ± 6 (6), N.S.	-4 ± 6 (6), DEC.
3	6 ± 5 (6)	-7 ± 5 (6), DEC.	1 ± 6 (6), DEC.	-3 ± 19 (5), N.S.
4	9 ± 3 (6)	-11 ± 5 (6), DEC.	1 ± 6 (6), DEC.	-3 ± 19 (5), DEC.
5	13 ± 5 (6)	-12 ± 3 (6), DEC.	0 ± 5 (6), DEC.	-16 ± 17 (5), DEC.
6	20 ± 7 (6)	-12 ± 4 (6), DEC.	7 ± 8 (6), DEC.	-12 ± 19 (5), DEC.
7	18 ± 7 (6)	-23 ± 7 (6), DEC.	4 ± 5 (6), DEC.	-13 ± 15 (4), DEC.
8	23 ± 11 (6)	-21 ± 7 (6), DEC.	9 ± 7 (6), DEC.	-7 ± 9 (2), DEC.
9	28 ± 11 (6)	-25 ± 13 (6), DEC.	14 ± 5 (6), DEC.	-7 ± 7 (2), DEC.
10	27 ± 6 (6)	-34 ± 15 (6), DEC.	9 ± 6 (6), DEC.	-19 ± 7 (2), DEC.
11	32 ± 12 (6)	-18 ± 4 (4), DEC.	18 ± 10 (6), DEC.	--
12	32 ± 11 (6)	-29 ± 5 (4), DEC.	18 ± 7 (6), DEC.	--
13	35 ± 12 (6)	-30 ± 6 (4), DEC.	20 ± 11 (6), DEC.	--
14	46 ± 14 (6)	-30 ± 8 (4), DEC.	28 ± 11 (6), DEC.	--
15	51 ± 17 (6)	-27 ± 9 (4), DEC.	34 ± 14 (6), N.S.	--

Table 6 (Continued)

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
16	47 ± 16 (6)	-37 ± 11 (4), DEC.	37 ± 16 (6), N.S.	--
17	47 ± 19 (6)	-51 ± 8 (3), DEC.	37 ± 18 (6), N.S.	--
18	63 ± 18 (6)	-40 ± 8 (3), DEC.	44 ± 21 (6), N.S.	--
19	61 ± 17 (6)	-49 ± 8 (3), DEC.	46 ± 19 (6), N.S.	--
20	52 ± 16 (6)	-53 ± 7 (2), DEC.	44 ± 17 (6), N.S.	--
21	64 ± 18 (5)	-58 ± 13 (2), DEC.	49 ± 21 (6), N.S.	--
22	68 ± 24 (5)	--	51 ± 19 (6), N.S.	--
23	65 ± 17 (4)	--	55 ± 23 (6), N.S.	--
24	77 ± 27 (4)	--	56 ± 20 (6), N.S.	--
25	74 ± 24 (4)	--	60 ± 24 (6), N.S.	--
26	81 ± 26 (4)	--	72 ± 23 (6), N.S.	--

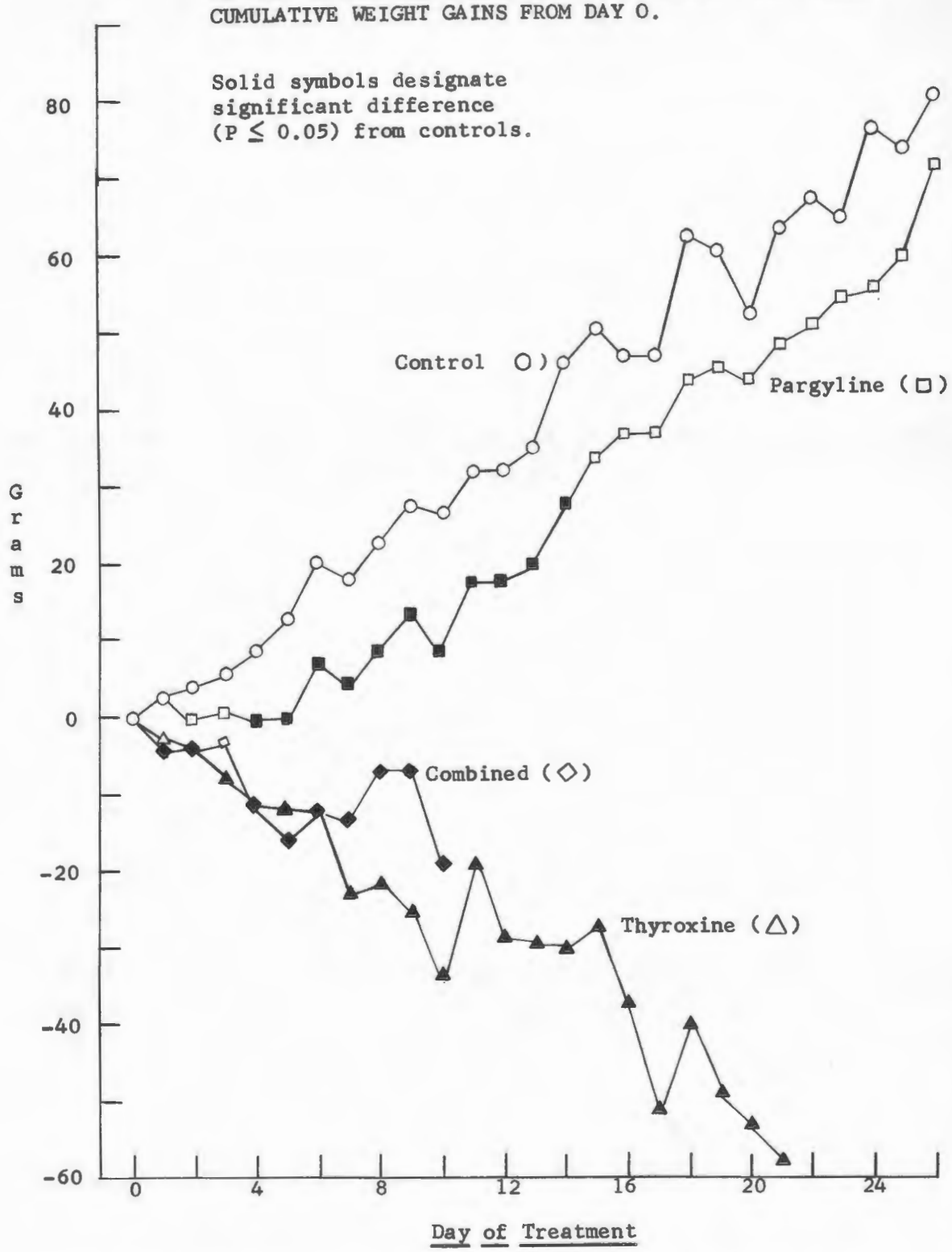
a: mean, ± S.D., cumulative weight gains from day 0.
b: number of rats.
c: at $P \leq 0.05$:

N.S. = not significantly different from vehicle-treated group.
DEC. = significantly decreased from vehicle-treated group.

Drug Doses, Daily, From Day 0: Na Thyroxine, 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-21. Pargyline HCl, 25 mg/kg. Combined, thyroxine as above + pargyline as above. Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

These animals form the principal group of phase 1 (see Page 28, Summary of Experimental Design).

FIG. 5. THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS. II. MEAN CUMULATIVE WEIGHT GAINS FROM DAY 0.



Doses (i.p.) daily from day 0: Na Thyroxine, 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-21. Pargyline HCl, 25 mg/kg. Combined, Na thyroxine and pargyline HCl as above. Control, glass-distilled water, 1 ml/kg.

TABLE 7

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS: III. DAILY WEIGHT GAINS

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
1	3 ± 3[a] (6)[b]	-2 ± 7 (6), N.S.[c]	3 ± 3 (6), N.S.	-4 ± 5 (6), DEC.
2	1 ± 4 (6)	-1 ± 5 (6), N.S.	-3 ± 5 (6), N.S.	0 ± 4 (6), N.S.
3	2 ± 5 (6)	-4 ± 6 (6), N.S.	1 ± 2 (6), N.S.	2 ± 13 (5), N.S.
4	3 ± 6 (6)	-4 ± 3 (6), DEC.	-1 ± 4 (6), N.S.	-9 ± 9 (5), N.S.
5	4 ± 4 (6)	-1 ± 3 (6), DEC.	0 ± 5 (6), N.S.	-5 ± 5 (5), DEC.
6	6 ± 5 (6)	0 ± 2 (6), DEC.	7 ± 4 (6), N.S.	4 ± 6 (5), N.S.
7	-1 ± 6 (6)	-10 ± 5 (6), DEC.	-3 ± 6 (6), N.S.	-9 ± 12 (4), N.S.
8	5 ± 5 (6)	2 ± 5 (6), N.S.	5 ± 3 (6), N.S.	-7 ± 5 (2), N.S.
9	5 ± 6 (6)	-5 ± 7 (6), DEC.	6 ± 5 (6), N.S.	-1 ± 2 (2), DEC.
10	-2 ± 3 (6)	-8 ± 4 (6), DEC.	-5 ± 3 (6), N.S.	-12 ± 0 (2), DEC.
11	5 ± 5 (6)	9 ± 12 (4), N.S.	8 ± 7 (6), N.S.	--
12	0 ± 3 (6)	-11 ± 6 (4), DEC.	1 ± 6 (6), N.S.	--
13	3 ± 4 (6)	-1 ± 1 (4), N.S.	2 ± 5 (6), N.S.	--
14	11 ± 5 (6)	-1 ± 3 (4), DEC.	8 ± 4 (6), N.S.	--
15	5 ± 5 (6)	3 ± 3 (4), N.S.	6 ± 5 (6), N.S.	--

Table 7 (Continued)

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
16	-4 ± 3 (6)	-9 ± 5 (4), N.S.	2 ± 3 (6), INC.	--
17	2 ± 4 (6)	-10 ± 12 (3), N.S.	1 ± 4 (6), N.S.	--
18	14 ± 2 (6)	11 ± 11 (3), N.S.	7 ± 3 (6), DEC.	--
19	-2 ± 3 (6)	-20 ± 2 (3), DEC.	2 ± 3 (6), N.S.	--
20	-9 ± 14 (6)	-8 ± 6 (2), N.S.	-2 ± 4 (6), N.S.	--
21	18 ± 21 (5)	-5 ± 6 (2), N.S.	5 ± 5 (6), N.S.	--
22	4 ± 15 (5)	--	3 ± 5 (6), N.S.	--
23	-11 ± 8 (4)	--	3 ± 6 (6), INC.	--
24	12 ± 14 (4)	--	1 ± 6 (6), N.S.	--
25	-3 ± 13 (4)	--	4 ± 5 (6), N.S.	--
26	7 ± 3 (4)	--	12 ± 3 (6), INC.	--

a: mean, ± S.D., daily weight gains.
b: number of rats.
c: at $p \leq 0.05$:

N.S. = not significantly different from vehicle-treated group.
INC. = significantly elevated over vehicle-treated group.
DEC. = significantly decreased from vehicle-treated group.

Drug Doses, Daily, From Day 0: Na Thyroxine, 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-21. Pargyline HCl, 25 mg/kg. Combined, thyroxine as above + pargyline as above. Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

These animals form the principal group of phase 1 (see Page 28, Summary of Experimental Design).

TABLE 8

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS: CUMULATIVE DAILY WEIGHT GAINS FROM DAY 0

DAY	VEHICLE	THYROXINE	PARGYLINE	COMBINED
1	5 ± 5[a] (26)[b]	-3 ± 10 (15), DEC.[c]	1 ± 7 (15), N.S.	-1 ± 5 (39), DEC.
2	11 ± 9 (15)	-13 ± 14 (15), DEC.	2 ± 12 (15), DEC.	-3 ± 10 (39), DEC.
3	9 ± 13 (25)	-5 ± 17 (12), DEC.	4 ± 15 (11), N.S.	-5 ± 9 (30), DEC.
4	17 ± 12 (11)	-1 ± 8 (10), DEC.	7 ± 8 (10), DEC.	-13 ± 13 (30), DEC.
5	13 ± 15 (20)	1 ± 10 (9), DEC.	13 ± 14 (10), N.S.	-12 ± 13 (20), DEC.
6	19 ± 11 (15)	4 ± 21 (7), N.S.	19 ± 9 (10), N.S.	-12 ± 16 (21), DEC.
7	29 ± 12 (13)	3 ± 21 (6), DEC.	23 ± 8 (10), N.S.	-11 ± 9 (15), DEC.
8	29 ± 9 (11)	-5 ± 22 (6), DEC.	25 ± 8 (10), N.S.	-17 ± 11 (12), DEC.
9	32 ± 11 (12)	3 ± 22 (6), DEC.	35 ± 9 (10), N.S.	-14 ± 9 (6), DEC.
10	37 ± 15 (12)	1 ± 18 (6), DEC.	36 ± 7 (10), N.S.	-10 ± 9 (4), DEC.
11	44 ± 11 (12)	1 ± 20 (6), DEC.	43 ± 7 (10), N.S.	-15 ± 13 (4), DEC.

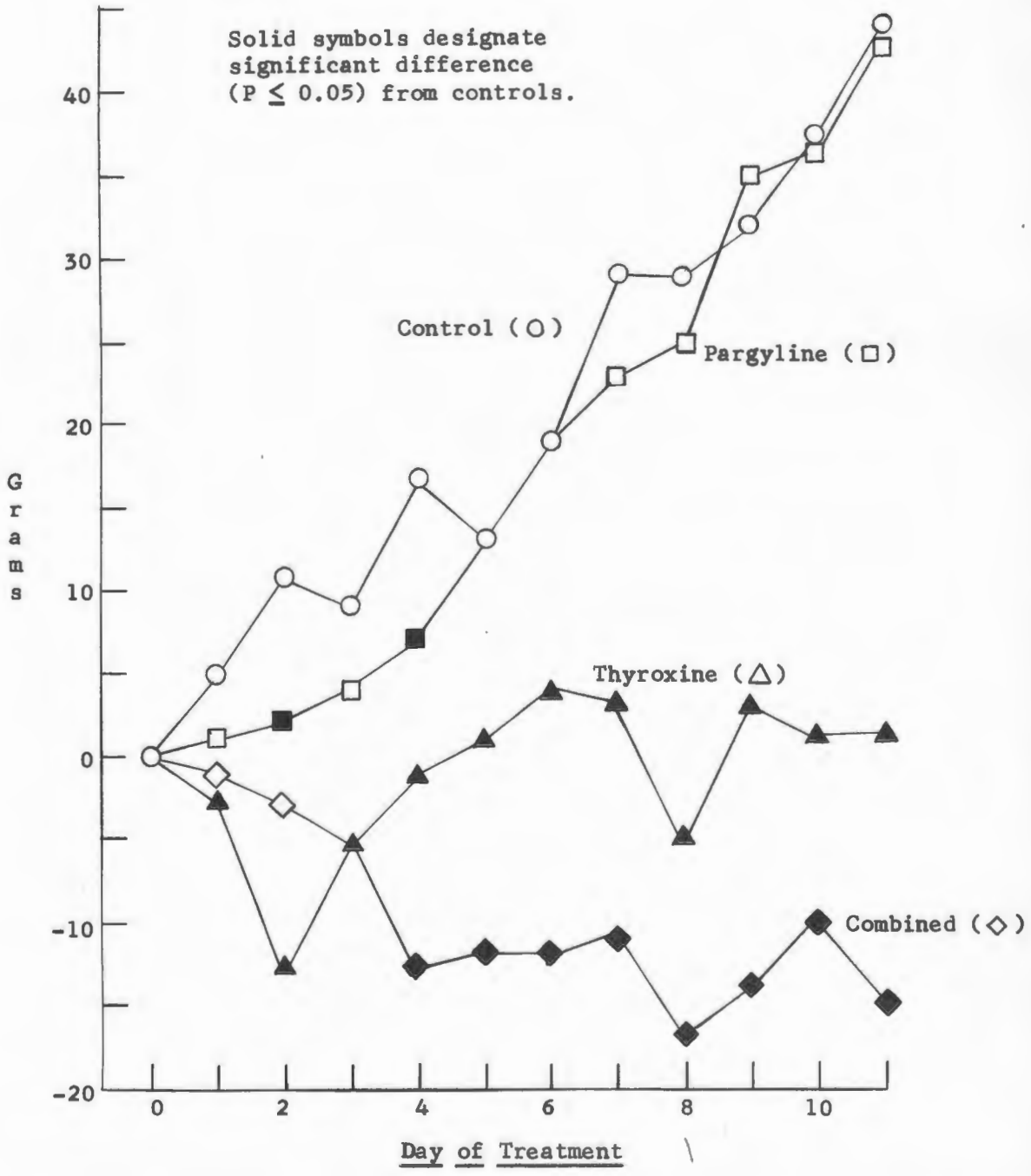
a: mean, ± S.D., cumulative weight gains from day 0.
b: number of rats.
c: at $P \leq 0.05$:

N.S. = not significantly different from vehicle-treated group.
DEC. = significantly decreased from vehicle-treated groups.

Drug Doses, Daily, From Day 0: Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-11. Pargyline HCl, 25 mg/kg. Combined, thyroxine as above + pargyline as above. Vehicle, glass-distilled water, 1 ml/kg. All injected i.p.

These animals form the principal group of phase 2 (see Page 28, Summary of Experimental Design).

FIG. 6. THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON THE WEIGHT GAINS OF MALE ALBINO RATS. MEAN CUMULATIVE WEIGHT GAINS FROM DAY 0.



Doses (i.p.) daily from day 0: Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-11. Pargyline HCl, 25 mg/kg. Combined, Na thyroxine and pargyline HCl as above. Control, glass-distilled water, 1 ml/kg.

TABLE 9

PARTIAL PURIFICATION OF MONOAMINE OXIDASE FROM BRAIN AND LIVER OF MALE ALBINO RATS

PREPARATION	MAO ACTIVITY[a], µmol kynuramine/hr/mg protein at 29°C	
	BRAIN	LIVER
Homogenate	0.39	1.78
Mitochondria	0.78	2.72
% Increase	200	150

a: Assayed as described in Methods section, Page 39.

TABLE 10

STABILITY STUDY: MONOAMINE OXIDASE ACTIVITY IN BRAIN AND LIVER OF MALE ALBINO RATS, MITOCHONDRIA STORED AT -40°C

DAYS IN FREEZER at -40°C	MAO ACTIVITY[a], µmol kynuramine/hr/mg protein, at 30°C[b]	
	BRAIN	LIVER
0 (fresh)	0.62	2.93
2	0.63	-
4	-	2.97
7	0.67	-
9	0.63	-
15	0.65	-
85	-	3.41
101	0.62	-

a: Mitochondria were assayed as described in Methods section, Page 39.
b: These assays were carried out at ambient room temperatures, 24.4-30°C. MAO activity was found to increase 0.0143 µmol kynuramine/hr/mg protein/°C in brain and 0.192 µmol kynuramine/hr/mg protein/°C in liver. Reported values are corrected for 30°C.

TABLE 11

TERMINAL BRAIN MONOAMINE OXIDASE ACTIVITY IN MALE ALBINO RATS

	RAT NUMBER	PROTEIN IN MITOCHONDRIAL SUSPENSION, mg/ml	PROTEIN IN MAO ASSAY CUVETTE, mg	TERMINAL MAO ACTIVITY, µmol kynuramine/ hr/mg protein
1.	DRUG TREATMENT: VEHICLE (glass-distilled water). 1 ml/kg, i.p., daily, days 0-11.			
	3-1-br	1.84	2.76	0.61
	3-3-br	1.73	2.60	0.69
	4-2-br	1.67	2.51	0.69
	4-3-br	1.47	2.21	0.84
2.	DRUG TREATMENT: VEHICLE. 1 ml/kg, i.p., daily, days 0-17.			
	S-2v2-br	0.96	1.44	1.36
	S-2v3-br	1.28	1.92	0.80
3.	DRUG TREATMENT: VEHICLE. 1 ml/kg, i.p., daily, days 0-27.			
	W-3-br	1.65	2.48	0.61
	W-4-br	1.29	1.94	0.95
	W-5-br	1.50	2.25	0.92
4.	DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-11. i.p. daily			
	18-1-br	1.43	2.15	0.84
	18-2-br	1.47	2.21	0.66
	18-3-br	1.43	2.15	0.65
5.	DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-28. i.p. daily			
	T-2-br	1.50	2.25	0.83
6.	DRUG TREATMENT: PARGYLINE HCl. 25 mg/kg, i.p., daily, days 0-11.			
	9-2-br	1.43	2.15	0
	10-1-br	1.79	2.69	0
	10-2-br	1.29	1.94	0
	10-3-br	0.73	1.10	0
	11-1a-br	0.84	1.26	0
	11-1b-br	2.61	3.92	0
	11-3-br	0.85	1.28	0
	12-1-br	1.50	2.25	0
	12-2-br	1.45	2.18	0
	12-3-br	1.28	1.92	0
	19-1-br	1.20	1.80	0
	19-2-br	1.39	2.09	0
	19-3-br	1.39	2.09	0

Table 11 (Continued)

RAT NUMBER	PROTEIN IN MITOCHONDRIAL SUSPENSION, mg/ml	PROTEIN IN MAO ASSAY CUVETTE, mg	TERMINAL MAO ACTIVITY, µmol kynuramine/ hr/mg protein
7. DRUG TREATMENT: PARGYLINE HCl. 25 mg/kg, i.p., daily, days 0-27.			
M-1-br	1.78	2.67	0
M-2-br	1.19	1.79	0
M-3-br	1.60	2.40	0
M-4-br	1.17	1.76	0
M-5-br	1.28	1.92	0
M-6-br	1.18	1.77	0
8. DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-11. + PARGYLINE HCl, 25 mg/kg, days 0-11, i.p. daily.			
20-1-br	1.77	2.66	0
20-3-br	1.39	2.09	0
20-4-br	2.13	3.22	0
9. DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-9. + PARGYLINE HCl, 25 mg/kg, days 0-9, i.p. daily.			
S-17c3-br	1.11	1.67	0
S-18c2-br	0.89	1.34	0
S-19c1-br	1.37	2.06	0
S-19c3-br	0.96	1.44	0

MAO assay described in Methods section, Page 39.

TABLE 12

TERMINAL LIVER MONOAMINE OXIDASE ACTIVITY IN MALE ALBINO RATS

	RAT NUMBER	PROTEIN IN MITOCHONDRIAL SUSPENSION, mg/ml	PROTEIN IN MAO ASSAY CUVETTE, mg	TERMINAL MAO ACTIVITY, µmol kynuramine/ hr/mg protein
1.	DRUG TREATMENT: VEHICLE (glass-distilled water). 1 ml/kg, i.p. daily, days 0-11.			
	3-1-L	14.15	1.42	3.33
	3-3-L	4.45	0.45	3.56
	4-2-L	9.90	0.99	1.54
	4-3-L	5.40	0.54	2.97
2.	DRUG TREATMENT: VEHICLE. 1 ml/kg, i.p. daily, days 0-17.			
	S-2v2-L	2.29	0.46	2.10
	S-2v3-L	1.87	0.19	4.06
3.	DRUG TREATMENT: VEHICLE. 1 ml/kg, i.p. daily, days 0-27.			
	W-2-L	1.53	0.15	3.29
	W-3-L	2.08	0.62	2.49
	W-4-L	2.65	1.33	2.43
4.	DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-11. i.p. daily			
	18-1-L	6.60	0.66	2.43
	18-2-L	3.35	0.34	2.54
	18-3-L	8.20	0.82	1.55
5.	DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-19; 0.5 mg/kg, days 20-28. i.p. daily			
	T-2-L	2.18	1.09	1.04
6.	DRUG TREATMENT: PARGYLINE HCl. 25 mg/kg, i.p. daily, days 0-11.			
	9-2-L	2.00	0.20	0
	10-1-L	1.36	0.14	0
	10-2-L	3.90	0.39	0
	10-3-L	7.45	0.75	0
	11-1a-L	10.00	0.10	0
	11-1b-L	6.85	0.69	0
	11-3-L	7.00	0.70	0
	12-1-L	5.50	0.55	0
	12-2-L	6.10	0.61	0
	12-3-L	7.45	0.75	0
	19-1-L	2.06	2.06	0
	19-2-L	1.65	0.17	0
	19-3-L	1.94	0.19	0

Table 12 (Continued)

RAT NUMBER	PROTEIN IN MITOCHONDRIAL SUSPENSION, mg/ml	PROTEIN IN MAO ASSAY CUVETTE, mg	TERMINAL MAO ACTIVITY, µmol kynuramine/ hr/mg protein
7. DRUG TREATMENT: PARGYLINE HCl. 25 mg/kg, i.p. daily, days 0-27.			
M-1-L	1.49	0.15	0
M-2-L	1.93	0.19	0
M-3-L	3.50	0.35	0
M-4-L	3.50	0.35	0
M-5-L	1.53	0.15	0
M-6-L	2.65	0.27	0
8. DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-11. + PARGYLINE HCl, 25 mg/kg, days 0-11, i.p. daily.			
20-1-L	2.65	0.27	0
20-3-L	3.95	0.40	0
20-4-L	1.85	0.19	0
9. DRUG TREATMENT: Na THYROXINE. 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-9. + PARGYLINE HCl, 25 mg/kg, days 0-9, i.p. daily.			
S-17c3-L	1.87	0.19	0
S-18c2-L	1.45	0.15	0
S-19c1-L	1.58	0.16	0
S-19c3-L	1.87	0.19	0

MAO assay described in Methods section, Page 39.

TABLE 13

SUMMARY OF TABLES 11 AND 12

TERMINAL MONOAMINE OXIDASE ACTIVITY IN MALE ALBINO RATS

TISSUE	DRUG TREATMENT GROUP				
	Group 1[c] Vehicle-treated	Groups 1,2,&3 All Vehicle-treated	Group 4 Thyroxine-treated	Groups 6&7 Pargyline-treated	Groups 8&9 Combined Thyroxine + Pargyline-treated
Brain					
N[a]	4	9	3	19	7
$\bar{X}$ ± S.D.[b]	0.71 ± 0.10[a]	0.83 ± 0.24	0.72 ± 0.11	0	0
Liver					
N	4	9	3	19	7
$\bar{X}$ ± S.D.	2.85 ± 0.91	2.86 ± 0.79	2.17 ± 0.77	0	0

a: N = number of rats.
b: Indicates mean ± S.D. of MAO activity in units of µmol kynuramine/hr/mg protein, at 30°C.
c: Groups are the drug-treatment groups of Tables 11 and 12.

STATISTICAL COMPARISONS

TISSUE	COMPARISON[d]	RESULT
Brain	Group 1, Vehicle, vs. Group 4, Thyroxine.	N.S.[d]
Brain	Groups 1,2,&3, All Vehicles, vs. Group 4, Thyroxine.	N.S.
Liver	Group 1, Vehicle, vs. Group 4, Thyroxine.	N.S.
Liver	Groups 1,2,&3, All Vehicles, vs. Group 4, Thyroxine.	N.S.

d: Comparisons were made by means of Student's "t" test (Snedecor, 1956). N.S. = no significant difference, $P \leq 0.05$.

TABLE 14

PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS

TYPICAL DATA GENERATED BY TWO UNTREATED GROUPS OF RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. INCREMENTAL COUNTS PER 30 MINUTES.

DATA FROM CAGE I LENGTH OF STUDY: 12 DAYS

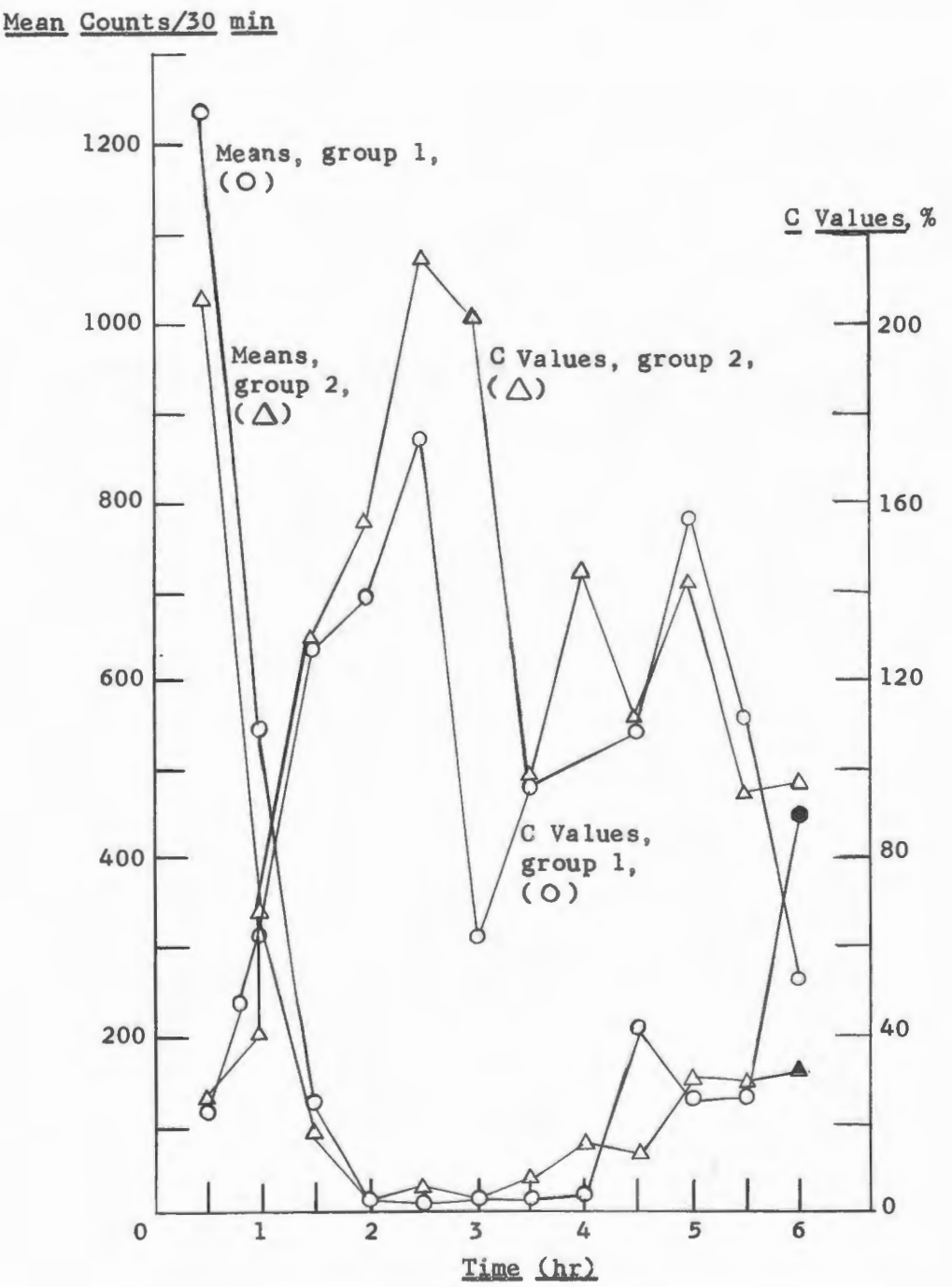
TIME (hr)	GROUP 1 (odd days)			GROUP 2 (even days)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
½	1241	296	24	1030	223	22	N.S.
1	553	346	63	336	137	41	N.S.
1½	125	161	128	91	121	133	N.S.
2	7	10	140	9	13	156	N.S.
2½	16	27	174	36	78	215	N.S.
3	14	9	62	20	40	202	N.S.
3½	9	9	101	45	43	96	N.S.
4	26	28	107	80	116	145	N.S.
4½	211	240	114	72	77	107	N.S.
5	127	180	142	156	245	157	N.S.
5½	130	182	111	144	171	94	N.S.
6	447	238	53	161	158	98	SIGN.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of counts generated during each of the 12 half-hour periods by group 1, three rats run in the cage on the odd days (days 1,3, 5,7,9,11) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by group 2, three rats run in the cage on the even days (days 2,4,6,8,10,12). Comparisons were made by means of Student's "t" test (Snedecor, 1956):

N.S. = not significant ($P \leq 0.05$).
SIGN. = significant difference, ($P \leq 0.05$).

FIG. 7. PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS. TYPICAL DATA GENERATED BY TWO UNTREATED GROUPS OF RATS. INCREMENTAL COUNTS PER 30 MINUTES FOR 6 HOURS.



Solid symbols designate significant difference ($P \leq 0.05$), group 1 vs. group 2.

TABLE 15

PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS

TYPICAL DATA GENERATED BY TWO UNTREATED GROUPS OF RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. TOTAL CUMULATIVE COUNTS PER HOUR.

DATA FROM CAGE I LENGTH OF STUDY: 12 DAYS

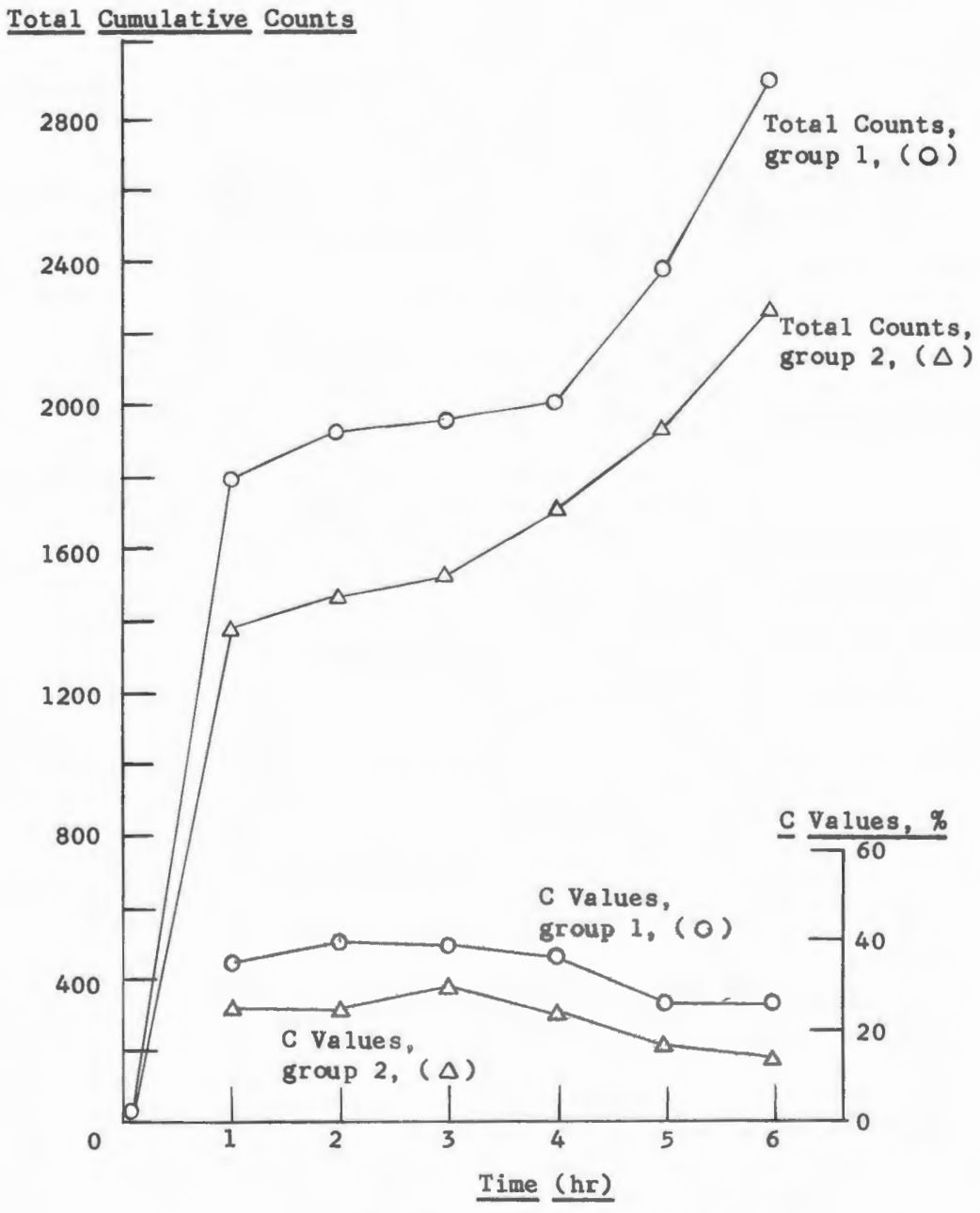
TIME (hr)	GROUP 1 (odd days)			GROUP 2 (even days)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs group 2
1	1794	630	35	1367	340	25	N.S.
2	1926	764	40	1466	350	24	N.S.
3	1956	764	39	1522	449	30	N.S.
4	1991	743	37	1647	374	23	N.S.
5	2329	599	26	1924	305	16	N.S.
6	2907	745	26	2267	302	13	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of total cumulative counts generated at each of the 6 hours by group 1, three rats run in the cage on the odd days (days 1, 3,5,7,9,11) was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by group 2, three rats run in the cage on the even days (days 2,4,6,8,10,12). Comparisons were made by means of Student's "t" test (Snedecor, 1956):

N.S. = not significant ($P \leq 0.05$).

FIG. 8. PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS. TYPICAL DATA GENERATED BY TWO UNTREATED GROUPS OF RATS. TOTAL CUMULATIVE COUNTS PER HOUR FOR 6 HOURS.



No significant difference ($P \leq 0.05$), group 1 vs. group 2.

FIG. 9. PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS. MEANS ± STANDARD DEVIATIONS OF ALL C VALUES IN 22-DAY STUDY.

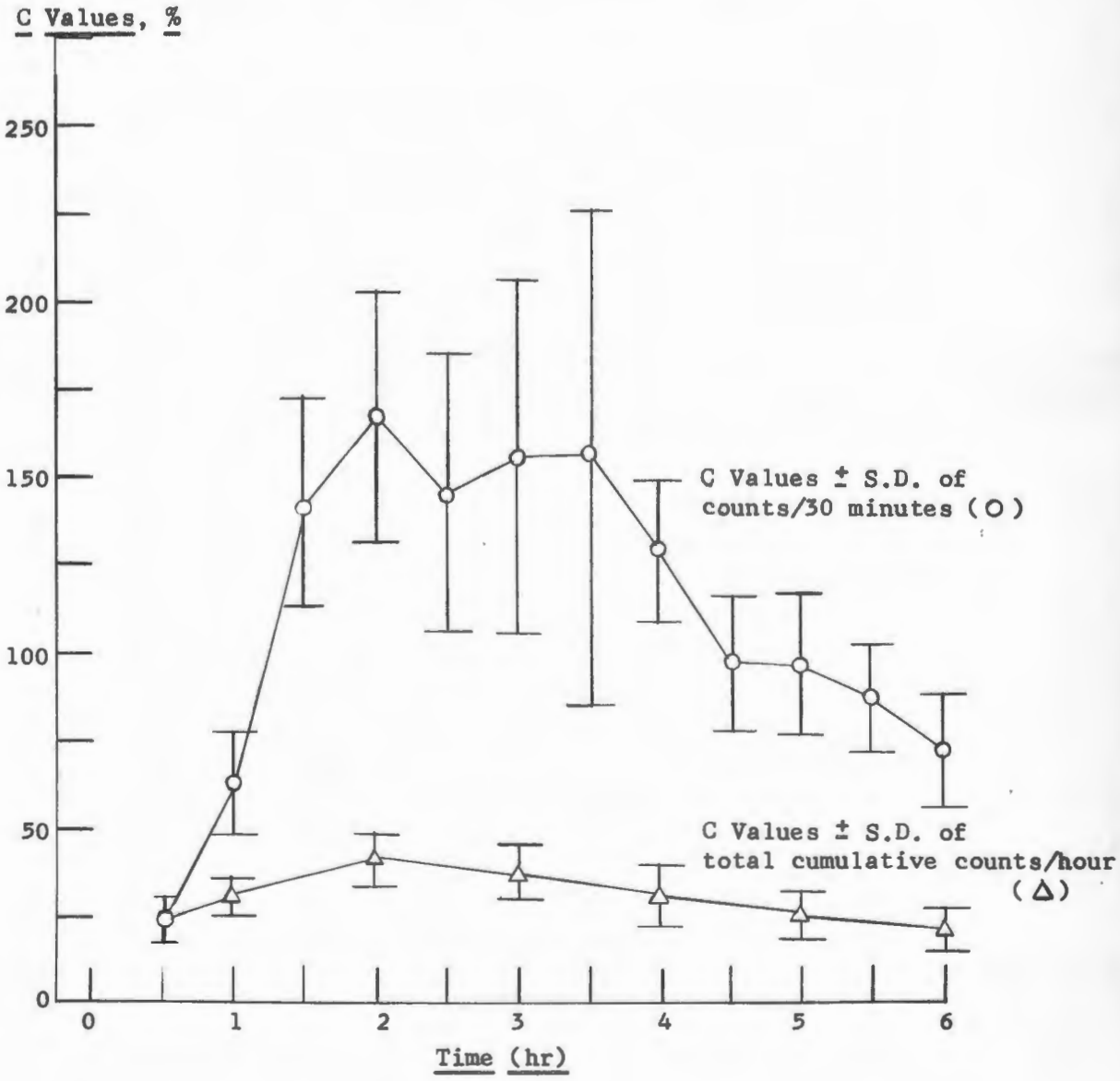


TABLE 16

PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS

INCIDENCES OF SIGNIFICANT DIFFERENCES BETWEEN TWO UNTREATED GROUPS OF RATS, USING INCREMENTAL COUNTS PER 30 MINUTES FOR 6 HOURS.

LENGTH OF STUDY: 22 DAYS

TIME (hr)	PORTION OF STUDY ANALYZED		
	DAYS 1-22 11 runs/group	DAYS 1-12 6 runs/group	DAYS 9-22 7 runs/group
½	I, III[a]	III	I,
1	I,	-	I, II, III
1½	-	-	-
2	-	-	-
2½	-	-	-
3	-	-	-
3½	-	-	-
4	-	-	-
4½	II	-	I, II
5	-	-	I
5½	-	-	-
6	I	I	I

a: Roman numerals indicate the actophotometer cage in which the significant change occurred. Four cages (I-IV) were used. In each cage the mean ± S.D. of counts generated during each of the 12 half-hour periods by one group of 3 rats run on the odd days (days 1,3,5,7,...) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by a similar group of 3 rats run in the same cage on the even days (days 2,4,6,8,...). Comparisons were made by means of Student's "t" test (Snedecor, 1956) and significant differences ($P \leq 0.05$) are noted above, by indicating the cage and the time-interval in which the change occurred.

TABLE 17

PRELIMINARY STUDY: ACTOPHOTOMETRIC METHOD FOR MEASURING GROSS ACTIVITY IN MALE ALBINO RATS

INCIDENCES OF SIGNIFICANT DIFFERENCES BETWEEN TWO UNTREATED GROUPS OF RATS, USING TOTAL CUMULATIVE COUNTS PER HOUR FOR 6 HOURS.

LENGTH OF STUDY: 22 DAYS

TIME (hr)	PORTION OF STUDY ANALYZED		
	DAYS 1-22 11 runs/group	DAYS 1-12 6 runs/group	DAYS 9-22 7 runs/group
1	I[a]	-	I
2	-	-	I
3	-	-	I
4	-	-	I, II
5	I, II	II	I, II
6	I, II	II	I, II

a: Roman numerals indicate the actophotometer cage in which the significant change occurred. Four cages (I-IV) were used. In each cage the mean ± S.D. of total cumulative counts generated at each of the 6 hours by one group of 3 rats run on the odd days (day 1,3,5,7,...) was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by a similar group of 3 rats run in the same cage on the even days (days 2,4,6,8,...). Comparisons were made by means of Student's "t" test (Snedecor, 1956) and significant differences ($P \leq 0.05$) are noted above, by indicating the cage and the hour in which the change occurred.

TABLE 18

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

A. SUMMARY OF DATA GENERATED BY TWO VEHICLE-TREATED GROUPS OF RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. INCREMENTAL COUNTS PER 30 MINUTES.

DATA FROM CAGE I LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (vehicle-treated)			GROUP 2 (odd days) (vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
½	881	123	14	1139	250	22	N.S.[a]
1	106	53	50	284	253	89	N.S.
1½	10	6	60	127	208	164	N.S.
2	3	2	67	4	5	25	N.S.
2½	6	4	67	4	4	100	N.S.
3	22	38	173	66	141	214	N.S.
3½	19	21	110	29	41	141	N.S.
4	44	48	109	6	8	133	N.S.
4½	9	9	100	6	6	100	N.S.
5	9	8	89	43	69	160	N.S.
5½	23	30	130	5	5	100	N.S.
6	107	192	179	75	92	123	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of counts generated during each of the 12 half-hour periods by group 1, three rats injected daily with vehicle and run in the cage on the even days (days 0,2,4,6,8,10) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by group 2, a similar group of 3 rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: N.S. indicates that for the given time-interval of 30 minutes there was no difference in the activity of the two groups of rats ($P \leq 0.05$).

Dose: Vehicle (glass-distilled water), 1 ml/kg daily, i.p.

TABLE 19

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

B. SUMMARY OF DATA GENERATED BY A GROUP OF VEHICLE-TREATED RATS AND BY A GROUP OF THYROXINE-TREATED RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. INCREMENTAL COUNTS PER 30 MINUTES.

DATA FROM CAGE II LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Thyroxine-treated)			GROUP 2 (odd days) (Vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
½	707	211	30	1558	493	32	DEC.[a]
1	134	61	46	623	455	73	N.S.
1½	42	37	88	181	158	88	N.S.
2	60	45	75	30	29	97	INC.
2½	77	34	44	10	10	100	INC.
3	64	52	81	85	161	189	N.S.
3½	73	37	51	41	48	117	N.S.
4	61	43	70	46	35	76	N.S.
4½	99	89	90	121	149	123	N.S.
5	132	133	101	20	16	80	N.S.
5½	84	71	85	45	59	131	N.S.
6	105	89	85	282	262	93	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of counts generated during each of the 12 half-hour periods by group 1, three rats injected daily with thyroxine and run in the cage on the even days (days 0,2,4,6,8,10) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by group 2, a similar group of 3 rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: For the given time-interval of 30 minutes the activity of the thyroxine-treated rats relative to the vehicle-treated rats was DEC. (decreased), N.S. (not significantly different), or INC. (increased). ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Daily, i.p.

TABLE 20

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

C. SUMMARY OF DATA GENERATED BY A GROUP OF VEHICLE-TREATED RATS AND BY A GROUP OF PARGYLINE-TREATED RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. INCREMENTAL COUNTS PER 30 MINUTES.

DATA FROM CAGE III. LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Pargyline-treated)			GROUP 2 (odd days) (Vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
½	660	395	60	1705	600	35	DEC.[a]
1	290	225	78	651	408	63	N.S.
1½	152	87	57	297	347	117	N.S.
2	208	133	64	39	35	90	INC.
2½	346	423	122	58	46	79	N.S.
3	218	276	127	166	242	146	N.S.
3½	134	173	129	73	57	78	N.S.
4	81	111	137	64	47	73	N.S.
4½	267	403	151	177	235	133	N.S.
5	321	491	153	126	148	117	N.S.
5½	191	234	123	28	21	75	N.S.
6	639	730	114	153	123	80	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./ $\bar{X}$ x 100%.

The mean ± S.D. of counts generated during each of the 12 half-hour periods by group 1, three rats injected daily with pargyline and run in the cage on the even days (days 0,2,4,6,8,10) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by group 1, a similar group of 3 rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: For the given time-interval of 30 minutes the activity of the thyroxine-treated rats relative to the vehicle-treated rats was DEC. (decreased, N.S. (not significantly different) or INC. (increased). ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Pargyline HCl, 25 mg/kg. Daily, i.p.

TABLE 21

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

D. SUMMARY OF DATA GENERATED BY A GROUP OF VEHICLE-TREATED RATS AND BY A GROUP OF RATS TREATED WITH PARGYLINE AND THYROXINE, RUN IN THE ACTO-PHOTOMETER FOR 6 HOURS. INCREMENTAL COUNTS PER 30 MINUTES.

DATA FROM CAGE IV LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Pargyline + Thyroxine-treated)			GROUP 2 (odd days) (Vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
½	626	251	40	1105	329	30	DEC.[a]
1	212	46	22	342	374	109	N.S.
1½	216	169	78	83	132	159	N.S.
2	219	150	68	29	38	131	INC.
2½	171	135	79	27	33	122	INC.
3	186	174	94	17	8	47	N.S.
3½	213	238	111	35	46	131	N.S.
4	140	125	89	33	28	85	N.S.
4½	233	295	127	96	171	178	N.S.
5	192	250	130	40	39	98	N.S.
5½	105	63	60	77	99	129	N.S.
6	218	136	62	158	242	153	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./ $\bar{X}$ x 100%.

The mean ± S.D. of counts generated during each of the 12 half-hour periods by group 1, three rats injected daily with pargyline and thyroxine and run in the cage on the even days (days 0,2,4,6,8,10) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by group 2, a similar group of 3 rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: For the given time-interval of 30 minutes the activity of the rats given pargyline and thyroxine, relative to the activity of the vehicle-treated rats, was DEC. (decreased), N.S. (not significantly different) or INC. (increased). ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Combined-treatment: Pargyline HCl, 25 mg/kg; Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Daily, i.p.

TABLE 22

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

E. SUMMARY OF DATA GENERATED BY TWO VEHICLE-TREATED GROUPS OF RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. TOTAL CUMULATIVE COUNTS PER HOUR.

DATA FROM CAGE I LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Vehicle-treated)			GROUP 2 (odd days) (Vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
1	1423	484	34	987	133	14	N.S.[a]
2	1554	558	36	1000	135	14	N.S.
3	1625	570	35	1028	167	16	N.S.
4	1659	595	36	1091	204	19	N.S.
5	1709	618	36	1109	208	19	N.S.
6	1769	570	32	1239	264	21	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of total cumulative counts generated at each of the 6 hours by group 1, three rats injected daily with vehicle and run in the cage on the even days (days 0,2,4,6,8,10) was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by group 2, a similar group of three rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: N.S. indicates that for the given interval of total lapsed time there was no difference in the activity of the two groups of rats. (≤ 0.05).

Dose: Vehicle (glass-distilled water), 1 ml/kg. Daily, i.p.

TABLE 23

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

F. SUMMARY OF DATA GENERATED BY A GROUP OF VEHICLE-TREATED RATS AND BY A GROUP OF THYROXINE-TREATED RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. TOTAL CUMULATIVE COUNTS PER HOUR.

DATA FROM CAGE II LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Thyroxine-treated)			GROUP 2 (odd days) (Vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
1	842	257	31	2181	939	43	DEC.[a]
2	944	260	28	2391	967	40	DEC.
3	1084	242	22	2387	1095	44	DEC.
4	1217	274	22	2574	1146	42	DEC.
5	1448	275	19	2715	1094	40	N.S.
6	1637	345	21	3042	1064	35	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of total cumulative counts generated at each of the 6 hours by group 1, three rats injected daily with thyroxine and run in the cage on the even days (days 0,2,4,6,8,10) was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by group 2, a similar group of three rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: For the given total lapsed time-interval the activity of the thyroxine-treated rats relative to the vehicle-treated rats was DEC. (decreased) or N.S. (not significantly different). ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Daily, i.p.

TABLE 24

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

G. SUMMARY OF DATA GENERATED BY A GROUP OF VEHICLE-TREATED RATS AND BY A GROUP OF PARGYLINE-TREATED RATS RUN IN THE ACTOPHOTOMETER FOR 6 HOURS. TOTAL CUMULATIVE COUNTS PER HOUR.

DATA FROM CAGE III LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Pargyline-treated)			GROUP 2 (odd days) (Vehicle treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
1	951	605	64	2355	964	41	DEC.[a]
2	1310	640	49	2692	1291	48	N.S.
3	1874	650	35	2916	1397	39	N.S.
4	2090	717	34	3053	1397	46	N.S.
5	2677	1212	45	3356	1682	50	N.S.
6	3507	1134	32	3536	1736	49	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of total cumulative counts generated at each of the 6 hours by group 1, three rats injected daily with pargyline and run in the cage on the even days (days 0,2,4,6,8,10), was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by group 2, a similar group of three rats injected daily with vehicle and run on the odd days (days 1,3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: For the given total lapsed time-interval the activity of the pargyline-treated rats relative to the vehicle-treated rats was DEC. (decreased) or N.S. (not significantly different). ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Pargyline HCl, 25 mg/kg, Daily, i.p.

TABLE 25

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

H. SUMMARY OF DATA GENERATED BY A GROUP OF VEHICLE-TREATED RATS AND BY A GROUP OF RATS TREATED WITH PARGYLINE AND THYROXINE, RUN IN THE ACTO-PHOTOMETER FOR 6 HOURS. TOTAL CUMULATIVE COUNTS PER HOUR.

DATA FROM CAGE IV LENGTH OF STUDY: 11 DAYS

TIME (hr)	GROUP 1 (even days) (Pargyline + Thyroxine-treated)			GROUP 2 (odd days) (Vehicle-treated)			COMPARISON
	$\bar{X}$	S.D.	C(%)	$\bar{X}$	S.D.	C(%)	group 1 vs. group 2
1	838	270	32	1448	667	46	N.S.[a]
2	1274	380	30	1560	768	49	N.S.
3	1631	636	39	1604	761	48	N.S.
4	1984	962	49	1672	727	42	N.S.
5	2409	1478	61	1808	715	40	N.S.
6	2732	1617	59	2044	686	34	N.S.

$\bar{X}$ = mean.
S.D. = standard deviation.
C = coefficient of variation, S.D./$\bar{X}$ x 100%.

The mean ± S.D. of total cumulative counts generated at each of the 6 hours by group 1, three rats injected daily with pargyline and thyroxine and run in the cage on the even days (days 0,2,4,6,8,10), was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by group 2, a similar group of three rats injected daily with vehicle and run on the odd days (days 1, 3,5,7,9). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

a: For the given total lapsed time-interval the activity of the rats given pargyline and thyroxine was N.S. (not significantly different) from the activity of the vehicle-treated rats. ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Combined-treatment: Pargyline HCl, 25 mg/kg; Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Daily, i.p.

TABLE 26

SUMMARY

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

INCIDENCES OF SIGNIFICANT DIFFERENCES BETWEEN DRUG-TREATED AND VEHICLE-TREATED GROUPS OF RATS, USING INCREMENTAL COUNTS PER 30 MINUTES FOR 6 HOURS.

LENGTH OF STUDY: 11 DAYS

TIME (hr)	I.[a] Vehicle vs. Vehicle	II. Vehicle vs. Thyroxine	III. Vehicle vs. Pargyline	IV. Vehicle vs. Thyroxine + Pargyline
½	-	DEC.[b]	DEC.	DEC.
1	-	-	-	-
1½	-	-	-	-
2	-	-	INC.[b]	INC.
2½	-	INC.	-	INC.
3	-	-	-	-
3½	-	-	-	-
4	-	-	-	-
4½	-	-	-	-
5	-	-	-	-
5½	-	-	-	-
6	-	-	-	-

a: Roman numerals indicate the actophotometer cage. Four cages (I-IV) were used. In each cage the mean ± S.D. of counts generated during each of the 12 half-hour periods by a group of three rats treated daily with vehicle and run on the odd days (days 1,3,5,7,9) was computed. Each of the 12 means ± S.D. was compared to the mean ± S.D. of counts generated during the same half-hour interval by a similar group of three rats, treated daily with vehicle (I), thyroxine (II), pargyline (III) and thyroxine + pargyline (IV), and run on the even days (days 0,2,4,6,8,10). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

b: DEC. or INC. indicate that for the given time-interval of 30 minutes the drug-treated rats were less active or more active, respectively, than the vehicle-treated rats in the same cage at the same time-interval. ($P \leq 0.05$).

Dose: Vehicle (glass-distilled water), 1 ml/kg. Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Pargyline HCl, 25 mg/kg. Daily, i.p.

TABLE 27

SUMMARY

THE EFFECTS OF CHRONIC THYROXINE AND PARGYLINE TREATMENT ON LOCOMOTOR ACTIVITY IN MALE ALBINO RATS

INCIDENCES OF SIGNIFICANT DIFFERENCES BETWEEN DRUG-TREATED AND VEHICLE-TREATED GROUPS OF RATS, USING TOTAL CUMULATIVE COUNTS PER HOUR FOR 6 HOURS.

LENGTH OF STUDY: 11 DAYS

TIME (hr)	I.[a] Vehicle vs. Vehicle	II. Vehicle vs. Thyroxine	III. Vehicle vs. Pargyline	IV. Vehicle vs. Thyroxine + Pargyline
1	-	DEC.[b]	DEC.	-
2	-	DEC.	-	-
3	-	DEC.	-	-
4	-	DEC.	-	-
5	-	-	-	-
6	-	-	-	-

a: Roman numerals indicate the actophotometer cage. Four cages (I-IV) were used. In each cage the mean ± S.D. of total cumulative counts generated at each of the 6 hours by a group of three rats treated daily with vehicle and run on the odd days (days 1,3,5,7,9) was computed. Each of the 6 means ± S.D. was compared to the mean ± S.D. of counts generated at the same hour by a similar group of three rats, treated daily with vehicle (I), thyroxine (II), pargyline (III), and thyroxine + pargyline (IV), and run on the even days (days 0,2,4,6,8,10). Comparisons were made by means of Student's "t" test (Snedecor, 1956).

b: DEC. indicates that for the given total lapsed time the drug-treated rats were less active than the vehicle-treated rats in the same cage at the same total lapsed time. ($P \leq 0.05$).

Doses: Vehicle (glass-distilled water), 1 ml/kg. Na Thyroxine, 1 mg/kg, days 0-4; 0.5 mg/kg, days 5-10. Pargyline HCl, 25 mg/kg. Daily, i.p.

V. DISCUSSION

A. Blood Pressure Studies

The systolic blood pressures of the thyroxine-treated rats was significantly increased over the control group on day 2 of drug treatment (Table 1 and Figure 1). Determinations were again made on days 7, 10, 12, 16 and 19, and during this period the mean systolic pressure ± S.D. of the thyroxine-treated rats increased steadily from 131 ± 3 mm Hg on day 7 to 184 ± 2 mm Hg on day 19. During the same period mean systolic pressures in the control group ranged from 113 ± 7 to 121 ± 7 mm Hg. Rats receiving pargyline exhibited no significant changes from the control group on day 2. On day 7, however, systolic pressure was significantly decreased in the pargyline-treated rats, and remained lowered for the rest of the study. The range was 83 ± 10 to 94 ± 13 mm Hg during days 7 to 19; from Figure 1 it can be seen that once the hypotension was established it remained constant within a range of 11 mm Hg. The range of the controls in this period was 8 mm Hg.

Rats receiving both thyroxine and pargyline showed no significant change in systolic blood pressure on day 2 of drug treatment. In a second study (Table 2), it was shown that no significant change in a similarly-treated group of rats had occurred by day 3 of treatment. Mortality in rats receiving both thyroxine and pargyline prevented, in both studies, the determination of blood pressures beyond days 2 or 3, respectively. This degree of mortality was not observed, however, in other rats receiving both drugs but not subjected to the blood pressure determination procedure. It is postulated, then, that in some way the blood pressure procedure was stressful enough to cause relatively early death in these animals but not in animals receiving thyroxine alone or pargyline alone. It is possible that the incubation at 40°C for 20 minutes precipitated the mortality. Hyperthyroid animals are

characteristically intolerant to heat (Morgans, 1964) but if this factor alone were causing the deaths then mortality in the thyroxine-treated rats would have also been high. This, however, did not occur.

Qualitative observations noted by the investigator in the course of this study appear to be of interest. No quantitative measurements were made of heart rate or of diastolic pressure. Nevertheless, in the thyroxine-treated group the hypertension was accompanied by a very obviously increased heart rate and greatly increased excursions between the systolic peak and the diastolic trough, as seen on the oscilloscope screen. An increase in the difference between systolic and diastolic pressures generally indicates increased stroke volume in the heart. If at the same time the heart rate is increased a net increase in cardiac output might be postulated. These two phenomena were repeatedly observed on each day that determinations were made. Furthermore, the same effects were seen in rats receiving both thyroxine and pargyline, but in these animals there was no elevation of systolic pressure. Finally, rats receiving pargyline alone appeared to exhibit slightly reduced heart rate and definite decreases in the excursions between systolic peaks and diastolic troughs. This group of rats exhibited hypotension during the times when these effects were noted.

In view of these observations it is interesting to note that Brest et al. (1964) found no change in the cardiac output of hypertensive patients treated with pargyline, and Sutnick et al. (1964) found no change in the pulse rates of hypertensive patients in whom pargyline administration produced a marked hypotensive response. Smith (1965) showed, however, that pretreatment with pargyline (100 mg/kg) 12 hours before sacrifice reduced the heart rate of isolated guinea pig atria.

It is the opinion of the investigator that thyroxine produced an increase in heart rate and cardiac output as well as the measured increase in

systolic pressure. This view is in accord with the hemodynamic effects seen in hyperthyroid conditions. These effects have been discussed above (Wurtman et al., 1964; Danowski et al., 1964; Barker et al., 1965; Bray, 1964).

Pargyline definitely prevented the hypertension induced by thyroxine. In rats receiving both drugs no increases over control values of blood pressure were seen. The observation that pargyline appears not to block the tachycardia and increased systolic-diastolic excursions in rats receiving both drugs favors the view that the hypotensive effect of MAO inhibitors is mediated by a decreased peripheral resistance (Kopin et al., 1964; Goldberg, 1964). But inasmuch as the rats receiving pargyline alone did appear to exhibit reduced heart rate and systolic-diastolic excursions, it cannot be stated categorically that pargyline had no effect at all on the heart.

It is hoped that no confusion will result from the inclusion of qualitative observations made in this work. Speculation based on these observations is intended only to provide a further basis for interpreting the measured hemodynamic effects obtained in this study.

B. Urinary Sodium and Potassium Studies

It can be seen from Tables 3 and 4 and Figures 2 and 3 that thyroxine treatment in rats was accompanied by significantly increased ($P \leq 0.05$) urinary output of potassium and sodium beginning on day 6 of treatment. The failure of sodium output to be significantly increased on day 8 of the study is probably unimportant in view of significant increases on days 6, 7 and 10. These results are in accord with the report of Williams and Bakke (1962) that both sodium and potassium excretion are increased in hyperthyroidism.

Treatment with pargyline reduced the mean values of urinary potassium and sodium when compared to the controls, but except for a decreased potassium

output on day 8 these lowered values were not statistically significant. It is very interesting, however, that rats receiving both thyroxine and pargyline did not exhibit a statistically significant increase in urinary sodium and potassium excretion. There was only one exception: sodium excretion on day 6 was significantly increased. Thus in these animals sodium and potassium excretion was increased, but not significantly so, over the controls.

Pargyline has been reported to moderately reduce the glomerular filtration rate in humans (Brest et al., 1964; Onesti et al., 1964). If this is also happening in rats receiving pargyline, less sodium and potassium would be expected to enter the kidney tubules and, presumably, less might be excreted. The data obtained from rats receiving both drugs might prompt one to speculate that by effecting a reduction in glomerular filtration rate, pargyline reversed the outflow of sodium and potassium caused by thyroxine. The slight, although insignificant, reduction in excretion of sodium and potassium in animals receiving pargyline alone lends support to this view. It must be emphasized, however, that these data in no way suggest that pargyline is causing a retention of sodium and potassium. Animals receiving pargyline exhibited sodium and potassium excretion patterns that although falling on one side or another of the control values were not different, statistically, from control values.

C. Weight Gain Studies

Many investigators have shown that animals treated with various forms of the thyroid hormones lose weight or do not gain weight at the same rate as untreated animals (Kekki, 1964; Evans et al., 1964; D'Angelo and Grodin, 1964; Moury et al., 1964; Logothetopoulos, 1963). In these and other studies the body weight status of the animals has been determined in various ways. Comparing gross weights of the treated animals to the gross weights of the untreated controls is valid only when starting weights of both groups do not differ (Jackson, 1962). Weight is sometimes expressed as per cent of animal

weight on the first day of drug treatment, or by subtracting beginning weight from final weight at the end of the study.

Because weight loss is the simplest indication of hyperthyroidism it was decided early in this work to use the weight parameter as a determinant of hyperthyroidism in the rats treated with thyroxine. The daily weights of the 24 animals in the blood pressure study[1] were accordingly used as a starting point for weight analysis.

There was no significant difference, on day 0 of the study, between the weights of the control group and each of the 3 experimental groups. Accordingly, the total weights of the control group were compared to the total weights of each experimental group on each day of the study. (Table 5 and Figure 4). It was found that a significant difference between the control group and the thyroxine-treated group occurred on day 7 of the study, and persisted until the end of the experiment. The animals receiving pargyline showed no change in total weight from the total weight of the controls throughout the study. Finally the total weights of the animals receiving both thyroxine and pargyline were not different from the controls on any day of the study.

The thyroxine-treated animals had exhibited significant hypertension on day 2 of the study. It seemed likely, then, that these animals were in fact hyperthyroid; yet the decrease in weight was not seen until day 7. Thus, it seemed to the investigator that the use of total weights for an indication of weight status in the animals might not be a particularly sensitive method.

Accordingly the cumulative weight gain from day 0 was calculated for each animals used in the study. This was done simply by subtracting the weight

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1. See Summary of Experimental Design, Page 28.

of the animal on day 0 from its weight on each successive day. In this way a record of weight gain from beginning of treatment was obtained for each animal. The means ± standard deviations of these values were determined and the cumulative weight gains of the control group were compared to the gains of each of the 3 experimental groups, for each day of the study.

These data are summarized in Table 6 and Figure 5. It is obvious that beginning on day 2 and persisting for the remainder of the experiment, weight gains in the thyroxine-treated group were significantly lower than control values. Moreover, these animals exhibited negative weight gains: that is, they lost weight. After 19 days of treatment these rats were 49 ± 8 g lighter than their individual starting weights; the control rats on day 19 were 61 ± 17 g heavier than their individual starting weights.

The fact that weight loss and hypertension in thyroxine-treated rats both became significant on day 2 is probably not of outstanding importance. But one might expect these 2 phenomena to occur within a fairly short period of time.

Rats receiving both thyroxine and pargyline exhibited significant weight loss from day 1 to the end of the study, except for day 3, for which the comparison to the controls showed no significant change. This one value is negligible, however, in view of the overall weight pattern. This weight loss in the animals receiving both drugs was completely obscured when total weights were used for comparison.

From day 3 to day 14 inclusive the pargyline-treated animals exhibited a significant decrease in weight gain. This disappeared on day 15. It is difficult to understand this curious phenomenon. Perhaps the stress of being subjected to the blood-pressure procedure resulted in a transient weight loss which disappeared as the rats adapted to the stress. Since all animals consumed all food offered to them, decreased food intake is ruled

out as a causative factor. In other pargyline-treated animals (to be discussed below) not subjected to blood pressure determinations, no weight loss occurred. It is tempting to speculate that stress is the factor. Guarino et al. (1964) showed that isolated rats treated with the same daily dose of pargyline as was used in this work showed irritability and 28% more brain serotonin than similarly-treated rats housed in pairs. Isolation was deemed to be stressful. Isolation was not a factor in the present work, however, and the rats showed no irritability.

A third method of weight analysis was carried out. Daily weight gains were computed for each animal by subtracting total weight on day 1 from total weight on day 2, then day 2 weight from day 3 weight, successively to day n-1 from day n. These data are summarized in Table 7. No trends could be established, and it is probable that because of the large standard deviations no useful information can be derived from this kind of weight analysis.

It was concluded, then, that only the cumulative weight gains from day 0 of treatment afforded a sensitive method for detecting drug-induced changes in weight. This method was accordingly used in the principal study of phase 2 of this work[1] to determine weight patterns in the animals used for electrolyte and activity studies. Results are summarized in Table 8 and Figure 6. Thyroxine-treated rats lost weight significantly; pargyline-treated rats showed no significant change from the controls, and rats receiving both pargyline and thyroxine lost more weight than the thyroxine-treated rats.

It should be emphasized that with very infrequent exceptions all rats in these studies consumed all the food offered to them. These weight patterns are, then, a reflection of food utilization rather than food consumption, inasmuch as each group of 3 rats was offered 60 g of food per day and was observed to consume all of it.

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1. See Summary of Experimental Design, Page 28.

The weight loss in hyperthyroid rats is a well-known phenomenon that need not be elaborated. Its appearance in this work confirms the efficacy of the dose of thyroxine in producing hyperthyroidism.

D. Monoamine Oxidase Studies

The decision to use mitochondria suspended in hypotonic phosphate buffer in the assay for MAO was based on several considerations. Earlier papers (Hawkins, 1952; Zile and Lardy, 1959) indicated that most of the MAO activity was to be found in the mitochondria. More recently, Oswald and Strittmatter (1963) indicated that 76.7% of the MAO activity of a rat liver homogenate was recovered in the mitochondria. They suggested that the minor MAO activities distributed among nuclear, lysosomal and supernatant fractions represented mitochondrial contamination; but the 12.6% activity found in the microsomes was in excess of mitochondrial contamination. Baudhuin et al. (1964) found that 74% of total MAO activity in rat liver homogenate was recovered in the mitochondria; 24% was found in the microsomes. Thus it appeared reasonable to use mitochondria for the assay of MAO.

In Table 9 the results of a purification study are reported. It can be seen that mitochondria from homogenates of liver and brain had 150% and 200% respectively greater MAO activity than the homogenates from which they were isolated. Guha and Murti (1965) reported a 210% increase of MAO activity in mitochondria isolated from rat liver homogenates.

Because of the large numbers of animals used in this study it was impossible to run MAO assays on freshly isolated mitochondria from brains and livers. Some method of preservation was therefore indicated. Seiden and Westley (1962) reported that lysed brain mitochondria stored in 0.05 M phosphate buffer (pH 7.4) at -40°C for two months did not lose MAO activity appreciably. Their assay was run, however, on soluble MAO isolated by sonification of the thawed lysed mitochondria. This method of obtaining soluble

MAO is appropriate only for *in vitro* studies. Thus it is not suitable for studying the effects on MAO, as assayed *in vitro*, of drugs administered *in vivo*. It was decided, then, to assay lysed mitochondria that were re-homogenized and suspended in 0.05 M phosphate buffer (pH 7.4). A stability study on this preparation is reported in Table 10. Aliquots of freshly prepared brain and liver mitochondria were assayed and the remaining aliquots were put into separate tube and frozen at -40°C for subsequent thawing and assay at various time intervals. It can be seen from Table 10 that the MAO activity of lysed brain mitochondria remained unchanged after 101 days of freezing. Frozen liver mitochondria were assayed as far as 85 days after freezing, and also appeared to be stable. Thus it was deemed feasible to individually prepare for freezing and subsequent assay the mitochondria from many animals. This factor permitted the design of a relatively large-scale experiment on the last day of which as many as 40 or 50 animals might be sacrificed for preparation of brain and liver mitochondria. The mitochondria could then be assayed for MAO activity at convenient times within several months following sacrifice.

In the preparation of liver mitochondria lower relative centrifugation force ("r.c.f." or "g" value) was employed than that used by Myers and Slater (1957). The three successive centrifugations at 300 x g, 2000 x g and 4600 x g instead of the 800 x g, 6000-7000 x g and 18000 x g used by Myers and Slater probably resulted in a lower mitochondrial yield, consisting primarily of "heavy" mitochondria (Baudhuin *et al.*, 1964) with some contamination by other cell components. Since the other cell components contain small or negligible amounts of MAO activity, as already seen, contamination by these would result only in a somewhat lower degree of enzyme activity per mg protein. The fact that MAO activity in the liver mitochondria prepared in this work was 150% of homogenate activity instead of the 210% reported by Guha and

Merti (1965) is probably a reflection of the presence of inactive cell components with the mitochondria.

In the present work MAO assays of brain and liver mitochondria were run within 45 days after termination of the experiment. Results are reported in Tables 11 and 12 and summarized in Table 13.

In Tables 11 and 12 it can be seen that daily administration of vehicle did not alter terminal MAO activity in brain or liver after 12, 18 or 28 days of treatment. Accordingly, mitochondrial MAO activity for all 9 animals treated with vehicle for the various periods of time was summarized in Table 13 as 0.83 ± 0.24 μmol kynuramine/hr/mg protein in brain and 2.86 ± 0.79 such units in liver. The livers of animals treated with thyroxine had a MAO activity of 2.17 ± 0.77 μmol kynuramine/hr/mg protein, and in the brains of these animals MAO activity was 0.72 ± 0.11 such units. Both in brain and liver there was no statistical difference ($P \leq 0.05$) between the MAO activity of the thyroxine-treated animals and the control animals.

That thyroxine treatment did not alter MAO activity in brain is in agreement with the work of Zile (1960). Moreover, Tata (1964) has reported that the brain concentrates radioactive thyroxine and radioactive triiodothyronine hardly at all. Thus it appears possible that thyroxine treatment might not alter brain MAO activity because of lack of sustained contact of the hormones with brain tissue.

Daily treatment with thyroxine did not significantly alter MAO activity in rat liver mitochondria. Wurtman et al. (1963) reported similar results using the same dose of Na thyroxine (1 mg/kg) as in the present work. These investigators did find, however, a small but significant decrease in liver MAO activity when the daily dose of Na thyroxine was increased to 2.5 mg/kg, or higher. Moreover, they showed that hepatic COMT activity was unaffected

after daily treatment with the lower dose of thyroxine but was similarly lowered after treatment with the higher dose. D'Iorio and Leduc (1960) had also reported decreased liver COMT activity after daily injection of rats with 2.5 mg/kg of Na thyroxine. Wurtman et al. suggested that these decreases in rat liver MAO and COMT activity seen only after daily treatment with very high doses of thyroxine might represent a toxic effect.

In view of the results of Wurtman et al. and of the present study wherein no decrease of rat liver MAO activity was seen after thyroxine treatment, it is difficult to understand the decreased liver MAO activity reported by several investigators and already reviewed above in the literature survey section. It appears that these investigators did not use toxic doses of thyroid hormones; consequently the method of MAO assay must be considered. It was seen above that in every study of decreased liver MAO activity following thyroid administration, manometric techniques were employed using tyramine as substrate. Moreover, in each study it appeared that enzyme preparations containing lysed, swollen or aged mitochondria were used. Aebi (1962) showed that when manometric techniques were used for the assay of MAO with tyramine as substrate greater O_2 utilization or NH_3 production was seen in disrupted mitochondria than in intact mitochondria. He also pointed out that it is very difficult to extrapolate results of MAO studies on lysed mitochondria to events in the intact cell. Thus it appears that the use of manometric techniques for assays of MAO may require that greater attention be paid to the physical state of the mitochondria used in the study.

The range of values obtained in manometric assays of rat brain MAO activity is apparently quite wide. Zile and Lardy (1959) reported that MAO activity in the liver mitochondria of 22 normal rats was 7.5 μatoms O_2/mg N/hr, $\pm$ 1.1 standard error (S.E.). Similar mitochondria from 19 thyroid-fed rats had MAO activity of 3.6 such units $\pm$ 1.2 S.E. One can calculate the standard deviations

in this work, using the formula: S.D = S.E. x the square root of the sample size (Snedecor, 1956). The recalculated MAO activity values (means ± S.D) in the work of Zile and Lardy are 7.5 ± 5.2 for controls and 3.6 ± 5.3 for thyroid-fed animals. The number of assays was apparently large enough, however, to show a statistically significant decrease (P = 0.015) in the liver activity of these thyroid-fed rats. Nevertheless the obviously wide range of individual values may reflect in part the variable state of the mitochondria in the preparation used by these investigators.

Wurtman et al. (1963) suggested that because thyroxine induces mitochondrial swelling and inhibits mitochondrial oxidative systems it could interfere with MAO activity by affecting mitochondrial structure and energy production. In the present study the investigator used lysed mitochondria because it was desirable to bypass the structural changes induced by thyroxine. In this way any influence that injected thyroxine might have on MAO might be interpreted as a direct action on the enzyme itself. It appears possible from this work that thyroxine does not directly inhibit the enzyme in vivo.

From Tables 11, 12 and 13 it is quite obvious that daily treatment with pargyline for as little as 11 days reduced the MAO activity in rat brain and liver mitochondria to 0. 100% inhibition of an enzyme after treatment in vivo with a potent inhibitor is not uncommon. Weber et al. (1965) reported that after a single dose of pargyline (50 mg/kg) or iproniazid (150 mg/kg) given subcutaneously brain MAO activity in rats was suppressed 100%. McNeill (1964) found that 3 hours after intraperitoneal administration of phenelzine (30 mg/kg) to rats brain MAO activity was suppressed 97.5% and liver MAO activity was significantly suppressed 32.6%. It was not unusual, then, to find that daily treatment with pargyline for 11 days or longer totally suppressed MAO activity in liver and brain.

Total suppression of MAO activity was also seen in brains and livers of rats receiving both pargyline and thyroxine (Tables 11, 12 and 13). Since treatment with pargyline alone completely suppressed MAO activity in brain and liver these results are not at all surprising.

E. Activity Studies: The Method.

The actophotometer was chosen for the evaluation of locomotor responses in rats treated with pargyline, thyroxine or both pargyline and thyroxine because use of this cage is completely objective in respect to generation of data.

Rats have not been extensively used in photoelectric recorder-activity cages. It was necessary, therefore, to first establish a method in which limitations and sensitivity were known. Watzman et al. (1964) carried out an extensive study evaluating different parameters involved in the programming of the actophotometer for activity studies. These investigators used groups of 5 mice per cage and took readings every 15 minutes for 2 hours. From their study the following facts emerged. These were used or adapted for use in the present study.

1. The use of 5 mice resulted in highest counts and lowest coefficient of variation (S.D./mean, x 100%, the "C" value).
2. The C value was lowest in the first half hour.
3. There was significant variation from cage to cage.
4. Use of different types of housing and lighting in the room appeared not to modify animal response to drugs as measured in the actophotometer.
5. Fasting mice generated higher counts than satiated mice.
6. No "warmup" period or equilibrating period was employed; counters were turned on immediately after mice were placed in the actophotometer cages.

In the present work a preliminary study utilizing untreated rats was first carried out. The method has been presented above but is summarized here for convenience.

Three rats were put into an actophotometer cage; the counters were turned on immediately and total counts appearing every 30 minutes for 6 hours were recorded. The group of 3 rats was placed in the cage every other day. On the alternate days another, similar group of 3 rats was placed in the cage and counts were recorded in similar fashion. In this way, after 12 days there resulted 6 sets of readings per group of rats. Means ± S.D. of total accumulated counts were calculated at each hour interval. On a given day incremental counts per 30 minutes were determined simply by subtracting a given reading from the next reading. Means ± S.D. of these values were calculated for each half-hour interval. The results of a typical study are given in Table 14 and 15 and Figures 7 and 8.

In Table 14 the means ± S.D. of the incremental counts per 30 minutes for 6 hours are tabulated for each of the 2 groups of rats. The C values are also presented and these data are graphed in Figure 7. It is quite obvious that at 30 minutes mean counts are highest and C values are lowest. As the time increases, mean values drop and C values increase greatly.

A comparison, at each given time level, of the results of group 1 against the results of group 2 showed that only at the $5\frac{1}{2}$-6 hour interval was there a significant change between the two untreated groups. At first glance it would appear, then, that since there were no differences between the 2 groups of rats except for the last 30-minute interval, any interval or all of the intervals may be used in studying the effects of drugs on locomotor activity. It is postulated, however, that this should not be done.

As the C value increases the randomness of values about a mean increases. For example, in Table 14, group 1, the reading at the half-hour level is 1241 ± 296, C = 24%. In this interval the range of the 6 counts was 1099-1812 as determined from the raw data. At the 2-hour interval the reading is 7 ± 10, C = 140%: that is, in the 30 minutes between $1\frac{1}{2}$ hours and 2 hours the same

rats registered, on 6 alternate days, counts ranging from 0 to 25. An inspection of the data generated by group 2 in the same cage at the same time intervals shows that the results are similar to group 1; the C value in the first half-hour interval is 22% and at the 2-hour point is 156%. Since at neither time interval was a comparison of group 1 against group 2 significant at the 0.05 level it would appear only of academic interest to use the interval with the lowest C value. But in measuring the effects of drugs on locomotor activity in rats one wants first to determine the ability of the method to detect a difference between the control group and the drug-treated group if a difference exists. How efficient, then, is the 2-hour reading in detecting a difference, at the 0.05 level, between 2 groups of rats as compared to the efficiency of the half-hour reading? A higher C value implies greater randomness and therefore reduced efficiency. Snedecor (1956) has indicated, however, that the following formula may be used to more exactly specify the efficiency of the experiment:

$$\text{delta} = \frac{\text{S.D.}\, t_{.05}}{\sqrt{N}} \quad .$$

S.D. is the standard deviation, N is the sample size, t is the t-value at the 0.05 level of significance and delta is the smallest difference in the mean that the experiment will detect. Using values from group 1 of Table 14 and the t-value indicated in the table of t-distribution (Snedecor, 1956) for 5 degrees of freedom at the 0.05 level, delta at the first 30-minute interval is 310. The mean is 1241; 310/1241 x 100% = 25%. Thus, at the first half-hour interval the smallest increase or decrease in activity that the method will detect is 25% of the mean. By contrast, at 2 hours one must wait for a change of 157% in the mean before a significant difference at the 0.05 level can be obtained.

Similar calculations carried out on the data from the other time-intervals indicated that experimental efficiency decreases as C values increase. Thus it is clear that only the first 30-minute reading need be considered in these studies.

In Table 15 and Figure 8 a similar presentation of total cumulative counts per hour from the same cage is seen. Both these data and the data presented above and in Tables 14 and Figure 7 were derived, of course, from the same set of raw data. In the total cumulative counts the C values at one hour are higher than the 30-minute count but lower than the C value of the 30-60 minute interval. The means ± S.D. of all C values from all 4 cages are plotted in Figure 9. It is obvious that the C values are lowest at the first 30-minute interval. Since maximum efficiency is desired, the use of only the first 30-minute interval is indicated.

This preliminary study was run for 22 days; each group of 3 rats spent 11 alternate days in the cage. Use of 4 cages required 24 animals. It was surprising, in analyzing the 22-day data, to find incidences of significant differences between 2 groups of untreated animals, both in the incremental counts per 30 minutes and in the total cumulative counts. These incidences are tabulated in Tables 16 and 17. After it was noted that two of these differences occurred at the first 30-minute interval (Table 16) where experimental efficiency is highest, it occurred to the investigator that perhaps similar animals do not always behave similarly. But it was recalled that near the end of the 22-day period the rats were observed to be jumping up to the grids at the top of the actophotometers. Consequently, different portions of the 22-day study were separately analyzed in order to determine if there was in fact increased randomness near the end of the study. Tables 16 and 17 show that most of the significant differences of the 22-day data disappeared when only the first 12 days were analyzed. Moreover, differences in the last 2 weeks of the

study, days 9-22, were much higher than in the first 12 days. Thus it may be postulated that after many exposures to the actophotometer rats adapted at different rates, revealing differences between groups. This is analogous to training rats for psychological studies: some always learn a given lesson faster than others. It is recommended, then, to use no more than 6 or 7 runs in the cage per group, if the added effect of varying degrees of adaptation to the cages would be avoided.

The one incidence of significant difference between two untreated groups of rats, appearing at the first 30-minute interval in cage III, must be accepted as evidence that similar animals do on occasion behave differently (Table 16). It is because of findings such as this that statistical analysis, indicating the degree of confidence to be placed in the reported results, must accompany any study of this nature.

Gylys (1964) reported that modaline HCl, a MAO inhibitor, induced increased locomotor activity in mice, as measured by total counts, after 5 hours in the actophotometer. For this reason the 6-hour activity period was used in the present work, even though the preliminary study showed that a single count at 30 minutes was more efficient than total counts over a period of 5 or 6 hours. Moreover, a 6-hour run with drugs afforded a check on the method.

In studies using actophotometer cages for analysis of locomotor activity a "warmup" or equilibration period is often used. A "warmup" is simply a delay of about 5 to 30 minutes between the time that animals are placed in the cage and the time that the counters are turned on. Watzman et al. (1964) indicated that such delays increase experimental error. This effect can readily be shown in the present work. Using the data in Table 14 it has been shown that the 30-minute count in group 1 has a C value of 24%. The delta or efficiency value, calculated using Snedecor's formula as indicated above, is 25%: that is, a change of 25% in the mean values is the smallest that can be detected at the

0.05 level. Now if a "warmup" of 30 minutes had been employed the incremental count from 30-60 minutes would have been the first reading obtained. At this interval (1 hour) the C value is 63% and the smallest significant detectable change from mean values is 66%.

Thus the use of a "warmup" period results only in decreased efficiency. In the work of Veldcamp et al. (1965) the C value was approximately 50%. These investigators used a "warmup" period of 10 minutes before turning on the counters for a 30-minute count.

It is the opinion of the investigator that the increase in C values and the accompanying decrease in efficiency seen after the first 30-minute interval can be accounted for by a gradual decrease in the exploratory activity of rats. It was repeatedly observed that when rats are placed in an unfamiliar environment they immediately explore it fully. This results, of course, in increased bodily activity. But after 30-45 minutes exploratory activity wanes, and after several hours the rats exhibit a pattern surely familiar to all workers housing these animals in cages: very little movement and a tendency to gather together in one area of the cage. After one hour in the actophotometers the rats exhibited this pattern.

These observations prompted the investigator to conclude that the high efficiency of the first 30-minute count in the actophotometer resulted because exploratory activity in the cage is more constant than normal rat activity in familiar surroundings. In using only one count after 30 minutes to test the effects of drugs it must be kept in mind that one is measuring the effects of drugs on exploratory activity and not on normal cage activity. It is probably not rash to conclude that with the actophotometers currently in use the measurement of normal cage activity is of little or no value in the screening of drugs.

F. Activity Studies: The Effects of Thyroxine and Pargyline.

The effects of daily treatment for 11 days with thyroxine, pargyline and combined thyroxine and pargyline on the locomotor activity of rats are given in Tables 18 to 25 and summarized in Tables 26 and 27.

In Tables 18 and 22 it is shown that there were no differences in the locomotor activity of two groups of rats treated with vehicle (glass-distilled water).

The effects of thyroxine on locomotor activity are seen in Tables 19 and 23. Thyroxine-treated animals showed a significant depression of activity at the first 30-minute level (Table 19). Significant increases in activity at the 2-hour and the 2½-hour intervals are not considered meaningful in view of what has been shown above concerning the efficiency of the method past the 30-minute interval. Total cumulative counts (Table 23) in the thyroxine-treated group were also significantly less than control values in the 1-4 hour period. Close scrutiny of the data shows, however, that the decrease occurred in the first hour only; after one hour activity in both groups was similar.

Pargyline treatment (Tables 20 and 21) elicited a decrease in activity at the 30-minute level (Table 20). Again, the single increase at 2 hours is not considered meaningful. Total cumulative counts in the pargyline-treated group of rats were depressed only at the 1-hour level (Table 24), again reflecting a carryover of the depression seen in the first 30 minutes.

Treatment with both thyroxine and pargyline (Tables 21 and 25) elicited depression of activity in the first 30 minutes (Table 21). The increased activity at the 2-hour and at the 2½-hour intervals is not considered meaningful. There was no significant change in cumulative counts (Table 25).

Tables 26 and 27 were drawn up from the data of the preceding 8 tables; all instances of significant changes elicited by drug treatment are summarized here for convenience. It is obvious that 11 days of treatment with thyroxine,

pargyline and both drugs in combination elicited over this period a decrease in exploratory cage activity in rats as measured in the first 30-minute interval (Table 26). In the rats receiving thyroxine alone or pargyline alone this depression was severe enough to affect the 1-hour cumulative count (Table 27) and in the thyroxine-treated rats the depression in the first half-hour was severe enough to show depression of total incremental counts for the next 3 hours. It should be emphasized again that this 4-hour decrease does not reflect actual depression of activity for 4 hours but only severe depression in the first 30 minutes. This finding tends to support the view of the investigator that in rats only exploratory activity can be consistently measured.

That thyroxine should depress rat exploratory activity is not surprising. The metabolic and clinical effects of hyperthyroidism have already been discussed; in view of the protein-reducing effects in skeletal muscle alone one might expect decreased activity.

The questionable status of pargyline as a psychic energizer has already been discussed in the literature survey. The finding in the present study that pargyline depresses exploratory activity in rats may possibly be in agreement with the work of Barsa and Saunders (1964) who found frequent increases in psychotic symptoms when MAO inhibitors alone were given to depressed patients. It is certainly not in agreement with the report of Kline (1961), who felt that MAO inhibitors were quite effective in lessening the symptoms of such patients. It is difficult to correlate these psychiatric findings and it is even more difficult to extrapolate to depressed patients the results of work with normal rats. In 1961, however, Kline did not have the accumulated clinical experience with MAO inhibitors that was available in 1964 to investigators such as Barsa and Saunders. Thus it is possible that the recent extensive psychiatric studies with MAO inhibitors and increased familiarity with their effects may result in

some revision of older views concerning the psychiatric efficacy of these drugs when used without supportive drug therapy. It is possible that the enigmatic increased apathy seen by Kline in psychotic patients receiving both MAO inhibitors and thyroid may have been a manifestation of the reported increases in psychotic symptoms seen after MAO inhibitor therapy alone. At any rate the depressed exploratory activity seen in the present study in rats receiving both thyroxine and pargyline is not at all enigmatic after it is observed that each drug separately also caused a similar depression.

G. Correlations

Depression of exploratory activity in rats receiving thyroxine or both thyroxine and pargyline was accompanied by significant weight loss in both groups. Depression was accompanied by increased sodium and potassium excretion and sustained hypertension in the thyroxine-treated animals; in the groups receiving pargyline or both thyroxine and pargyline depression was accompanied by total suppression of terminal MAO activity in brain and liver.

Thus any one of the several effects induced by thyroxine treatment can be accompanied by depression. Moreover, depression can be accompanied by total suppression of MAO activity in brain and liver. No one effect, however, accompanied depression in all three experimental groups, and thus it is concluded that depression of exploratory activity in rats can be accompanied by a variety of physiological changes.

VI. SUMMARY AND CONCLUSIONS

A. The effects of chronic treatment with thyroxine, pargyline and combined thyroxine and pargyline were studied on locomotor activity, systolic blood pressure, urinary sodium and potassium excretion, body weight gains and terminal monoamine oxidase activity in brain and liver of male albino rats.

B. A revised actophotometric method for measuring the effects of chronic drug treatment on the locomotor activity of rats was established. A single reading at 30 minutes was the most suitable measure of exploratory activity, since comparisons of 30-minute readings provided the most sensitive and reproducible index for measuring drug responses on activity. Readings beyond the 30-minute interval exhibited greatly reduced sensitivity in detecting drug-induced changes in locomotor activity. It appears that this actophotometric method for measuring the exploratory activity of rats during chronic drug treatment has not been previously described.

C. Analyses of body weights recorded in these studies indicated that comparisons of cumulative weight gains from day 0 of drug treatment provided the most sensitive and reproducible index for determining changes induced in body weights by various factors.

D. Daily administration of thyroxine induced a sustained hypertension and a suppression of cumulative weight gains, both commencing on day 2 and lasting for at least 17 further days of treatment. Thyroxine treatment induced significant increases in urinary sodium and potassium excretion beginning on day 6, and over 11 days of treatment thyroxine induced a depression in exploratory activity. Terminal MAO activity in brain and liver was not affected.

E. Pargyline administered daily induced sustained hypotension beginning between days 2 and 7 of treatment and lasting for at least 17 further days of treatment. No weight loss or changes in urinary sodium or potassium excretion were seen. Pargyline induced a depression in exploratory activity over the

11 days of treatment and completely suppressed monoamine oxidase activity in brain and liver after 11 days of treatment or longer.

F. Concurrent administration of both thyroxine and pargyline did not induce changes in blood pressure after 3 days of treatment. Cumulative weight gains were continually suppressed from day 1. Urinary sodium and potassium excretion was not significantly altered, but depression of exploratory activity over the 11 days of treatment was induced, and terminal monoamine oxidase activity in brain and liver was completely suppressed.

G. Qualitative observations indicated that thyroxine increased the heart rate and stroke volume. This effect was not abolished when pargyline was given together with thyroxine, but pargyline alone appeared to reduce heart rate and stroke volume.

H. The implications of these findings are discussed.

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