1961

The Influence of Thyroid Feeding on the Pharmacologic Actions of Certain Monoamine Oxidase Inhibitors

Richard N. Carrier
University of Rhode Island

Follow this and additional works at: http://digitalcommons.uri.edu/theses

Terms of Use
All rights reserved under copyright.

Recommended Citation
http://digitalcommons.uri.edu/theses/184

This Thesis is brought to you for free and open access by DigitalCommons@URI. It has been accepted for inclusion in Open Access Master's Theses by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons@etal.uri.edu.
THE INFLUENCE OF THYROID FEEDING
ON THE PHARMACOLOGIC ACTIONS OF CERTAIN
MONOAMINE OXIDASE INHIBITORS

BY

RICHARD N. CARRIER

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
PHARMACOLOGY

UNIVERSITY OF RHODE ISLAND
1961
ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. P. V. Buday, for his guidance given not only during this study and the preparation of this dissertation but also throughout the entire period of graduate study.

He also wishes to thank most sincerely the following: Dr. John J. DeFeo and Dr. Elizabeth Chase, members of the graduate committee; Mr. David R. DeFanti for his aid in the urinary dopamine studies.

The author wishes to express his sincere gratitude to Dorothy L. Carrier for her unfailing patience, understanding and assistance in preparation of this thesis.
ABSTRACT

Although a few disparate reports have been published to the contrary, accumulating evidence seems to indicate that the feeding of thyroid extract, or the parenteral administration of thyroxine, produces a decline in the monoamine oxidase activity in the livers of various animals. As a consequence this anti-enzymatic effect, and the resultant biologic sparing of catecholamines, has been incriminated as partly responsible for the hypersensitivity of hyperthyroid animals and isolated tissues, to the pharmacologic actions of sympathomimetic amines.

Rather recently a class of anti-melancholy drugs, or "psychic energizers", have been made clinically available. These agents seem to act as anti-depressants by virtue of their monoamine oxidase antagonizing properties.

It was strange, therefore, to note in the literature that a clinical psychotherapeutic incompatibility existed between concurrent thyroid and monoamine oxidase inhibitor medication. Peculiar changes in thyroid function, blood pressure, and blood glucose levels were also noted clinically with the latter medicaments. The present research was undertaken, using blood glucose, blood pressure, and acute and subacute toxicity studies as parameters, to evaluate
these paradoxical reports. Male, albino rats were employed as subjects.

Two chemically dissimilar, irreversibly acting, monoamine oxidase inhibitors, iproniazid (Marsilid) and MO-911, were selected as test compounds. The daily intraperitoneal injection of iproniazid (175 mg/kg) and MO-911 (10 and 25 mg/kg) for a three week period elicited only inconsequential blood sugar changes in both euthyroid and hyperthyroid animals. Systolic pressures became significantly elevated upon continued injection of either drug, and the pressor effects were enhanced by feeding a 2% thyroid diet.

Limited dopamine excretion studies in hyperthyroid rats revealed no particular changes, whereas massive, daily doses of iproniazid provoked increased excretion of the catecholamine approximately 200 to 300% after fifteen days of enzyme inhibitor treatment. These urinary results did not parallel the hypertensive effects.

Weight loss, sluggishness, and gross and microscopic evidence of vital organ damage occurred with massive, daily doses of iproniazid. The acute lethal effects of MO-911 were augmented by thyroid feeding.

These results seem to suggest strongly that the direct and indirect pharmacologic actions of iproniazid and MO-911, given in high daily doses to rats, are aggravated by a hyperthyroid state; these experimental findings have obvious clinical significance.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>111</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>tv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>2</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>3</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>4</td>
</tr>
<tr>
<td>SURVEY OF THE LITERATURE</td>
<td>6</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>17</td>
</tr>
<tr>
<td>RESULTS</td>
<td>25</td>
</tr>
<tr>
<td>SUBACUTE STUDIES</td>
<td></td>
</tr>
<tr>
<td>General Observations</td>
<td>25</td>
</tr>
<tr>
<td>Subacute Toxicity</td>
<td>25</td>
</tr>
<tr>
<td>Tissue Pathology</td>
<td>26</td>
</tr>
<tr>
<td>Systolic Blood Pressure and Blood Glucose</td>
<td>27</td>
</tr>
<tr>
<td>Urinary Analyses</td>
<td>29</td>
</tr>
<tr>
<td>ACUTE TOXICITY STUDIES</td>
<td>29</td>
</tr>
<tr>
<td>DISCUSSION OF RESULTS</td>
<td>42</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>52</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>55</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>64</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Results of subacute toxicity experiments with iproniazid and MO-911 administered daily</td>
</tr>
<tr>
<td>2</td>
<td>Urinary dopamine levels of iproniazid and thyroid treated albino rats</td>
</tr>
<tr>
<td>3</td>
<td>Acute toxicity of intraperitoneally administered MO-911</td>
</tr>
<tr>
<td>I-A</td>
<td>Concentration of standard dextrose solutions and corresponding colorimeter readings</td>
</tr>
</tbody>
</table>
## LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blood glucose standardization curve</td>
<td>34</td>
</tr>
<tr>
<td>2.</td>
<td>Graph of mean body weight changes of rats under MAO inhibitor and/or thyroid medication.</td>
<td>35</td>
</tr>
<tr>
<td>3.</td>
<td>Blood pressure, blood glucose, and urinary dopamine levels of rats fed 2% thyroid</td>
<td>36</td>
</tr>
<tr>
<td>4.</td>
<td>Effect of daily intraperitoneal doses of iproniazid (175mg/kg) on rat blood pressure, blood glucose, and urinary dopamine levels</td>
<td>37</td>
</tr>
<tr>
<td>5.</td>
<td>Effect of daily intraperitoneal doses of MO-911 (25mg/kg) on rat blood pressure and blood glucose levels</td>
<td>38</td>
</tr>
<tr>
<td>6.</td>
<td>Effect of daily intraperitoneal doses of MO-911 (10mg/kg) on rat blood pressure and blood glucose levels</td>
<td>39</td>
</tr>
<tr>
<td>7.</td>
<td>Effect of daily intraperitoneal doses of MO-911 (25mg/kg) + 2% thyroid feeding on rat blood pressure and blood glucose levels</td>
<td>40</td>
</tr>
<tr>
<td>8.</td>
<td>Effect of daily intraperitoneal doses of MO-911 (10mg/kg) + 2% thyroid feeding on rat blood pressure and blood glucose levels</td>
<td>41</td>
</tr>
<tr>
<td>I-A.</td>
<td>Graphic configurations of the MAO inhibitors employed in the study</td>
<td>66</td>
</tr>
</tbody>
</table>
INTRODUCTION

Accumulating evidence seems to indicate that the feeding of thyroid extract, or the parenteral administration of thyroxine, produces a decline in the monoamine oxidase activity of various animals. This anti-enzymatic effect may result in a biological sparing of catecholamines which might be partly responsible for the hypersensitivity of hyperthyroid animals and isolated tissues to the pharmacologic actions of sympathomimetic amines. It has been shown that monoamine oxidase is intimately involved in catecholamine metabolism and that its inhibition can alter the circulating levels of various pressor and glycogenolytic amines.

Recently a class of anti-melancholy drugs or "psychic energizers" has been introduced for clinical use. These compounds appear to act as anti-depressants by virtue of their ability to irreversibly inhibit monoamine oxidase activity. The clinical use of these substances is known to cause a noticeable elevation in the mood of depressed subjects.

Other recent evidence has been presented in the literature which indicates that a clinical psychotherapeutic incompatibility exists with the concurrent thyroid and anti-melancholy monoamine oxidase inhibitor medication. Peculiar changes
in thyroid function, blood pressure and blood glucose levels such as orthostatic hypotension and hypoglycemia were also noted clinically with many of the latter medicaments.

Such evidence indicates that a close relationship exists between the in vivo activity of the thyroid hormone and of the enzyme monoamine oxidase, and that when the activities of these substances are simultaneously altered a variety of peculiar effects are elicited.

The present research was undertaken to evaluate some of the possible effects of concurrent administration of both desiccated thyroid and monoamine oxidase inhibitors in laboratory rodents. Blood glucose, blood pressure, and acute and subacute toxicity studies were employed as parameters.
Elemental iodine was officially introduced into medicine for the relief of goiter by Coindet in 1820. However, it is believed that the Chinese, many centuries before the birth of Christ, had learned that certain substances, presently known to contain iodine, exerted beneficial effects in this disease. In 1891 Murray demonstrated that the parenteral use of an emulsion of sheep's thyroid provided satisfactory replacement therapy for hypothyroidism. Four years later, Fauman showed that about twenty-five percent of all the iodine in the mammalian body was found in the thyroid gland, and that it was firmly bound to a colloidal protein-like material. Oswald later identified this as a protein and named it thyroglobulin. By hydrolysis of this substance Kendall (1915a,b,c) obtained small quantities of a crystalline, iodinated material which was therapeutically active in hypothyroid patients; it was termed thyroxine. Later Harington (1926) identified thyroxine as a derivative of the amino acid tyrosine and assigned to the former the structural formula;
Many excellent reviews and papers have recently been published on the biosynthesis of thyroid hormones (Gross and Pitt-Rivers, 1951, 1952a, b; Pitt-Rivers, 1960), and the transport and distribution of this hormone (Gross and Pitt-Rivers, 1953; Ingbar, 1960). Significant advances have also been made in the chemistry and physiology of the thyroid stimulating hormone (TSH) (White, 1946; Albert, 1949; Rawson, 1949; Harris and Woods, 1947; Nelson and Bradley, 1960; Sonenberg and Money, 1960; Carsten and Wynston, 1960), and in the description of the role the nervous system plays in the control of thyroid function (Greer, 1957; Taurog et al., 1957; Greer et al., 1960). Unfortunately, the mechanism of action of thyroxine and its analogues has not yet been clearly defined. Blood borne thyroxine can be converted to triiodothyronine (TRIT) in the thyroidectomized rat (Gross and Leblond, 1951) and human (Pitt-Rivers et al., 1955), and there is some evidence to indicate that this conversion may be an essential step in forming the "active" hormone (Lardy et al., 1957). A number of compounds have been implicated as the peripherally acting hormone. TRIT is without question, at least in mammals, the more rapid acting (Barker, 1956, 1957), and with chronic dosage, the more potent (Gross and Pitt-Rivers, 1954; Roche and Michel, 1955). Several acetyl derivatives of thyroxine, viz., tetraiodothyroacetic acid (TETRAC) and triiodoacetic acid (TRIAC) have recently been isolated; they are less active biologically than thyroxine or TRIT (Trotter, 1955; Goolden,
1956). However, the actual chemical entity responsible for the observed hormonal effects still remains controversial.

It is thought that thyroid analogues may act directly or indirectly on the enzymatic activity of nervous tissue as they do on somatic tissues. This is significant as many facets of brain chemistry and metabolism are now being extensively studied. Recent investigators have outlined the possible metabolic pathways for the synthesis of dopamine, epinephrine (E), and norepinephrine (NE), catecholamines which are found extensively in nervous tissue. It is noteworthy in this respect that l-tyrosine is the amino acid precursor of thyroxine as well as of the catecholamines (Blaschko, 1959).

In order to understand the manifold enzymatic effects of the thyroid hormone, investigators have delved into the cellular metabolism of the hormone itself. Such studies have shown that there are three possible pathways for the metabolism of the iodothyronines: dehalogenation, oxidation of the alanine chain, and esterification of the phenolic group (Lissitzky and Bouchilloux, 1957; Roche and Michel, 1960). It is also believed that the iodothyronines are oxidatively deaminated to give a keto acid which is subsequently decarboxylated to give the corresponding acetic acid derivatives (Roche et al., 1957; Tomita et al., 1957). In vitro studies have shown these derivatives to be present in skeletal muscle as well as in the liver and kidney. These findings apparently demonstrate that the oxidative degradation of thyroxine is possible in all tissues (Albright
are regulated by the effect of iodothyronines on cytochrome C. Employing rat liver homogenates Tipton and Nixon (1946) have demonstrated that cytochrome oxidase activity is decreased following administration of TSH, desiccated thyroid, or thyroxine. However, other workers have been unable to duplicate these results (Smith and Williams-Ashman, 1949). It is difficult to show that thyroxine has a specificity of action on cytochrome C since adrenalectomy, like thyroidectomy, will produce a fall in total body cytochrome C (Drabkin, 1950b). Cytochrome oxidase has also been shown to oxidize epinephrine in the presence of cytochrome C (Blachko et al., 1940).

More recently the hypothesis has been presented that the iodothyronines may exert their metabolic effects by "decoupling" a specific oxidative phosphorylation thus allowing the oxidative system to function more rapidly but less effectively (Lardy and Maley, 1954), and this may account for the higher basal metabolic rate and decreased work efficiency of hyperthyroid animals (Lardy, 1957). Indeed, it has been found that thyroxine in vitro depresses the ratio of esterified, i.e., high energy, phosphorus to oxygen (Leblond and Grad, 1948; Niemeyer et al., 1951; Martius and Hess, 1951, 1952; Hoch and Lipmann, 1953). Diiodothyronine and iodine, but not iodide, have a similar action (Klemperer, 1951).

A number of additional facts support this oxidative
phosphorylation hypothesis. Of significance is the recent observation that mitochondrial swelling and contraction cycles are induced by thyroxine (Tapley et al., 1955). Evidence indicates that the mitochondrial swelling requires electron transport and this effect is promoted by thyroxine as well as by phosphate, calcium ions, and reduced glutathione, whereas contraction occurs in the presence of adenosine triphosphate (Lehninger, 1960). This becomes important in the light of the fact that there is a decreased oxygen consumption in enlarged mitochondria, which is in turn attributed to a decrease in adenosine triphosphate levels (Aebi, 1952).

However, a great deal of opposition has arisen to the oxidative phosphorylation hypothesis since the thyroxine concentration to produce these effects in vitro is several orders of magnitude greater than that normally present in vivo. In addition, in vitro responses have not been consistent and sufficiently reproducible (Rawson and Sonenberg, 1959).

Thyroid hormone exerts a significant action on the mammalian nervous system. The nervous instability of patients with Graves' disease (exophthalmic goiter) has been noted since the disease was first recognized (Grave, 1835; Parry, 1835; von Basedow, 1840). Similarly, the apathy and sluggishness of cretins has been known for centuries (Major, 1939). Furthermore, various psychoses have been associated with both hyperthyroidism and myxedema (Dunlap and Moersch, 1935; Jamieson and Wall, 1936). Voluntary muscle reflexes are diminished in thyroidectomized animals.
(Brody, 1941a,b), and electroencephalograms of athyreotic patients show diminished voltage, slow frequency, and an absence of cortical alpha waves. In hyperthyroid patients the alpha rhythm is increased (Bertrand et al., 1938). Some correlation has been found between the basal metabolic rate and the rate of alpha wave emanation from the nervous system (Ross and Schwab, 1939). In contrast to the effects on general metabolism, most investigators have been unable to detect a change produced by thyroxine in the rate of oxygen consumption of brain slices (Harington, 1944). It is felt that alpha wave frequency measurements during artificially induced hyperthyroidism are indirect measures of carbohydrate metabolism in the brain (Rubin et al., 1937). Bowman (1925) and Hoskins (1946) found lowered basal metabolic rates in a large number of schizophrenic patients. They also described unusually high clinical tolerance to the effects of dried thyroid. Brody and Man (1950), however, have reported normal serum protein-bound iodine levels in similar patients while Cranswick (1955) has described abnormally elevated thyroid uptakes of I_{131} in a majority of schizophrenic patients. The administration of TRIT to various psycho-pathologically affected, but euthyroid, patients evokes the appearance of formerly latent or absent hostile emotions and sexual unrest (Flach et al., 1960). Significant emotional changes, however, were not always observed (Flach et al., 1958).

Paralleling the advances in thyroid research, stimulated by the introduction of radioactive iodine, has been the progress
which has been made in neurochemistry as a consequence of the introduction of iproniazid, a "psychic energizer". This hydrazine derivative was originally synthesized by Roche Laboratories for the chemotherapy of tuberculosis. It was soon evident, however, that the greatest value of the drug lay in its ability to create a sustained feeling of "well-being" in psychically depressed patients (Crane, 1956). Selikoff and others (1953) attributed this effect to central stimulation. Zeller et al. (1952a) discovered that iproniazid was a potent inhibitor of the intracellular enzyme monoamine oxidase (MAO), an enzyme incriminated in the mode of action of certain anti-depressant drugs.

MAO has been found in significant amounts in liver, kidney, and other organs and tissues (Richter and Tingey, 1939), and is present chiefly in mitochondrial and microsomal cell fractions (Cotzias and Dole, 1951; Hawkins, 1952). Enzymatic studies have shown that iproniazid irreversibly blocks MAO activity in the autonomic nervous system, brain, liver, and kidney, both in vitro and in vivo (Zeller and Barsky, 1952; Zeller et al., 1952b).

The psychiatric value of iproniazid and other MAO inhibitors has been clearly demonstrated and has led to the introduction of a large number of chemically heterogeneous anti-depressants (Delay and Buisson, 1958; Geyer, 1958; Tobin et al., 1959). Evidence indicates that MAO is important in the metabolism of serotonin (5-HT) and the catecholamines, and that the anti-depressant effects elicited by the MAO
inhibitors are related to the central nervous system changes in these monoamines (Pletcher, 1957; Udenfriend et al., 1957; Spector et al., 1958). The relation of MAO to catecholamine metabolism was first recognized by Blaschko et al. (1937) who demonstrated that the enzyme oxidatively deaminated epinephrine. Brodie et al. (1959) have shown that after single, large, oral doses of MAO inhibitors both 5-HT and NE levels increase significantly in dog and rabbit brain. However, contrary to previous reports it has been found that 0-methylation probably constitutes the major pathway for the catabolism of this amine (Armstrong et al., 1956; Axelrod, 1957). MAO has also been incriminated in the metabolism of tyramine and dopamine (Spector et al., 1958; Resnick, 1959).

It is known that thyroid hormone potentiates the action of epinephrine on oxygen consumption and basal metabolic rate (Lardy, 1957), and heart rate (Gravenstein and Thier, 1960). This potentiation of the activity of epinephrine on the resting blood pressure (Gerlei, 1938) and on isolated arteries (Smith, 1954) has been explained as the ability of thyroxine to elicit a fall in tissue amine oxidase levels. Likewise, Burn and Marks (1935) showed that thyroidectomized rabbits were slightly less responsive to epinephrine induced hyperglycemia than normal animals. More recent experiments have shown that feeding of desiccated thyroid causes hepatic MAO of rabbits to diminish, whereas thyroidectomy causes MAO activity to rise. The rise in blood glucose caused by injected epinephrine is greater in rabbits fed thyroid extract,
and this hyperglycemic effect has been explained by the fall of amine oxidase which thyroid feeding produces. Similarly, the increased tolerance to epinephrine induced hyperglycemia in thyroidectomized rabbits has been explained by the rise of MAO levels elicited by thyroid removal (Burn and Spinks, 1952). To the contrary, however, hyperthyroidism has been reported to elevate liver MAO in rats and guinea pigs (Clark, 1950). Dubnick et al. (1960) report that rat liver MAO activity is only slightly augmented by thyroidectomy while brain levels remain unchanged.

Similar studies by Zile and Lardy (1959) have indicated that thyroid fed rats have elevated circulating E and NE levels, and that hypothyroid animals possess high levels of hepatic mitochondrial MAO activity. Activity of the enzyme under hyperthyroidism was shown to reach a minimum only after twelve days of treatment. Termination of thyroid feeding allowed MAO activity to return to normal within fourteen days, and consequently the reversible decrease in MAO activity by thyroid hormone was considered to be an indirect suppression of the enzyme, perhaps through a regulation of synthesis of the enzyme. This supposition is also shared by others (Ozaki et al., 1960). In contrast to the work of Spinks and Burn (1952), it has been shown that there is no correlation in MAO activity and blood sugar levels in the frog (Smith, 1960).

The distinct psychotropic actions of thyroxine and iproniazid proves interesting when one considers the clinical
observation of Bailey et al. (1959) that thyroid extract apparently does not combine well pharmacologically with simultaneous iproniazid medication. Milne (1967) has amplified this report and has reported that a paradoxical apathy and sluggishness supervenes in depressed patients receiving both a psychic energizer and thyroid extract.

Another significant link between thyroxine and the MAO inhibitor action is concerned with their respective efficacy in the acceleration of angina pectoris. Total thyroidectomy has proved an effective therapeutic procedure in the treatment of this disease, and this procedure alters the response of the cardiovascular system to epinephrine (Reab, 1965). The salutary anti-anginal effects of iproniazid were first reported in 1957 and confirmed in various clinics (Cesarman, 1956; Cossaio, 1959; Schweizer, 1959; Masters and Donoso, 1959). Several hypotheses have been postulated as to the mechanism of the anti-anginal effects of the MAO inhibitors. Masters and Donoso (1959) find that iproniazid causes an elevation of myocardial 5-HT, and that relief from angina is mediated through myocardial and cerebral autonomic nerves. Cesarman (1957,1959) proposes that iproniazid may influence cardiac metabolism through some enzymatic mechanism. Pletcher and Pellmont (1958) have demonstrated that a striking rise in myocardial catecholamines occurs in iproniazid treated guinea pigs, and they have related these changes to the therapeutic effects of this compound. In certain hypothyroid patients, however, thyroid in doses as low as eight to sixteen
milligrams has produced severe anginal pain. With concomitant therapy with an MAO inhibitor, it was found that thyroid dosage could be gradually increased to relieve the myxedema without aggravating the cardiovascular complaint (Wolffe and Shubin, 1959).

The administration of desiccated thyroid has been shown to elevate the cardiac output whereas myxedematous patients show a subnormal output (Scheinberg et al., 1950). Hyperthyroidism appears to increase the mean systolic ventricular pressures and the resting blood pressure of rabbits (Fullerton and Harrop, 1930; Spinka, 1952). In contrast, iproniazid regardless of its monoamine sparing effects, has been shown to cause severe orthostatic hypotension (Randall, 1958) possibly through ganglionic blockade (Gertner, 1959). It has, for this reason, been suggested for the treatment of hypertension (Harnes, 1958).
MATERIALS AND METHODS

Subacute Studies

Sixty male, albino rats of the Wistar strain, ranging in weight from 170-210 g, were divided into 10 groups of 6 animals each (Table 1). Each group was housed separately. The environmental temperature was maintained between 250-270°C. Initially, all 60 rats were placed on a control diet consisting of water ad lib. and specially repelleted tablets of Purina Laboratory Chow1 for 7 days. On the eighth day, groups I through V were continued on this control diet while groups VI through X were placed on a similar diet which, in addition, contained 2% powdered Thyroid U.S.P.2 The animals were then maintained on these respective diets for an additional 21 days. To prevent vitamin A deficit, due to increased utilization of this vitamin during the thyroid treatment (Hague et al., 1948) and to retard weight loss (Logaras and Drummond, 1938), all animals were given 3 drops of Oleum Percomorphum3 (vitamin A and D) orally every third day of the study. On the eighth day, normal blood pressure and blood glucose levels were taken. Subsequent blood pressure levels were recorded daily, and blood sugars were measured every third day.

1 Ralston Purina Co., St. Louis 2, Missouri.
2 Armour Laboratories, Kankakee, Illinois.
3 Mead Johnson and Co., Evansville 21, Indiana.
Diet. The incorporation of thyroid extract into the diet was considered the most satisfactory method of hormone treatment (Anderson et al., 1943), although it presented certain technical difficulties which required the extemporaneous repelleting of the animal feed. The food product as manufactured contains no excipient to bind the fibrous meal. Instead, moisture in the form of steam is added directly to the mix, and the moistened meal is forced through a die under high pressure. Our facilities did not permit high compression tableting so that moisture alone was not a sufficient adhesive for the fibrous and resilient meal.

Starch, dextrin, sucrose, glucose, lactose, or other similar carbohydrate-like excipients commonly employed to prevent crumbling, were inappropriate to use as these would have a tendency to modify certain metabolic effects of the thyroid treatment. A method found simple and expeditious to produce an acceptable remade chow tablet, using limited and easily accessible equipment, was to crush the hard, brittle pellets by use of a Wiley laboratory mill, passing the coarse material thence through a Mikro Sampl mill (model 579A) to give a number 60 powder or finer. To each 100 g of this powder was added 40 ml of a warm,

---

4 Letter from Special Chows Research Division, Research Department, Ralston Purina Co., St. Louis 2, Missouri.


6 Pulverizing Machinery Co., Summit, New Jersey.
freshly prepared, gelatin-acacia solution made by dissolving 10 g of Gelatin (granular) U.S.P. and 1 g of Acacia (powder) U.S.P. in 100 ml of boiling distilled water. The binder and powders were then mixed thoroughly to form a damp mass. The latter was then sifted through a number 10 mesh brass screen to form granules. These particles were then passed through a number 20 mesh screen to obtain a finely granulated mass.

To prepare a 2% thyroid diet it was necessary to readjust the formulation so that 100 g of the mass contained 90.76 g powdered chow, 2.00 g powdered thyroid, and 7.24 g of excipient. These substances were prepared and mixed as previously described. Using a hand driven, single punch, Stokes tablet machine (model A3-Eureka)7 with a 1.27 cm standard concave punch and die set for maximum fill (1.09 cm), the damp granulation was compressed into 0.5 g convex tablets having 2.0 mm edge thickness. The pressure on the punch approximated 7.5 tons per square inch. The tablets were allowed to dry and harden thoroughly at ambient room temperature (approximately 21°C) for 36 hours or longer.

Drugs. Eight of the 10 groups of rodents received daily intraperitoneal injects of a MAO inhibitor for the 21 days concomitant with the thyroid or control diets. The remaining two groups were used as controls (cf. Table 1). Animal groups I, II, VII, and VIII were injected with

isonicotinyl-2-isopropylhydrazine phosphate (iproniazid)\textsuperscript{8}, a hydrazide enzyme inhibitor, and groups III, IV, IX, and X were injected with N-benzyl-N-methyl-2-propynylamine hydrochloride (M0-911)\textsuperscript{9}, a non-hydrazide inhibitor, in the doses as outlined in Table 1. The structural formulae of these drugs are given in Figure I-A (Appendix). The solutions for injection were prepared by dissolving the respective salts in isotonic saline. The pH of the solutions approximated 3.5.

Methods. Indirect systolic blood pressures were determined by the method of Kersten et al. (1947) using a Photoelectric-Tensometer\textsuperscript{10}. All readings were taken to the closest 5 mm Hg and were recorded as direct instrument values.

Blood glucose levels were determined by the colorimetric method of Nelson (1944) as modified by Somogyi (1945). Samples were read in optical density units on a Bausch and Lomb Spectronic 20 colorimeter, type 39-24-10\textsuperscript{11}, using the special \(\frac{3}{4}\) inch diameter cuvettes as provided by the manufacturer\textsuperscript{12}. To prevent reading errors, the cuvettes were calibrated using a standard \(\text{CuSO}_4\) solution. The colorimeter was standardized by using known amounts of dextrose diluted

\textsuperscript{8} Generously supplied as Marsilid by Dr. Robert E. Dixon, Roche Laboratories, Nutley 10, New Jersey.

\textsuperscript{9} Generously supplied by Dr. G.M. Everett, Abbott Laboratories, North Chicago, Illinois.

\textsuperscript{10} Metro Industries, Long Island City 6, New York.

\textsuperscript{11} Bausch and Lomb Optical Co., Rochester, New York.

\textsuperscript{12} Spectronic 20 test tubes # 33-29-27, Bausch and Lomb Optical Co., Rochester, New York.
to 100 ml with glass distilled water. Seven concentrations were employed and ranged from 60 to 210 mg/100 ml (Table I-A). Five readings of each concentration were made by taking 0.1 ml of the sample and adding it to 1.5 ml of distilled water. To this mixture was added 0.2 ml of 5% ZnSO₄ and 0.2 ml of 0.3 N Ba(OH)₂. All chemicals were of reagent quality. The suspension was centrifuged for 5 minutes at 4000 rpm's. One ml of the supernatant liquid was then pipetted into a Folin-Wu sugar tube which had been previously filled with 1.0 ml of alkaline copper tartrate solution. The mixture was heated in boiling water for 20 minutes and then cooled in running tap water for 2 minutes. To the cooled solution was added 1.0 ml of arsenomolybdate reagent and the solution was mixed and allowed to stand for approximately 2 minutes. After adjusting the volume of the resulting colored solution to 8.0 ml with distilled water the solution was placed into the cuvette. A blank determination was prepared in the same manner using 0.1 ml of glass distilled water in lieu of the dextrose. The blank cuvette was then placed in the colorimeter and the instrument adjusted to an optical density of zero and a wavelength of 500 μm. The sugar sample was then substituted for the blank and the resultant optical density read. The mean of the 5 readings for each sample was plotted against the concentration (Table I-A; Figure 1). The resultant plots were then adjusted using the line regression method as described by Ostle (1956) and the mathematically derived line was plotted.
Blood samples were obtained by direct intracardiac puncture with a 0.25 ml syringe and a number 27 gauge-1 inch hypodermic needle. In order to obviate blood sampling difficulties and possible epinephrine release (Weil, 1952), the samples were taken while the animals were lightly anesthetized with pentobarbital sodium (20 mg/kg). To eliminate variances in blood glucose levels, due to differences in the food intake of the animals, all animals were fasted for 17 hours prior to the blood collections. The blood samples were processed in the aforementioned method employing a blank with each group of samples. The blood glucose concentration of each sample was determined from instrument readings and the respective concentration in mg/100 ml read from the line graph (Figure 1).

**Histologic Studies.** Samples of liver, kidney, spleen, and thyroid tissue were removed from one representative animal in each group studied. These tissues were fixed in Bouin's solution, dehydrated, cleared in xylol, mounted in paraffin, and sliced into 10 μ sections. The sections were stained with hematoxylin and eosin and examined for general pathology.

**Urinary Dopamine Studies.** Twenty-four hour urine samples from groups II, V, and X were collected in 2 N H₂SO₄ on the eighth and fifteenth day of the studies. The pooled samples were filtered and processed for extraction of catecholamines (Crawford and Law, 1958). An aliquot of the urine extract was passed through a strongly acidic, cationic
exchange resin (Dowex 50W-X8, Na+ form) and the dopamine was eluted with 8.0 ml of 2 N HCl (Bertler et al., 1958). Urinary dopamine levels were determined by the trihydroxyindole method of Carlsson and Waldeck (1958) employing an Amresco-Bowman spectrophotofluorometer.

Acute Toxicity Studies

As a consequence of the subacute MAO inhibitor and thyroid interaction studies, an apparent augmentation was observed in the mortality of hyperthyroid rats receiving high daily parenteral doses of the hydrazide (iproniazid) or the non-hydrazide (MO-911) enzyme inhibitor (Table 1). To evaluate this possible hormonal sensitization, 73 male rats (Wistar strain) ranging in initial weight from 133-190 g were employed in an acute mortality study. Four groups of fasted animals (172 g mean weight), 8 animals to a group, previously fed on a 7 day control diet ad lib., were injected intraperitoneally with doses of MO-911 differing by .03 log units (Table 4). Four other groups (127 g mean weight), previously made hyperthyroid by a 7 day desiccated thyroid (2%) ad lib. diet, were treated in an identical manner. Nine additional hyperthyroid rats (130 g mean weight) were injected solely with normal physiological saline solution and served as controls. All animals were housed at a temperature of 25°-27°C.

---

14 American Instrument Co., Inc. Silver Spring, Maryland.
15 Control diet was identical to that used in the subacute study.
16 Thyroid diet was identical to that used in the subacute study.
and were provided prior to MAO inhibitor medication with oral vitamin A and D supplementation at 3 day intervals.
RESULTS

SUBACUTE STUDIES:

General Observations. The MAO inhibitor medicated animals exhibited somewhat less motor activity and seemed easier to handle than control animals. Within 3 to 4 days, all thyroid fed rodents showed considerable irritability, whereas the hyperexcitability was less apparent when thyroid was combined with the enzyme inhibitor medication. The augmented apathy in the latter instances became increasingly pronounced with continued drug administration.

Food and water consumption decreased with iproniazid therapy but was less pronounced with MO-911. Thyroid fed controls seemed to increase their intake twofold, while MO-911 and iproniazid treated, hyperthyroid animals seemed to be restrained in their feeding habits. The demise of drug treated animals was always preceded by complete cessation of food consumption. Mean body weights of euthyroid subjects under iproniazid medication fell steadily while the weights of animals receiving MO-911 showed either slight decreases or moderate increases. Weight decline also occurred in the hyperthyroid groups; this was most marked in those animals also receiving a MAO inhibitor (Figure 2). Untreated animals maintained on the control diet showed normal weight gains.
Sporadic incidence of diarrhea was common to all the enzyme inhibitor treated rodents and was particularly prevalent in those animals receiving desiccated thyroid. The feces of MAO inhibitor treated animals were coated with considerable intestinal mucus. With iproniazid therapy, gross observations of urine samples showed considerable hemoglobin; blood specimens used for glucose determinations contained laked erythrocytes. Reddish colored ocular discharges, resembling dried blood, were also regularly apparent with iproniazid, but they were present less frequently with K0-911. An abnormal degree of salivation occurred with both iproniazid and K0-911 treatments in the euthyroid and hyperthyroid animals, but it appeared more prevalent in those individuals treated with the latter compound.

**Subacute Toxicity.** Symptoms of iproniazid toxicity rapidly developed. A number of euthyroid rodents given the 250 mg/kg dose of iproniazid died on the second day of injection and all died within 8 days (Table 1); concurrent administration of thyroid to a similar group produced expiration within the 21 day study period whereas concomitant thyroid feeding and iproniazid treatment resulted in all deaths within 6 days. Although no deaths followed thyroid feeding (group V), or K0-911 administration (groups III and IV), a combination of these substances caused all animals to die within 15 days (groups VIII and IX).

**Tissue Pathology.** Livers removed from 21 day, iproniazid treated animals displayed small lesions; kidneys appeared dark
red while spleens of all the subjects were almost black. Histologic examination of these organs revealed hepatocellular damage, renal tubule necrosis, and considerable erythrocyte fragmentation in the spleen. Rodents sacrificed after 21 days of MO-911 medication exhibited none of these organic changes, although several of the spleens appeared dark red in color. Thyroid and MO-911 tissues were unable to be procured.

Systolic Blood Pressure and Blood Glucose. Blood pressure and blood glucose determinations are plotted in Figures 3 to 8. Standard deviations were derived from the formulae:

\[ \bar{Y} = \frac{EY}{n} ; \quad s^2 = \frac{E(Y-Y)^2}{n-1} \]

where \( Y \) represents the individual reading, \( \bar{Y} \) the sample mean, \( n \) the sample number, \( s^2 \) the variance, and \( s \) the standard deviation with \( n-1 \) degrees of freedom (Ostle, 1958). A t-distribution formula ( \( \bar{Y} = Y+ t \frac{s}{\sqrt{n}} \) ) was applied to the normal mean blood pressure and glucose levels of the sixty samples employed using a t value of 2.660 (Dixon and Massey, 1957). \( \bar{Y} \) represents the mean value plus or minus the resultant deviation within 99% confidence limits with 59 degrees of freedom. The calculated normal, mean blood pressure and glucose levels were determined as 110 \( \pm \) 2.65 mm Hg and 104 \( \pm \) 3.87 mg % respectively. The standard blood pressure deviation of the 60 samples was calculated to be \( \pm \) 7.66 mm Hg and is represented in the figures by the
solid, horizontal lines. The glucose mean standard deviation was calculated to be \( \pm 11.20 \text{ mg} \% \) and is represented by the dashed lines. All readings, inclusive of the greater percentage of their individual standard deviations, when present outside of these areas, are noted as statistically significant changes.

Three week daily treatment with either enzyme inhibitor and/or desiccated thyroid elicited no significant changes in blood glucose levels, and only in those animals near death did the concentrations fall (Figures 1 to 8).

Blood pressures were elevated by both thyroid and MAO inhibitor treatments. Elevations in mean arterial pressure were witnessed with thyroid after 48 hours of feeding, rose to peak levels by the sixteenth day, with a plateau occurring at approximately 157 mm Hg. Blood pressures of euthyroid subjects after 48 hours of iproniazid (175 mg/kg) medication produced significant elevations, followed by a sharp increase to a peak reading of about 165 mm Hg within 5 days. Pressures remained stable until the eighth day when they fell precipitously below normal; readjustment to normal values occurred by the thirteenth day (Figure 4) even with continued daily medication.

A similar blood pressure pattern was exhibited in MO-911 treated euthyroid rats. In contrast to iproniazid, MO-911 treatment produced an initial transient hypotensive effect lasting 3 to 4 days. This hypotensive pattern appeared similar at both dose levels, but was of shorter duration at
the 10 mg/kg dose. Within 7 to 8 days of daily MO-911 treatment blood pressures of both dosed groups rose to approximately 155 mm Hg. This marked hypertensive state, as with iproniazid treated subjects, persisted for several days. The post-hypertensive pressures with MO-911, however, did not fall below normotensive levels but stabilized slightly above the mean normal pressures (Figures 5 and 6).

Treatment of hyperthyroid animals (3 day thyroid feeding) with MO-911 produced a marked hypertensive response after 2 days of drug administration, with higher peak systolic pressures prevailing than during enzyme inhibitor or thyroid administration alone (Figures 7 and 8). Blood pressure levels fell erratically after the eleventh day of treatment, and all animals expired either on the fourteenth or fifteenth day.

Urinary Analyses. Urinary dopamine values appeared to be within normal values in rodents made hyperthyroid by the 21 day feeding of 2% desiccated thyroid (Table 2). To the contrary, however, these values were seemingly elevated in hypertensive, euthyroid animals treated with daily doses of iproniazid (175 mg/kg). An extremely high value was observed at day 15 where peculiarly enough the blood pressure values were within normal limits (Figures 3 and 4).

**ACUTE TOXICITY STUDIES**

The acute toxicity results are presented in Table 3. Because a perfect statistical analysis of the respective LD50's was not possible, due to the limited number of animals
employed and the one group aberrantly responding to MO-911 at 175 mg/kg, only an approximation to his dose for the enzyme inhibitor in the euthyroid animals could be made; this value was found to be 183.5 ± 15.0 (S.E.) mg/kg by using a graphic method of analysis (De Beer, 1945). It should be noted that in the thyroid-MO-911 treated group all deaths, save one at the 152.4 mg/kg level, occurred within 2 hours. These results show rather conclusively that the intraperitoneal toxicity of MO-911 in hyperthyroid rats is markedly increased and that the LD$_{50}$ of the drug lies well below 152 mg/kg under these circumstances.
TABLE 1

Results of subacute toxicity experiments with iproniazid and MO-911 administered daily

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of MAO Inhibitor</th>
<th>Diet</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Iproniazid 250 mg/kg</td>
<td>Control</td>
<td>All in 8 days</td>
</tr>
<tr>
<td>II</td>
<td>Iproniazid 175 mg/kg</td>
<td>Control</td>
<td>2 in 21 days</td>
</tr>
<tr>
<td>III</td>
<td>MO-911 25 mg/kg</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td>IV</td>
<td>MO-911 10 mg/kg</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>None</td>
<td>2% Thyroid</td>
<td>None</td>
</tr>
<tr>
<td>VI</td>
<td>Iproniazid 250 mg/kg</td>
<td>2% Thyroid</td>
<td>All in 2 days</td>
</tr>
<tr>
<td>VII</td>
<td>Iproniazid 175 mg/kg</td>
<td>2% Thyroid</td>
<td>All in 6 days</td>
</tr>
<tr>
<td>VIII</td>
<td>MO-911 25 mg/kg</td>
<td>2% Thyroid</td>
<td>All in 14 days</td>
</tr>
<tr>
<td>IX</td>
<td>MO-911 10 mg/kg</td>
<td>2% Thyroid</td>
<td>All in 15 days</td>
</tr>
<tr>
<td>X</td>
<td>None</td>
<td>Control</td>
<td>None</td>
</tr>
</tbody>
</table>
TABLE 2

Urinary dopamine levels of iproniazid and thyroid treated albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of Collection</th>
<th>Urinary Dopamine Concentration (μg/l)</th>
<th>Mean Arterial Blood Pressure mm Hg ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>362</td>
<td>110 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>475</td>
<td>112 ± 7.9</td>
</tr>
<tr>
<td>Daily i.p. Iproniazid 175 mg/kg Control Diet</td>
<td>8</td>
<td>835</td>
<td>164 ± 13.8</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2159</td>
<td>116 ± 27.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>482</td>
<td>140 ± 9.0</td>
</tr>
<tr>
<td>Thyroid (2%) Fed</td>
<td>15</td>
<td>517</td>
<td>161 ± 5.0</td>
</tr>
</tbody>
</table>
### TABLE 3

**Acute toxicity of intraperitoneally administered MO-911**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Mean Weight (g)</th>
<th>Mean Weight at Injection (g)</th>
<th>Dose (mg/kg)</th>
<th>Response (12 hrs)</th>
<th>% Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO-911, i.p.</td>
<td>165</td>
<td>185</td>
<td>187.5</td>
<td>4/8</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>180</td>
<td>175.0</td>
<td>2/8</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>163</td>
<td>163.0</td>
<td>3/8</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>161</td>
<td>152.4</td>
<td>2/8</td>
<td>25.0</td>
</tr>
<tr>
<td>MO-911, i.p. + Thyroid (2%) Diet</td>
<td>153</td>
<td>122</td>
<td>187.5</td>
<td>8/8</td>
<td>100.0*</td>
</tr>
<tr>
<td></td>
<td>154</td>
<td>129</td>
<td>175.0</td>
<td>8/8</td>
<td>100.0*</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>127</td>
<td>163.0</td>
<td>8/8</td>
<td>100.0*</td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>129</td>
<td>152.4</td>
<td>8/8</td>
<td>100.0*</td>
</tr>
<tr>
<td>Thyroid Diet Controls +0.14 ml Normal Saline i.p.</td>
<td>153</td>
<td>130</td>
<td>--</td>
<td>0/9</td>
<td>0</td>
</tr>
</tbody>
</table>

* All deaths within 2 hr of dosing.
Fig. 1. Blood glucose standardization curve. Mean instrument values ▲; mean calculated values ■.
Fig. 2. Graph of mean body weight changes of rats under MAO and/or thyroid medication.

- Thyroid diet •
- Control diet •
- MO-GIL (10mg/kg) + control diet ▲
- Iproniazid (175mg/kg) + control diet ▲
- MO-11 (10mg/kg) + 2% thyroid diet ▲

35
Fig. 3. Blood pressure, blood glucose, and urinary dopamine levels of rats fed 2% thyroid.

Blood pressure ●; blood glucose ▲; ■ = 482 ug/liter urinary dopamine; △ = 517 ug/liter urinary dopamine.
Fig. 4. Effect of daily intraperitoneal doses of iproniazid (175 mg/kg) on rat blood pressure, blood glucose, and urinary dopamine levels.

Blood pressure ●; blood glucose ●; ▲ = 835 ug/liter urinary dopamine; △ = 2159 ug/liter urinary dopamine.
Fig. 5. Effect of daily intraperitoneal doses of MC-911 (25mg/kg) on rat blood pressure and blood glucose levels.

Blood pressure •; blood glucose ▲.
Fig. 6. Effect of daily intraperitoneal doses of M0-911 (10mg/kg) on rat blood pressure and blood glucose levels.
Blood pressure •; blood glucose ▲.
Fig. 7. Effect of daily intraperitoneal doses of MC-911 (25mg/kg) + 2% thyroid feeding on rat blood pressure and blood glucose levels.
Blood pressure •; blood glucose ▲.
Fig. 8. Effect of daily intraperitoneal doses of MO-911 (10mg/kg) + 2% thyroid feeding on rat blood pressure and blood glucose levels. Blood pressure •; blood glucose ▲.
DISCUSSION OF RESULTS

The ostensible reduction of motor activity observed in the MAO inhibitor medicated rodents, at first glance, appears contradictory to the clinical evidence indicating that these compounds are effective psychomotor stimulants (Loomer et al., 1957; Tobin et al., 1959; Voelkel, 1959). One must consider, however, the ability of these inhibitors to reduce the duration of sleep (Kline, 1958) and produce insomnia and weakness (Ayd, 1959; Hawkins et al., 1959; Tobin et al., 1959). Employment of high, subacute doses can thus produce a rapid and profound elevation of central nervous system activity causing generalized body fatigue; a fatigue condition which would effectively mask its cause.

In the animals where thyroid was combined with enzyme inhibitor medication, fatigue and weakness were undoubtedly reflections of apathy. This is a plausible conclusion since hyperthyroidism itself is capable of producing increased central nervous system activity characterized by nervousness and hyperexcitability (Rawson, 1959). Although Dongier et al., (1956) indicate that there is no correlation between the degree of anxiety and the rate of thyroid secretion, James (1941) is of the opinion that the level of thyroid activity does a great deal to determine the relative excitability.
of all nervous processes. Again, such gross psychomotor stimulation, incurred through hyperthyroidism, often produces fatigue and general weakness (Rawson, 1959). Wang (1927) has actually demonstrated a depression of spontaneous activity in rats after two weeks oral medication with desiccated thyroid which has been attributed to increased fatigue of these animals. An apparent corollary then is that the increased apathy observed in hyperthyroid rodents medicated with MAO inhibitors may be a result of continuous, immense, potentiated central excitability which brings about a rapid generalized fatigue. Whether or not this potentiated fatigue occurs through a combined effect of thyroid and MAO inhibitors upon amine oxidase systems is an intriguing question.

The apparent increase in food and water intake of thyroid fed rats, irrespective of their continued weight loss, agrees well with the reports of Carlson (1941) and Barker (1951) but not completely with that of Richter (1933). No doubt the use of vitamins A and D as dietary supplements offset, to a degree, certain of the adverse effects of high doses of the thyroid substance. Interestingly, vitamin A has been reported to protect animals against the rise in oxygen consumption caused by desiccated thyroid (Sadu and Brody, 1947).

The weight gains experienced with MO-911 medicated animals correlates well with clinical observations that MAO inhibitors produce increased appetites resulting in significant weight increases (Tobin et al., 1959). On the other hand, the peculiar weight loss observed with iproniazid may be a
result of the induced toxicity caused by the high subacute doses employed (250 and 175 mg/kg). The combination treatment with thyroid and inhibitor, which caused both a drastic weight reduction and loss of appetite, is difficult to interpret, but it certainly testifies to a potentiation of toxicity.

Toxic manifestations of iproniazid in euthyroid rodents were clearly evident as deaths occurred in both the 175 and 250 mg/kg dose levels during the 21 day study. Among these animals a significant erythrocyte destruction was observed. This is not surprising in as much as hydrazine and its analogues are known to possess hemolytic properties. Benson et al. (1952) have shown that short term iproniazid administration causes increased destruction and formation of erythrocytes. Moreover, histological investigations of animals treated with high doses of hydrazide inhibitors often reveal increased erythrocytosis as evidenced by hepatic, renal, and splenic hemosiderosis, profuse bone marrow cellularity, and hyperemia of the spleen (Binden and Studer, 1959). The lack of gross hemolytic changes observed with MO-911 offers added evidence that such toxicological syndromes are most likely the result of the hydrazine moiety of hydrazide MAO inhibitors.

A singular toxicological observation was the apparent reddish, ocular discharges which occurred with subacute medication with both iproniazid and MO-911. This effect has a parallel in the report (Gray, 1961) that ocular discharge of a porphyrin-like material occurs in rats under subacute
medication with etryptamine, a new indole MAO inhibitor.

The feces of MAO inhibitor treated rats were often coated with considerable mucus. This observation parallels the gastrointestinal hypermucosity noted with the psychiatric use of similar psychic energizers (Sainz, 1959); this side effect may reflect irritation of the alimentary canal induced by the medication.

In order to evaluate the possible blood glucose responses of MAO inhibitors in euthyroid and hyperthyroid rodents, it became necessary to demonstrate the possible effect of both a hydrazide and a nonhydrazide inhibitor on blood sugar levels, particularly since Underhill (1911) has stated that subcutaneous injection of hydrazine sulphate into dogs produces a fall in blood glucose within 24 hours. Also, Underhill (1911, 1914) reports that this compound when administered to dogs depletes both liver and skeletal glycogen and prevents glycosuria of depancreatized animals through inhibition of sugar excretion by the kidney. These observations tend to indicate that the hypoglycemic effect seen clinically with iproniazid medication (O'Connor et al., 1953; Alexander and Berkeley, 1959; Greenblatt and Kahn, 1959; Weiss et al., 1959) could be in part the contribution of the hydrazide moiety (\(-\text{N-N-}\)) in this compound. Surprisingly, however, subacute administration of iproniazid and MO-911 to both euthyroid and hyperthyroid rodents evoked no significant blood glucose changes.

Of further interest was the lack of significant blood glucose changes resulting from 2% desiccated thyroid feeding.
treated rodents.

Although extensive investigation tends to support the hypothesis that iproniazid is not a practical in vivo potentiator of the effects of peripheral E and NE (Cahen et al., 1953; Schayer and Smiley, 1953; Schayer et al., 1953; Balzer and Holtz, 1956; Kamijo et al., 1956; Corne and Graham, 1957; and others), evidence indicates that breakdown of E and NE is depressed in iproniazid treated rats (Schayer, 1953; Schayer et al., 1955). The resultant peripheral vasopressor effects of these hormones may, in part, explain the hypertension. Goldberg (1959) has reported that pheniprazine, a chemical analogue of iproniazid, causes a marked pressor effect in anesthetized, vagotomized dogs when given intravenously.

Rodents fed 2% iesthesiostated thyroid presented no such inconsistent vascular effects; the hypertension developed rapidly and was maintained. This confirms the findings of Samiy (1952) with thyroxine in rats and rabbits. Although hypertension is not a normally reported syndrome in hyperthyroidism, Blumgart et al. (1930) have stated that blood volume increases in this condition. This together with a probable increase in cardiac output can elevate blood pressures during thyroid overactivity. Although it is simple to accept the supposition that elevated cardiac output is primarily a function of increased metabolism, Rasmussen (1941) declares that induced hyperthyroidism results in cardiac changes that cannot be directly correlated with metabolic rate. In addition,
Leblond and Hoff (1944) indicate a possible direct effect of thyroid on the heart. Whether these effects are directly responsible for elevated blood pressures is difficult to ascertain. A noteworthy observation is the fact that when thyroid and inhibitor administration are combined in rodents all hypotensive reactions as exhibited with MAO inhibitor treatment alone are obliterated. Whether such responses are a direct potentiation of combined inhibitor and thyroid treatment requires further investigation.

Since MAO inhibitors are capable of inhibiting a normal catabolic pathway of dopamine in vivo (Holtz, 1959), one would expect an increase of this substance in certain body tissues. After prolonged inhibitor treatment in rodents (14 days) with high, intraperitoneal doses of iproniazid, a significant rise in urinary dopamine levels did occur. Dopamine acts as a pressor in the rat (Vogt, 1959), although it is decidedly less potent than other catecholamines (Pennefather and Rand, 1960). Samiy (1952) noted that hyperthyroidism in rats augmented the pressor response to intravenous doses of dihydroxyphenylalanine, the precursor of dopamine; his results inferred that this effect was due to enhanced receptor sensitivity to the formed dopamine. Although it was not possible to determine urinary levels of dopamine from MAO inhibitor treated, hyperthyroid rats in the present experiments, it might very well be that Samiy's findings explain, in part, the rapid onset and marked pressor effects recorded in these treated animals. It is difficult,
on the other hand, to explain the apparent lack of correlation between high urinary dopamine levels and the pressor responses in response to high daily doses of iproniazid. It may simply be a case of cardiovascular adaptation of the drug treated animal.

The original intention was to administer both iproniazid and MO-911 to both hyper- and euthyroid rats in equimolar concentrations per kilogram of body weight. Since we originally employed iproniazid at 250 and 175 mg/kg dose levels, this necessitated the use of equimolar MO-911 concentrations at 175 and 123 mg/kg. In our preliminary observations, the subacute administration of MO-911 at these doses produced total mortality after the second injection. Subsequent information received from Abbott Laboratories indicated that subacute intravenous administration of 25 and 10 mg/kg of MO-911 was well tolerated in rats. Therefore, these new doses were utilized and found to be satisfactory.

Of significant interest was the acute and subacute potentiation of MAO inhibitor toxicity in hyperthyroid animals. The administration of desiccated thyroid or thyroxine is known to modify the susceptibility of animals to the poisonous effects of a number of substances. Thyroid hormone increases the toxicity of alloxan (Houssay and Sara, 1945), cocaine (Glaubach and Pick, 1931), morphine (Hunt and Seidell, 1910), dinitrophenol (Glaubach and Pick, 1934), epinephrine

17 Letter from Dr. G.M. Everett, Abbott Laboratories, Chicago, Illinois.
(Kroneberg and Huter, 1951; Kroneberg, 1952), and other substances. Large doses of thyroid substance may produce liver damage (Barker, 1951), and this finds parallel in the ability of certain MAO inhibitors to intensify liver damage caused by certain hepatotoxic agents (Zbinden and Studer, 1959). However, the cause of the augmented lethality with iproniazid and MO-911 is not easily understood, but because of the early onset of malaise and rapid demise a direct sensitivity to the toxic effects to the MAO inhibitor molecule per se seems to be the most likely explanation. These effects, on the other hand, may possibly be related to MAO inhibition coupled with the similar enzymatic in vivo effects of thyroid feeding (Spinks and Burn, 1952; Zile and Lardy, 1959) resulting in an acute liberation of unbound catecholamines, a diminished ability of the animal to cope with stress, and a predisposition to fatal thyrotoxicosis. The reported psychotherapeutic incompatibility of concurrent MAO inhibitor and thyroid medication (Bailey et al., 1959; Kline, 1961) may possibly be explained by this hormone induced sensitivity to drug toxicity.
SUMMARY AND CONCLUSIONS

1. The present research was undertaken using blood glucose, blood pressure, and the acute and subacute toxicity studies in rodents as parameters, to evaluate the possible psychotherapeutic incompatibility which has been reported to exist with concurrent thyroid and MAO inhibitor medication.

2. Daily intraperitoneal injection of iproniazid or MO-911 for three weeks reduced the spontaneous motor activity of male albino rats. Concomitant feeding of two percent desiccated thyroid augmented this apathy.

3. The subacute, intraperitoneal administration of either iproniazid or MO-911 in high doses caused severe loss of body weight; this weight loss was aggravated by concurrent thyroid feeding.

4. The sluggishness, aggravated weight decline, and symptoms of malaise from combined thyroid and MAO inhibitor medication may be explained by an increased sensitivity of hyperthyroid rats to acute or subacute MAO inhibitor toxicity.

5. Limited gross and histologic observations revealed that high, daily doses of iproniazid, for three weeks, caused hepatic and renal damage and erythrocyte hemolysis in
the spleen. MO-911 in daily, parenteral doses of ten or twenty-five milligrams per kilogram did not produce any apparent organic changes, although splenic darkening was noted.

6. In the doses employed, the MAO inhibitors alone or with thyroid treatment, failed to elicit any significant changes in blood sugar. Feeding of a two percent thyroid diet for three weeks likewise failed to modify the blood glucose picture.

7. The systolic blood pressures of iproniazid treated, euthyroid animals rose significantly above normotensive levels, beginning after forty-eight hours of medication, to a peak on the eighth day; whereupon they suddenly and precipitously fell to hypotensive levels.

8. MO-911, in daily doses of ten or twenty-five milligrams per kilogram body weight, elicited an initial and marked fall in blood pressure. This response soon gave way in both instances to a slowly developing hypertension which persisted until the tenth or sixteenth day of treatment.

9. The feeding of thyroid substance to MO-911 treated rats obliterated the initial hypotension and effected a sharp pressor response within three days of continued inhibitor medication. These pressor effects were greater and more acute in onset than those noted with either MO-911 or thyroid administration.

10. Urinary dopamine levels following one or two week treatment of high, daily parenteral doses of iproniazid rose about
three fold. The urinary levels of this biogenic catecholamine could not be correlated with pressor responses.

11. Although admittedly limited in extent, studies of urinary dopamine levels did not reveal any increase after three week feeding of rats with two percent desiccated thyroid.

12. These results suggest strongly that the direct and indirect pharmacologic actions of iproniazid and M0-911, given in high daily doses to rats, are aggravated by a hyperthyroid state; these experimental findings have obvious clinical significance.
LIST OF REFERENCES


Greer, M. A.: Ciba Symp. 10: 72, 1957.


Underhill, F. P.: ibid., 17: 293, 1914.


TABLE I-A
Concentration of standard dextrose solutions and corresponding colorimeter readings

<table>
<thead>
<tr>
<th>Concentration of Dextrose (mg/100ml)</th>
<th>Sample Number</th>
<th>Optical Density Reading</th>
<th>Mean Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>Blank 1</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blank 2</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blank 3</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blank 4</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blank 5</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blank 6</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blank 7</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>
Iproniazid

(1-isonicotinyl-2-isopropylhydrazine phosphate)

(N-benzyl-N-methyl-2-propynylamine hydrochloride)

FIG. I-A. Graphic configurations of the MAO inhibitors employed in the study.
THE INFLUENCE OF THYROID FEEDING ON THE PHARMACOLOGIC ACTIONS OF SOME MONOAMINE OXIDASE INHIBITORS

By

Richard N. Carrier(2) and Paul V. Buday(3)

(1) This investigation was supported in part by a U.S. Public Health Service research grant (MY-4435) from the National Institute of Mental Health.

(2) Submitted by R. N. Carrier to the Division of Graduate Studies of the University of Rhode Island in partial fulfillment for the degree of Master of Science (Pharmacology), 1961.

(3) Present address: Medical Communication Department, Medical Research Section, Lederle Laboratories, a Division of American Cyanamid Company, Pearl River, New York, U.S.A.
It is well-established that the calorigenic, metabolic, and hemodynamic effects of norepinephrine and epinephrine are diminished or lacking in hypothyroid animals (Sawyer and Brown, 1935; Raab, 1945; Brewster, 1956). On the other hand, although there are some contraindications as to an enhancement of native sympathomimetic amine activity during hyperthyroidism (Macmillan and Rand, 1962), thyroid hormone generally potentiates the action of epinephrine on oxygen consumption and basal metabolic rate (Lardy, 1957), and heart rate (Sawyer and Brown, 1935; Hoffmann et al., 1957; Gravenstein and Thier, 1960). The potentiation of epinephrine's activity on the resting blood pressure (Gerlei, 1938; Spinks, 1952) of rabbits and on isolated swine arteries (Smith, 1954) and rabbit duodenum (Trendelenburg, 1953) has been interpreted in terms of a decrease in tissue monoamine oxidase (MAO) levels or a reduction in the enzyme's activity engendered by thyroid hormone.

Similarly, Burn and Marks (1925) have found that feeding thyroid to rabbits increases their sensitivity to epinephrine-induced hyperglycemia. This hormonal "priming" effect was later ascribed to the ability of thyroid hormone to diminish hepatic MAO activity (Spinks and Burn, 1952; Trendelenburg, 1953). Although there have been some reports to the contrary (Westermann, 1956; Holtz et al., 1956), this in vivo inhibitory action of thyroid hormone on hepatic MAO in rodents seems reasonably well-established by the work of Zile and Lardy (1959, 1960), Calvert and Brody (1961), and Novick (1961). The hypothesis of Zile and Lardy (1959) that thyroid hormone regulates the biosynthesis of hepatic MAO has been supported by others (Ozaki et al., 1960).
Further, whether the action of thyroid hormone is primarily upon MAO, 0-methyltransferase, or both, the fact remains that the administration of thyroid elevates catecholamine blood levels and increases their urinary excretion in animals (Zile and Lardy, 1959; D'Iorio and Leduc, 1960) and man (Diwani et al., 1960).

Bailey et al. (1959), Kline (1961), and Saunders (1962) have found that melancholic patients medicated for several days with both thyroid and iproniazid, an irreversible MAO inhibitor and antidepressant, paradoxically became apathetic and sluggish, ashen and cyanotic in appearance, and showed evidence of a fall in blood pressure. These adverse behavioral and gross physical or somatic effects necessitated withdrawal of the antidepressive agent.

In light of these seemingly peculiar and contradictory effects, the present investigation was designed in preliminary fashion to determine what action thyroid feeding had, if any, on the pharmacodynamic actions of irreversible acting MAO inhibitors, and to attempt to explain, at least on a gross somatic basis, the reason for the reported psychotherapeutic incompatibility between concurrent thyroid and MAO inhibitor medication.

MATERIALS AND METHODS

Animals.—

Sixty male albino Wistar rats, ranging in weight from 170-210 gm, were randomly divided into 10 groups of 6 animals each. All groups were caged separately and the environmental temperature was kept between 25-27° C throughout the study.
Drugs and Dosage Protocol.—

Iproniazid (1-isonicotinyl-2-isopropylhydrazine) phosphate (Marsilid<sup>(1)</sup>) and pargyline (N-benzyl-N-methyl-2-propynylamine hydrochloride; MO-911; Eutonyl<sup>(2)</sup>) were used as irreversible MAO inhibitors. Both drugs were freshly prepared by dissolving the respective salts in physiological saline solution. Injections were given intraperitoneally. Starting on the eighth day of drug diet feeding, animal groups I and VII received 250 mg/Kg of iproniazid phosphate, and groups II and VIII received 175 mg/Kg daily. Groups III and IX received 25 mg/Kg of pargyline hydrochloride, and IV and X received 10 mg/Kg daily. These latter doses were selected because pargyline in vitro is almost 8 times (Taylor et al., 1960) and in vivo (intraperitoneally) about 12 times more potent an irreversible MAO inhibitor in the rodent than iproniazid (Everett, 1962).

The remaining two groups (V and VI) were not medicated with the synthetic enzyme inhibitors.

Diet and Feeding Protocol.—

Control group V, which received neither an MAO inhibitor nor thyroid substance, was given Purina Lab Chow (Ralston Purina Co.) ad libitum in a special retabletted form. A 2% thyroid drug diet was prepared by homogeneously combining desiccated thyroid U.S.P., with the powdered lab chow

<sup>(1)</sup>Registered trademark of Hoffmann-La Roche, Inc., U.S.A.

<sup>(2)</sup>Registered trademark of Abbott Laboratories, U.S.A.
and retabletting the granulation using suitable excipients. Incorporation of thyroid substance into the diet of the rats was felt most suitable in that it would allow gradual medication and continuous absorption to take place.

Initially, all 60 rats were fed the control diet for 7 days. On day 8, groups I through V were continued on this diet while groups VI through X were fed the thyroid ration. Consequently, groups VII and VIII receiving iproniazid also received 2% thyroid, while groups IX and X were fed the thyroid diet and received daily injections of pargyline. All animals were maintained on their respective diets for an additional 21 days or, where fatalities occurred, until the entire group died. To prevent or retard vitamin A deficit and weight loss normally evoked by hyperthyroidism, as well as for purposes of dietary standardization, all animals were given orally 3 drops of Oleum Percomorph (Mead Johnson) every third day. The animals had free access to water at all times.

**Histopathologic Studies.** —

Immediately after death or sacrifice, samples of liver, kidney, and spleen were removed from one or more representative animals of groups II, III, IV, and V. The tissues were fixed in Bouin's solution and, after preparation and section (10 microns), were stained with hematoxylin and eosin and examined for general morphologic changes.

**Blood Pressure Studies.** —

Indirect systolic blood pressures of each animal were taken daily using
a precalibrated photoelectric tensometer; the technique of Kersten et al., (1947) was followed. Fright and other like interfering stresses were avoided by training all animals to accommodate the pressure cuff before beginning the drug studies. Direct instrument values were read in mm Hg; in no case were they read greater than the closest 5 mm Hg. When fatalities due to drug treatment occurred, readings were taken for not less than 3 surviving animals of the particular group. The normal, mean systolic blood pressure and standard deviation of the entire 60 rats was calculated. Confidence intervals were calculated according to standard statistical procedures (Ostle, 1954) for the true mean pressure of such untreated animals.

**Urinary Dopamine Studies.**

Twenty-four hour urine samples from groups II, V, and VI were collected in 2N sulfuric acid on the eighth and fifteenth days of drug administration. The pooled samples were filtered and processed for the extraction of catecholamines (Crawford and Law, 1958). An aliquot was passed through a strongly acidic, cationic exchange resin (Dowex 50W, X-8, Na+ form) column, and the dopamine was eluted from the resin by 8 ml of 2N hydrochloric acid (Bertler et al., 1958). Dopamine concentrations were determined by using the trihydroxyindole-fluorimetric method of Carlsson and Waldeck (1958) and an Aminco-Bowman spectrophotofluorometer (American Instrument Co.).

**Fasting Blood Glucose Determinations.**

Blood samples were obtained every third day by direct intracardiac puncture
and were made, immediately following blood pressure recordings, from lightly anesthetized (pentobarbital sodium, 20 mg/Kg i.p.) rats. All animals were fasted for 17 hours prior to the blood sampling. Blood glucose concentrations for each animal were determined colorimetrically (Nelson, 1944; Somogyi, 1945) using a precalibrated Spectronic 20 colorimeter (Bausch and Lomb) and 12.7 mm diameter cuvettes. Individual concentrations were read as optional density units, and these were transformed into mg/100 ml by referring to a calibration curve. The individual values were averaged to obtain group means, and standard deviations were calculated. Confidence intervals, as before, were calculated for the true, untreated, rat mean blood glucose concentration.

RESULTS

General Observations.—

The MAO inhibitor-treated animals exhibited a somewhat diminished random motor activity and were easier to handle than unmedicated controls. Within 3-4 days, all thyroid-fed rats became considerably irritable, but in those groups where both enzyme inhibitor and thyroid were administered the hyperexcitability was less apparent. In fact, the sluggishness and asthenia of these dually medicated animals became increasingly pronounced as the drug treatments continued.

Food and water consumption was generally decreased in the MAO inhibitor-treated groups, but this effect was less pronounced with the pargyline treatments. Thyroid-fed animals ate and drank avidly, while hyperthyroid rats given either of the enzyme inhibitors seemed restrained in their
feeding habits. Death of the MAO inhibitor-treated animals was always preceded by complete inanition. Mean body weights of euthyroid rats under iproniazid medication fell steadily especially during the first 9 days of injection, while the body weights of similar animals under pargyline showed either slight decreases or moderate increases. A relatively slow, but progressive weight decline also occurred in the thyroid-fed rats after an initial 5-day increase. Untreated animals maintained on the control diet showed normal weight gains, and at the end of 3 weeks weighed, on the average, 45 gm more than the hyperthyroid control animals. A sporadic incidence of diarrhea was common in all enzyme inhibitor-treated rats; it was particularly evident in thyroid-fed animals. The feces of MAO inhibitor-treated rats were coated with considerable intestinal mucus. Considerable blood was noted in urine samples from iproniazid-treated rats; blood specimens contained laked erythrocytes. Iproniazid-medicated rats regularly showed reddish-colored ocular discharges that resembled dried blood; this effect was less frequently observed with pargyline. An abnormal degree of salivation occurred with both iproniazid and pargyline treatments in euthyroid and hyperthyroid animals, but this effect appeared more prevalent with the nonhydrazine compound.

Subacute Toxicity.—

Symptoms of iproniazid toxicity rapidly developed. In group I, given 250 mg/Kg of iproniazid daily, a few rats died on the second day of injection; all animals of this group died within 8 days (Table I).
The lower dose of iproniazid (175 mg/Kg) was considerably less toxic. The feeding of 2% desiccated thyroid concurrently with injected iproniazid at both dose levels (groups VII and VIII), however, hastened their deaths 3- to 4-fold. Although the feeding of thyroid alone (group VI) or the administration of pargyline at 10 (group IV) and 25 mg/Kg (group III) alone caused no fatalities, the concurrent administration of both thyroid and MAO inhibitor (groups IX and X) resulted in the deaths of all animals within 15 days.

Histopathologic Studies.—

Livers removed from rats which had received iproniazid for 21 days showed small superficial lesions. The kidneys of these animals appeared abnormally dark red in color; spleens, on the other hand, were almost black. Histologic examination of these organs revealed hepatocellular damage, renal tubule necrosis, and considerable erythrocyte fragmentation in the spleen.

Rats sacrificed after 21 days of pargyline dosing exhibited none of these tissue changes; several of the spleens in these groups, however, appeared abnormally dark red in color. Tissues from thyroid-MAO inhibitor-treated animals were not examined.
<table>
<thead>
<tr>
<th>Group</th>
<th>Daily Intraperitoneal Dose of MAO Inhibitor (mg/Kg)</th>
<th>Diet</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Iproniazid PO(_4) 250</td>
<td>Control</td>
<td>All within 8 days</td>
</tr>
<tr>
<td>II</td>
<td>Iproniazid PO(_4) 175</td>
<td>Control</td>
<td>2 within 21 days</td>
</tr>
<tr>
<td>III</td>
<td>Pargyline HCl 25</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td>IV</td>
<td>Pargyline HCl 10</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>None</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td>VI</td>
<td>None</td>
<td>2 % Thyroid</td>
<td>None</td>
</tr>
<tr>
<td>VII</td>
<td>Iproniazid PO(_4) 250</td>
<td>2 % Thyroid</td>
<td>All within 2 days</td>
</tr>
<tr>
<td>VIII</td>
<td>Iproniazid PO(_4) 175</td>
<td>2 % Thyroid</td>
<td>All within 6 days</td>
</tr>
<tr>
<td>IX</td>
<td>Pargyline HCl 25</td>
<td>2 % Thyroid</td>
<td>All within 14 days</td>
</tr>
<tr>
<td>X</td>
<td>Pargyline HCl 10</td>
<td>2 % Thyroid</td>
<td>All within 15 days</td>
</tr>
</tbody>
</table>
Blood Pressure and Urinary Dopamine Studies.

The normal, mean systolic blood pressure and standard deviation (S.D.) of the 60 rats were calculated to be $110 \pm 7.66$ mm Hg. Confidence limits (99%) of 107.4-112.6 mm Hg were calculated for the true mean in the untreated rats.

It can be seen from Figures 1-4 that a slight time factor exists in the response to drug or thyroid treatments. Moreover, most animals in the MAO inhibitor groups did not show synchronous changes in blood pressure so that the intragroup variability, for any given day, was generally more marked than that observed in control or pretreatment periods. Therefore, the following very conservative statistical procedure and reasoning was adopted: the S.D. of the mean of a sample of 6 animals, drawn from the same population as the 60 control observations, would be the S.D. of the general population divided by the square root of 6, i.e., 2.4495. Any mean of a sample of size 6 that differed from the population mean by more than 2.58 times the S.D. divided by 2.4495 would be significantly different at the 1% level. When, as in the present situation, the true S.D. is not known, the estimated value (7.66 mm Hg) may be used if the normal distribution critical value of 2.58 is replaced by the critical value of Student's "t" with the appropriate number of degrees of freedom, i.e., d.f. = 59; "t" = 2.660. Thus, making the conservative assumption that the true mean is as likely to be at the upper boundary of the confidence interval as at its center, any mean of a sample of 6 that lies outside the calculated confidence interval by more than 2.660 times the S.D. divided by 2.4495 is significantly different from the true mean of the control population,
at the 1% level, if indeed the confidence interval does contain the true mean. Since we have 99% confidence that it does contain the mean, we can say with not less than 98.01% confidence that any such outlying small sample (6) mean comes from a population other than that of the untreated rats, and is, therefore, a true difference. Applying these calculations, 98% "critical limits" were determined for the control mean blood pressure and are represented by the continuous, horizontal lines in Figures 1-4.

The 24-hour pooled urinary dopamine concentrations of the nonmedicated controls (group V) were estimated on days 8 and 15; these quantities were found to be 362 and 475 µg/l of urine respectively. In our laboratory, these amounts are considered representative. The mean arterial blood pressures taken on these same days were 110 ± 8.5 and 112 ± 7.9 mm Hg respectively.

As shown in Figure 1, iproniazid at the 175 mg/Kg daily dose level (group II) uniformly caused statistically significant pressor effects after day 1.

Blood pressures continued to rise until day 5 when they plateaued. On days 9 and 10, the systolic pressures fell precipitously and, except for a rise on day 11 of treatment, became rather erratic and remained only barely above or within normotensive levels for the remainder of the treatment period. The urinary dopamine level for this same group was almost
twice the normal on day 8. On day 15, where the mean blood pressure, surprisingly enough, was almost normal (116 ± 27.0 mm Hg), the excretion was approximately 5 times greater than customary.

The blood pressure effects of pargyline at the 25 mg/Kg daily dose level are shown in Figure 2.

In contrast with iproniazid, pargyline produced an initial, transient hypotensive effect that reached a maximum on day 3. After day 6, mean pressures rose for 3 days and attained a mean peak of about 155 mm Hg on day 9. Pressures fell on day 10, but they stabilized slightly above normotensive levels thereafter. At the lower dose (10 mg/Kg daily, group IV), pargyline elicited somewhat similar blood pressure effects, but the mean, peak pressor effect (163 mm Hg) did not occur until day 13. On days 16 and 17, mean pressures fell to about 127 mm Hg, whereupon a pressor effect (up to 145 mm Hg) again supervened on day 18 that persisted until the end of the treatment period.

The feeding of desiccated thyroid caused a definite, gradually developing pressor effect first noted on day 4; peak pressures of about 160 mm Hg occurred on day 16 (Figure 3).
Individual blood pressures in this group were much less variable than in any of the MAO inhibitor-treated animals. There was no evidence of a return to normotensive levels while thyroid feeding was continued.

Irrespective of the pressor responses, pooled dopamine excretion values on days 8 and 15 were 482 and 517 µg/l of urine respectively. These values for the thyroid-fed group were not considered abnormally high.

The feeding of 2% thyroid to iproniazid-medicated animals (groups VII and VIII) decimated the respective groups in such rapid fashion (Table I) that the recording of the erratic blood pressures and blood glucose concentrations was made almost impossible and gave little basis for interpretation.

Figure 4 shows the blood pressure effects in rats treated with 25 mg/Kg of pargyline and 2% thyroid.

It is obvious that although the variation in blood pressures among the individual animals of the group was large, a definite pressor effect began
after the second day and, except for a fall on day 9, was maintained until day 12 when the blood pressures of the surviving animals of the group fell sharply thereafter to normotensive or slightly hypotensive levels. No evidence of any initial transient fall in blood pressure as in the pargyline-treated rats was detected.

The collateral treatment of rats with 10 mg/Kg of pargyline and 2 % thyroid gave effects roughly equivalent to the higher dose of pargyline and thyroid feeding. The peak mean pressure occurred on day 6 and thereafter fell slowly, but erratically, to a minimum of 88 mm Hg on day 12. The blood pressures stabilized at about 105 mm Hg thereafter. The entire group died between days 14 and 15.

Fasting Blood Glucose Studies.–

The blood glucose mean and standard deviation of the 60 control measurements were calculated to be 104 ± 11.20 mm %. Confidence limits (99 %) of 107.9-100.1 mg % were calculated for the true mean in the untreated rats. Employing the same statistical reasoning as adopted for the blood pressure studies, the 98 % "critical limits" were determined for the control mean and are represented by the broken, horizontal lines in Figures 1-4.

As readily evident from the figures, 3-week daily treatment with either one of the enzyme inhibitors and/or desiccated thyroid generally failed to statistically modify mean blood glucose concentrations although fluctuations did occur; only in those animals near death from MAO inhibitor treatment did the concentrations fall.
DISCUSSION

Although not quantitated, both hydrazide and nonhydrazide MAO inhibitors in the doses employed did not elicit any gross evidence of hyperexcitability or an increase in general random motor activity. Certainly one explanation for the generalized depression of activity may be simply a reflection of subacute toxicity. The hyperirritability of the hyperthyroid rats, as well described by a number of other workers (French, 1912; Watanabe and Nomura, 1937), was completely reversed in those animals receiving both thyroid and an MAO inhibitor. The somato-motor weakness and sluggishness of these animals most likely is a reflection of general apathy and an index of an accelerated toxicity in animals whose general metabolism has already been seriously disturbed.

The general increase in food and water intake of the thyroid-fed rats, irrespective of their hampered weight gains, agrees with the reports of Carlson et al. (1912), French (1912), and Barker (1951); these effects are undoubtedly accounted for by an increased metabolic rate and the constant need to satisfy such a demand. No doubt the collateral use of vitamins A and D offset, to an extent, some of the adverse effects of high doses of thyroid. The exaggerated weight decline in the animals dosed with both hormone and an MAO inhibitor is most likely a nonspecific symptom of drug-induced toxicity.

The diarrhea noted in almost all thyroid-fed rats is a commonly observed untoward effect (gastrointestinal hypermotility) in thyroid-sensitive animals (Carlson et al., 1912; French, 1912). The marked diarrhea in
the MAO inhibitor-treated rats is difficult, however, to reconcile with the frequently reported constipating effect of iproniazid in man (O'Connor et al., 1953; Alexander and Berkeley, 1959; Weiss et al., 1959). The elimination of fecal matter coated with mucus was also a common finding in the iproniazid- and pargyline-medicated rats. Hypermucosity has been reported with the clinical use of other MAO inhibitor antidepressants (Sainz, 1959); this side effect in both man and rat may possibly indicate incipient gastroenteritis.

Hematuria and the presence of laked erythrocytes were commonly found in blood samples from iproniazid - but not pargyline-treated rats. The hemolytic effects of hydrazine and its analogues are well-documented, and the above toxicity we observed may be related to the hydrazine moiety of iproniazid. Hemolytic anemia has only rarely been reported to occur in man (O'Connor et al., 1953) with iproniazid use. Moreover, histopathologic studies in other rodents medicated with relatively high doses of hydrazides have shown signs of increased erythrocyte destruction, as evidenced by hemosiderosis of the kidney, liver, and spleen, and hyperemia or darkening of the latter organ (Benson et al., 1952; Zbinden and Studer, 1959). These reports, the hemolysis noted in our study, and the reported hepatic and splenic accumulation of iproniazid after parenteral administration (Koechlin and Iliev, 1959) may explain the organ darkening and injury observed by us. The lack of gross hemolytic changes with pargyline contributes presumptively to the impression that such toxicity is most probably due to the hydrazine moiety of iproniazid. A rather singular toxic effect was the reddish ocular discharges (chromodacryorrhealike) that occurred with subacute administration of both iproniazid and
pargyline. The only comparable effect, known to us, is the report of an ocular discharge of a porphyrin-like material in rats given subacute doses of tryptamine acetate, a new indole MAO inhibitor (Gray, 1961).

The rapid onset of iproniazid toxicity was not particularly surprising as the intraperitoneal doses used were very high. What was unexpected, however, were the rapidity and high incidence of fatalities in the simultaneously enzyme inhibitor- and thyroid-medicated rats. The administration of desiccated thyroid or thyroxine is known to modify the susceptibility of animals to the toxic effects of a number of drugs. Although large doses of thyroid may produce liver damage (Barker, 1951), the rapid onset of toxicity and high incidence of fatality in those animals receiving either one of the synthetic MAO inhibitors and thyroid seems to imply that the metabolic effects of the latter accentuate the direct, subacute toxicity of large doses of the antidepressants. This assumption is further strengthened by our previous findings that hyperthyroidism increases the acute toxicity of pargyline in rats (Carrier and Buday, 1961). Whether these toxic effects, acute or subacute, are in any way related to a combination of the in vivo MAO inhibitory effects of thyroid hormone and the synthetic enzyme inhibitors with a resultant elevation of catecholamines (Leduc et al., 1955; Zile and Lardy, 1959) or serotonin (Put and Hogenhuis, 1962), no conclusion can be drawn at this time; further work (behavioral and enzymatic), however, is in progress to study these possible causal relationships. In any event, the stress of impending thyrotoxicosis in the MAO inhibitor-treated animals in our study may, in some superficial fashion, parallel the clinical reports of a pharmacotherapeutic incompatibility during
simultaneous medication with thyroid extract and irreversible MAO inhibitors (Bailey et al., 1959; Kline, 1961).

The blood pressure responses to prolonged administration of both iproniazid and pargyline showed large variations and may be explained on the basis of oscillating responses and the small sample size. Because of the great dose difference between iproniazid and pargyline, little comparative importance can be given to the latter drug's minor primary hypotensive effects. What is perhaps of some importance is the fact that a pressor effect induced by repeated dosing with both drugs did occur. Hypotension is a common side effect during protracted medication with the MAO inhibitors and when given acutely to animals in relatively low doses (Cahn and Herold, 1962). Single, massive parenteral doses of iproniazid, however, cause a rise in rabbit blood pressure (De Palol and Lemberg, 1962). Dopamine, the biogenic precursor of norepinephrine and epinephrine, is a weak pressor material in the rat (Vogt, 1959). Its metabolism is reduced or blocked by MAO inhibitors (Horwitz et al., 1962), and we speculated that the pressor effects persisting from the second to eighth day with iproniazid dosing might be correlated with the urinary excretion of the catecholamine. No such correlation was able to be made, however. Dopamine excretion values were elevated approximately 100% on day 8 when the mean systolic blood pressure was some 55 mm Hg above the normal, but on day 15, when dopamine excretion values were increased about 400%, the mean blood pressure was within normal limits.

Hypertension is a frequent concomitant of clinical hyperthyroidism (Logan,
1930). In rats, thyroxine-induced hypertension was explained by Samiy (1952) as due to an altered receptor sensitivity to dopamine. Dopamine excretion was not significantly elevated in our thyroid-fed rats, however, and hence the pressor effects in the hyperthyroid rats may have no relationship to this catecholamine. Furthermore, hyperthyroidism inhibits hepatic dopa decarboxylase, the enzyme responsible for the rapid conversion of 3,4-dihydroxyphenylalanine (dopa) to dopamine (Clark, 1959). Considering this, and the avidity of MAO for dopamine, it is tempting to speculate about these mutually antagonistic properties of thyroid hormone on those enzymes playing a significant role in the biotransformation of dopamine and the reason for the normal dopamine excretion values in hyperthyroid rats.

Whereas the daily injection of pargyline alone uniformly elicited a slight, primary depressor response lasting as long as the fourth day of medication, the administration of pargyline to thyroid-fed rats obliterated this initial effect; a pressor effect was evoked as early as day 3 and although temporary, the pressor effects were somewhat greater than that with either hormone or drug given alone. The sudden and precipitous fall in blood pressure occurring on days 11 and 12 in those groups (IX and X) receiving both pargyline and thyroid cannot be readily explained. Certainly cardiovascular collapse, a prelude to death, is a possibility.

Parenteral administration of hydrazine sulfate in large doses elicits hypoglycemia in animals (Underhill, 1911), and clinical reports of diminished blood glucose levels during iproniazid therapy have been published (Weiss et al., 1959; Aksel, 1960). Contrary to expectations, however, no statistically
significant blood glucose changes occurred with iproniazid; no significant changes occurred with pargyline. Similarly, thyroid feeding elicited no blood glucose changes in rats; ordinarily the feeding of large quantities of thyroid to dogs and rats (Houssay, 1944) or hyperthyroidism in man (Geyelin, 1915) results in a definite hyperglycemia. The reason for our inability to duplicate these effects in our study is obscure.

SUMMARY

Daily intraperitoneal injections of massive doses of iproniazid phosphate and high doses of pargyline hydrochloride for as long as three weeks markedly reduced the general motor activity and body weight of male rats. Both of these effects, the general malaise, and the incidence of mortality was increased by the daily feeding of a high concentration of desiccated thyroid. Limited gross and microscopic studies showed that iproniazid (175 mg/Kg daily) caused hepatic, splenic, and renal injury, and hemolysis. Pargyline (10 and 25 mg/Kg daily), other than causing splenic darkening, showed no comparable effects.

Although exhibiting large fluctuations, systolic blood pressures rose rapidly, but only temporarily, in iproniazid-treated rats. Significant pressor effects in pargyline-dosed rats began only after 7 days. The collateral feeding of thyroid to pargyline-treated animals accelerated the onset and accentuated the pressor effects. The feeding of thyroid alone elicited a slowly developing and uniform pressor effect. Limited dopamine studies in normal, iproniazid- and thyroid-medicated rats showed that there was no apparent correlation between the pressor responses and the excretion
of dopamine. No significant changes in fasting blood glucose concentrations occurred during subacute administration of the MAO inhibitors or thyroid alone, nor when the treatments were concurrent.
ACKNOWLEDGMENTS

The iproniazid phosphate was generously supplied by Dr. Robert E. Dixon, Hoffmann-La Roche, Inc., and the pargyline hydrochloride was furnished through the courtesy of Dr. Guy M. Everett of Abbott Laboratories. The assistance of Dr. David R. DeFanti in performing the urinary dopamine studies is gratefully acknowledged. The authors are also grateful to Mr. William J. Dewey, Lederle Laboratories, for statistical advice.
REFERENCES


8. — Burn, J.H. and Marks, H.P. J. Physiol. (Lond.), 1925, 60, 131.


30. — Kline, N.S. J. Neuropsychiat., 1961, 2, 315


49. — Spinks, A. J. Physiol. (Lond.), 1952, 117, 35P.


60. — Zile, M. and Lardy, H.A. Arch. Biochem., 1959, 82, 411.
A method found simple and expeditious to produce an acceptable remade-chow tablet, using limited and easily accessible equipment, was to crush the hard, brittle pellets by use of a Wiley laboratory mill, passing the coarse material thence through a Mikro Samplmill (model 579A) to give a number 60 powder or finer. To each 100 g of this powder was added 40 ml of a warm, freshly prepared, gelatin-acacia solution made by dissolving 10 g of Gelatin (granular) U. S. P. and 1 g of Acacia (powder) U. S. P. in 100 ml of boiling distilled water. The binder and powders were then mixed thoroughly to form a damp mass. The latter was then sifted through a number 10 mesh brass screen to form granules. These particles were then passed through a number 20 mesh screen to obtain a finely granulated mass.

Using a hand driven, single punch, Stokes tablet machine (model A-3 Eureka) with a 1.27 cm standard concave punch and die set for maximum fill (1.09 cm), the damp granulation was compressed into 0.5 g convex tablets having 2.0 mm edge thickness. The pressure on the punch approximated 7.5 tons per square inch. The tablets were allowed to dry and harden thoroughly at ambient room temperature (approximately 21°C) for 36 hours or longer.