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The Effects of Monoamine Oxidase Inhibitors Upon Urinary Excretion of Dopamine and Changes in Arterial Blood Pressure

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THE EFFECT OF MONOAMINE OXIDASE INHIBITORS UPON URINARY
EXCRETION OF DOPAMINE AND CHANGES IN
ARTERIAL BLOOD PRESSURE

BY

CHARLES MARTIN VACCA

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

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MASTER OF SCIENCE THESIS
OF
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ABSTRACT

The relationship between urinary dopamine levels, and arterial blood pressure was studied in male albino rats.

The experimental rats were subjected to a right nephrectomy and received a monoamine oxidase inhibitor, either tranylcypromine or pargyline.

The blood pressure of the group receiving tranylcypromine was elevated to approximately 120 mm Hg during the first two weeks. During weeks seven and eight the blood pressure was further elevated to approximately 130 mm Hg but dropped back gradually to 115 mm Hg for a net drop of 3 percent where it remained to the end of the study. The blood pressure of the pargyline group, as expected, dropped steadily from approximately 115 mm Hg to approximately 84 mm Hg for a net drop of 34 percent.

Dopamine was extracted from paired urine samples by alumina and measured by spectrophotofluorimetry. Dopamine levels (mean from paired urine samples) of the experimental animals receiving pargyline or tranylcypromine were approximately 6 ug/ml/24 hour urine sample and 5 ug/ml/24 hour urine sample. These were lower than the levels of the control groups.

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I. INTRODUCTION

The catecholamines, epinephrine, norepinephrine, and dopamine have at times been implicated in producing or maintaining hypertension. Of primary interest in this study is 3-hydroxytyramine (dopamine), a precursor in the biosynthesis of norepinephrine and epinephrine; and monoamine oxidase (MAO), the enzyme which catalyzes the metabolic transformation of dopamine to norepinephrine. It was postulated that if monoamine oxidase was inhibited, the result would be an increase of the catecholamines such as dopamine, in the body, and that this increase or accumulation of dopamine conceivably helps to maintain arterial hypertension.

Goldblatt et al. (1934) revealed that ischemia in the kidneys produced an elevated systolic blood pressure. Ischemia in one kidney, while the other remains normal produces a temporary elevation of blood pressure about three or four days after the operation. However, if one kidney is removed and the artery of the remaining kidney constricted then permanent hypertension occurs.

DeFanti and DeFeo (1963) have shown a possible relationship between blood pressure and urinary dopamine, and a correlation between increased urinary dopamine levels and a decreased monoamine oxidase activity.

How, then could one of these catecholamines come to exist in excess in the body? Bing (1941) demonstrated that a pressor substance, possibly dopamine, formed by decarboxylation of DOPA, occurred in extracts of guinea pig kidneys

incubated under anerobic conditions. Schroeder (1942) showed that deamination but not decarboxylation of some amino acids is incomplete in kidneys deprived of their normal oxygen supply. Giordano et al. (1960) confirmed the finding of Schroeder when he found a decrease in MAO activity in the kidneys of rabbits rendered hypertensive by renal ischemia. DeFanti (1963) demonstrated a decrease in MAO activity in the kidneys of rats rendered hypertensive by renal ischemia.

This study will attempt to demonstrate that when chemicals such as MAO inhibitors are administered to unilaterally nephrectomized rats in the absence of renal constriction, a rise in blood pressure and an increase in urinary dopamine occurs. The issue is approached by studying the relationship between arterial blood pressure and urinary dopamine levels in rats receiving a monoamine oxidase inhibitor.

II. REVIEW OF LITERATURE

The disease hypertension has been known for centuries and has been actively studied for the past thirty years. It is of considerable interest that the etiology of the disease remains unknown to this day.

The catecholamines epinephrine, norepinephrine and dopamine and the disease hypertension have long been associated with each other. The discovery that dopamine is a precursor in the biosynthesis of norepinephrine and epinephrine (Blaschko, 1957), and the discovery that monoamine oxidase is an enzyme primarily involved in the metabolic transformation of dopamine, has led to the investigation of the catecholamines and particularly dopamine as a possible factor in the cause and maintenance of arterial hypertension.

The biochemical pathway of the formation of epinephrine is shown in figure 1. According to this accepted pathway, norepinephrine is the immediate precursor of epinephrine. It is clear then, that norepinephrine, a neurohormone in its own right is also an intermediate in epinephrine formation. The role of dopamine is not yet clearly understood as to whether it is a neurohormone as well as a precursor in the biosynthesis of norepinephrine and epinephrine. The fact that dopamine disappears from brain tissue after administration of reserpine (Carlsson, et al. 1962) might give some indication of a functional role for dopamine.

Studies of the circulatory effects of dopamine have been carried out in animals, but the results appear to differ with the species. Dopamine acts as a pressor substance

Fig. 1. The enzymatic synthesis of norepinephrine and epinephrine.

when given intravenously in the cat (Holtz et al. 1942) and also in the dog (Goldberg et al. 1959) whereas in the guinea pig and rabbit it is a depressor substance (Holtz et al. 1951).

It has recently been demonstrated that in the dog, dopamine is a depressor substance when administered in small doses but a pressor substance when administered in larger doses (McDonald et al. 1962).

Although in 1951 Von Euler demonstrated the presence of dopamine in the urine of patients afflicted with phaeochromocytoma, very few studies of the circulatory effects of dopamine have been carried out in man. (Horwitz, et al. 1962) infused a 1 percent solution of dopamine in healthy subjects for up to thirty minutes and concluded that a dose of 5 to 11 ug/kg/minute increased the systolic blood pressure, increased the cardiac output and effected a change in the heart rate. To confirm the findings of Horwitz et al., Allwood and Ginsberg in 1962 infused dopamine at a rate of 1 ug/minute and found a corresponding increase in systolic blood pressure up to two-hundred millimeters of mercury.

The local effect of dopamine in the hand was also studied (Allwood et al. 1962). They concluded that since dopamine exerted different responses in the forearm, it therefore reacted with both alpha and beta receptors in the forearm vascular bed and that this preference was dependent upon the concentration of dopamine present. It is not known whether these peripheral vascular responses resulted from the direct effect of dopamine or whether dopamine is converted to another catecholamine. The peripheral vascular effects of

dopamine in man is approximately one-fiftieth that of norepinephrine when infused at a corresponding dose rate. According to Horwitz et al. (1962) the effect of dopamine upon systolic blood pressure is evident within two to four minutes after starting the infusion. This is much longer than the time required for changes in systolic blood pressure to become evident with infusions of epinephrine or norepinephrine. This slight delay (one minute) is probably caused by the rate of conversion of dopamine to norepinephrine in vivo, however, it appears since the effects of dopamine and norepinephrine are different, e.g. on heart rate, that the effects of dopamine upon heart rate are not dependent upon the formation of norepinephrine. Dopamine also differs from other catecholamines in another respect inasmuch as Allwood & Ginsberg (1962) described piloerection in the forearm during intra-arterial dopamine infusions. Other catecholamines cause piloerection in man when introduced intradermally but they do not when infused intra-arterially in physiological concentrations. As piloerection is part of an emotional or fright response in man, it has been suggested that the role of dopamine in the body is not solely as a precursor of norepinephrine.

Monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) are of great importance in the metabolic transformation of epinephrine, norepinephrine and dopamine. MAO also oxidatively deaminates serotonin (5-HT). It appears that MAO metabolically transforms intracellular catecholamines while COMT metabolically transforms circu-

lating catecholamines. The enzyme MAO is located almost entirely in the mitochondrial fraction of cells.

The intracellular concentration of dopamine differs from other catecholamines. The cytoplasm contains an abundance of dopamine whereas much less is found in storage granules (Jarvik, M.E. 1964). Dopamine is also distributed differently in the CNS than other catecholamines.

MAO inhibitors produce an irreversible inactivation of MAO by forming a stable inhibitor-enzyme complex (Jarvik, M.E. 1964). Since the synthesis of biogenic amines is not inhibited, and since MAO which limits the level of intracellular amines is inhibited, amine concentration within the cell will increase. It is not known as yet to what extent the therapeutic action of MAO inhibitors is due to increases in intracellular concentration of biogenic amines.

After administration of a single dose of a MAO inhibitor endogenous norepinephrine, epinephrine, dopamine and 5-HT levels increase in various tissues, including the heart, brain, and blood. MAO inhibitors also enhance the effect of exogenous amines such as 5-HT and norepinephrine, as well as their precursors, 3,4-dihydroxyphenylalanine (DOPA) or 5-hydroxytryptophan. These amino acids are capable of penetrating the blood-brain barrier and are decarboxylated in the brain to their corresponding amines. In contrast to the precursors, these amines penetrate the barrier with difficulty.

The degree to which MAO is inhibited in a particular organ of the body varies with the route of administration, the MAO inhibitor and the substrate used for determining the

degree of inhibition (Zeller and Fouts, 1963).

Tranylcypromine which is (Parnate^R)¹ was synthesized in 1948 as an amphetamine analog (Burger, A., and Yost, W.: J. Am. Chem. Soc. 70:2198, 1948). The structures for tranylcypromine and amphetamine are shown in figure 2.

These agents are psychomotor stimulants and tranylcypromine is used clinically in the treatment of psychoneurotic and psychotic depression. Tranylcypromine is also a potent inhibitor of MAO. The pharmacology of tranylcypromine is rather scanty but it appears to resemble the pharmacology of the hydrazines; e.g. tranylcypromine elicits signs of CNS stimulation and sympathomimetic activity. Because tranylcypromine interferes with various enzymes such as MAO, it is able to prolong and intensify the action of other drugs and to interfere with steps in the metabolic pathways of several naturally occurring substances.

It is believed that the action of sympathomimetic amines is potentiated following the administration of MAO inhibitors. This effect is mainly upon indirectly acting amines such as tyramine and amphetamine rather than upon directly acting amines (norepinephrine or epinephrine). Administered catecholamines are metabolically transformed by the enzyme COMT, hence, MAO inhibitors will have little or no effect upon prolonging and intensifying their action. However, certain sympathomimetic amines act peripherally, primarily by releasing catecholamine stores in nerve endings, and

1. Registered tradename, Smith, Kline & French Laboratories, Philadelphia, Pennsylvania.

Fig. 2. Structures for tranylcypromine and amphetamine.

since the level of amines is raised by MAO inhibitors profound potentiation of effects such as pressor action may be expected.

III. INVESTIGATION

A. OBJECTIVES

The object of this investigation was to determine the effect of monoamine oxidase inhibitors upon urinary excretion of dopamine and to correlate this effect with changes in arterial blood pressure.

The specific objectives of this investigation were:

1. To determine if there is a decrease in the biological metabolism of dopamine as evidenced by an increase in dopamine in the urine of male albino rats receiving a monoamine oxidase inhibitor and unilateral nephrectomy.

2. To determine if the arterial blood pressure is elevated during this period and if there is a correlation between this elevated arterial blood pressure and the increased urinary dopamine level.

B. MATERIALS AND METHODS

1. General Considerations and Daily Protocol

In this study rats were divided into six groups for daily intraperitoneal injection of either tranylcypromine, (Parnate^R)¹, pargyline, (Eutonyl^R)² or the vehicle.

Male, albino rats of the Sprague-Dawley strain³ weighing 90-100 g were distributed two to a cage in standard metabolism cage banks⁴ and housed in a room maintained at 22 $\pm$ 1 degree centigrade and approximately 50 percent relative

-
1. Registered tradename of Smith, Kline & French Laboratories, Philadelphia, Pennsylvania.
 2. Registered tradename of Abbott Laboratories, North Chicago, Illinois.
 3. Charles River Breeding Farms, North Wilmington, Massachusetts.
 4. Wahmann Mfg. Co., Baltimore, Maryland.

humidity. The room was lighted by daylight through two windows from sunrise to sunset. All studies except blood pressure determinations were carried out in this room.

Each pair of animals was given 30 g of Purina¹ rat chow daily at 5 PM. Fresh tap water was allowed ad libitum from two jars of approximately 250 ml capacity in each cage. All animals were weighed and injected daily from 9 AM to 1 PM. Water jars, containers for the collection of urine and cages were cleaned and reset at this time.

Drugs were prepared for intraperitoneal injection as follows: 500 mg of tranylcypromine sulfate was dissolved in sufficient glass distilled, de-ionized water to make 500 ml of solution to give a concentration of 1.0 mg/ml. 5000 mg of pargyline hydrochloride was dissolved in sufficient glass distilled, de-ionized water to make 500 ml of solution to give a concentration of 10.0 mg/ml.

Drug Doses were as Follows:

tranylcypromine sulfate 3.3 mg/kg body weight
pargyline hydrochloride 25 mg/kg body weight
vehicle (glass distilled water) equal volume

This investigation was carried out in two separate parts.

In Part 1: 74 animals were divided into six groups as follows and received the following treatments:

Group I: Unilateral nephrectomy and tranylcypromine

1. Ralston Purina Co., St. Louis, Missouri.

sulfate 3.3 mg/kg of body weight.

Group II: Unilateral nephrectomy and pargyline hydrochloride, 25 mg/kg of body weight.

Group III: Unilateral nephrectomy and sham injection.

Group IV: Sham operation on right kidney and tranylcypromine sulfate, 3.3 mg/kg of body weight.

Group V: Sham operation on right kidney and pargyline hydrochloride, 25 mg/kg of body weight.

Group VI: Sham operation on right kidney and vehicle.

In addition, body weights, 24 hour urine sample and blood pressures were determined.

In Part 2, 592 urine samples were assayed for 3-hydroxytyramine (dopamine). The animals were sacrificed and the remaining kidney or kidneys removed and immediately frozen in liquid nitrogen for future analysis.

A preliminary study involving four rats was undertaken to determine gross toxicity effects of a daily injection of 3.3 mg/kg of tranylcypromine sulfate.

2. Preparation of Unilaterally Nephrectomized Rats

Male albino rats of the Sprague Dawley strain¹ weighing 90-100 g were used for this study. To remove the right kidney, a dorsolateral incision approximately one-half inch in length was made midway along the spinal column on the dorsal side. The kidney was located retroperitoneal to the abdominal cavity and lateral to the incision. The kidney was forced up through the incision by manipulating it with the

1. Charles River Breeding Farms, North Wilmington, Massachusetts.

fingers and exposed for surgical removal. At this point the adrenal gland was stripped from the kidney and left intact. The renal artery and vein were then ligated with nylon thread, the kidney was removed and immediately frozen in liquid nitrogen for future analysis.

The incision was closed with stainless steel wound clamps. Ether was the anesthetic used throughout the operation.

3. Collection of Urine Sample

The experimental rats were left for two weeks in a cage to recover and adjust to the nephrectomy. After the two week convalescent period, the animals were paired and placed in standard metabolism cages equipped with stainless steel funnels for collecting the urine. Urine collections were made three times weekly for the first few weeks then weekly for the remainder of the study. Twenty-four hour urine samples were collected in graduated cylinders containing 1.0 ml of 2.0 N sulfuric acid and immediately frozen at minus twenty degrees centigrade for future analysis.

4. Determination of Blood Pressure

All blood pressure studies were carried out in an Environrol constant temperature chamber at 28 ± 1 degree centigrade which maintained low humidity by a rapid exchange of air within the chamber. To determine the blood pressure, the indirect systolic blood pressure tail-cuff method was employed. The rats were removed from their cages, placed

into a plastic carrying cage, and heated in an incubator-box equipped with two 25 watt electric bulbs which maintained the temperature in the incubator at approximately 40 ± 2 degrees centigrade. The rats were exposed to this temperature for approximately fifteen minutes. After warming, each rat was encased in a plastic chamber about 23 cm long by 7 cm wide and 5 cm in height. The plastic holder is closed at one end, but by manipulating a movable piston, the internal length of the chamber was adjusted to fit each rat. The detachable partition was secured in place, leaving only the rat's tail exposed.

An inflatable tail-cuff connected to a physiograph manometer system was placed around the base of the rat's tail. The tail was passed through a plastic holder¹ so designed that when the screwclamp arrangement was tightened, the tail was pressed into contact with a Beckman Infraton² signal divider set to receive a maximum impulse. The Infraton output lead was plugged into the direct current input of a Hewlett-Packard oscilloscope Model 130B³ set at a sweep time of 20 msec/cm.

With this system the systolic pressure pulsations in the rat's tail were converted to peaks on the oscilloscope screen. When the rubber bulb of the manometer system was manually squeezed, the pressure within the system increased,

-
1. Constructed by Dr. John J. DeFeo, Dept. of Pharmacology, University of Rhode Island.
 2. Beckman Co., Palo Alto, California.
 3. Hewlett-Packard Co., Palo Alto, California.

hence, the cuff became inflated and the peaks on the oscilloscope screen disappeared, as the circulation in the tail was depressed. The pressure within this system was regulated by opening the pressure escape valve on the manometer. As the circulation in the tail increased, the peaks reappeared upon the screen. When this occurred the systolic blood pressure was considered to be present because the pressure in the arteries is greater than the pressure in the manometer system.

The rat blood pressure recorded on a particular day was the mean of at least three successive determinations not varying by more than approximately five mm of Hg.

5. Preparation of Chromatographic Column

Pyrex glass tubing approximately seven cm in length and one cm in diameter was drawn to a point approximately one-half cm in diameter. One end was fitted with a piece of polystyrene tubing approximately three cm in length attached to a funnel. A vinyl tube three cm long and one-half cm in diameter conducted the eluate into a beaker. These glass columns permitted a flow rate of approximately fifteen drops per minute.

6. Preparation of Aluminum Oxide

One-hundred g of aluminum oxide¹ was washed with 500 ml of 2.0 N hydrochloric acid for approximately twenty minutes at eighty degrees centigrade on a Temco controlled heat stir

1. Aluminum Oxide for Chromatographic Adsorption Analysis, The British Drug Houses Ltd. B.D.H. Laboratory Chemical Division, Poole, England. Distributed in the U.S.A. by Gallard-Schlesenger Chemical Mfg. Corp., Carle Place, L.I., N.Y.

plate. The alumina was then filtered through a sintered glass filter and washed again with 500 ml of hot 2.0 N hydrochloric acid. The alumina was suspended in glass distilled water and the supernatant liquid decanted carrying with it fine particles; about twenty seconds was allowed for the alumina to settle before decanting. This procedure was repeated ten times. The alumina was heated in an oven at one-hundred degrees centigrade until dry.

7. Extraction of Catecholamines from Urine

The urine sample was thawed and centrifuged for fifteen minutes. A 5 ml or 10 ml sample was transferred by volumetric pipet to a clean pyrex test tube. One to two drops of concentrated hydrochloric acid and 2 ml of 0.25 M ethylenediaminetetracetic acid, di-sodium salt (EDTA) were added to the sample; the tubes were stoppered, heated in a water bath at one-hundred degrees centigrade for twenty minutes and cooled in a continuously flowing cold water bath. The sample was transferred with three 5 ml washings of glass distilled water to appropriate beakers. The pH of the sample was adjusted to 8.4 by titrating with 1 N sodium hydroxide; during the titration 1 ml of sodium bisulfite solution containing 10 mg/ml was added. The sample was then ready to be passed through a chromatographic column containing approximately 700 mg of acid washed alumina.

8. Extraction of Dopamine from the Urine

The pH of 700 mg of acid washed alumina in 10 ml of ammonium acetate buffer pH 8, was adjusted to a pH of 8.3 by adding 0.3 ml or five drops of 1 N sodium hydroxide. The

columns were packed and washed with five ml of glass distilled water. The above prepared urine sample was passed through the column, followed by 5 ml of 0.1 M sodium acetate, and 5 ml of glass distilled water. At this point the dopamine had been taken up by the alumina. Elution was accomplished with two consecutive 5 ml volumes of 0.2 N acetic acid which were collected in a clean dry beaker.

9. Preparation of Eluate for Development

A 3 ml aliquot of eluate was transferred to a clean calibrated pyrex tube. One ml each of 0.3 M potassium carbonate and 0.1 M phosphate buffer pH 6.5, solution was added.

10. Development of 3 ml Eluate

Two drops of 0.25 M freshly prepared iodine solution was added to each tube containing the 3 ml eluate, phosphate buffer and potassium carbonate and mixed on a Vortex mixer allowing three minutes for completion of the iodine addition. After a total time of exactly three minutes from addition of iodine to the first tube, 1 ml of alkaline sulfite reagent was added and again mixed. Exactly four minutes from the addition of the alkaline sulfite solution, 1 ml of 6 N acetic acid was added. All volumes were adjusted to 10 ml. These sample eluates were heated in a water bath at one-hundred degrees centigrade for five minutes, cooled and centrifuged for ten minutes. A sufficient quantity was poured into clean, clear fused quartz cells and irradiated for ten minutes under short wave ultra-violet light.

The fluorescence of the samples was read on an Aminco-

Bowman Spectrophotofluorometer.¹ The activation and fluorescent wavelengths were 325 mμ and 380 mμ (uncorrected instrument values) respectively.

A standard curve of known concentrations of dopamine was run with each set of samples assayed. Dopamine concentrations of 0.5 ug, 1.0 ug, 2.0 ug, and 3.0 ug respectively were used. These known dopamine concentration samples were treated experimentally as the urine samples with the exception of passing the urine samples through the alumina columns.

With each group of urine samples, two samples of known dopamine concentration were assayed to ascertain per cent recovery. The known dopamine concentration samples were prepared by adding 2.0 ug of dopamine to 10 ml of distilled water and treated throughout the assay exactly as the urine samples.

A linear relationship such as reported by Carlsson and Waldeck (1958) was found to exist between dopamine concentrations of 0.05 ug, 0.1 ug, 0.2 ug, 0.3 ug, 0.4 ug, and 0.5 ug/ml and fluorescent intensity (figure 3).

11. Weight Gain Studies

The effects of daily treatment with tranylcypromine, pargyline, unilateral nephrectomy and sham operation upon the daily body weights of each rat was determined.

All animals were weighed daily prior to treatment, to the nearest gram on a Ohaus small animal balance². Each rat was marked for identification and individual records were

1. American Instrument Company, Inc., Silver Spring, Maryland.
2. Ohaus Scale Corp., Union, New Jersey.

Fig. 3 Fluorescence intensity at varying concentrations of dopamine.

Fluorescence is given in arbitrary units
(meter multiplier value $\times$ % transmission
 $\times$ 100)

Activating wavelength 325 m μ ; fluorescent
wavelength 380 m μ .

TABLE 1

Percent recovery ^a of dopamine added to samples.

Dopamine Added ug/ml	Total Dopamine Calculated ug/ml	Number of Samples	Percent Recovery
2	1.46	51	73
4	3.32	4	83
6	4.95	4	82

^aAverage recovery equals seventy-nine percent.

C. RESULTS

All results, including values from the control group, values from the individual test groups, and statistical analyses are presented under this section.

1. Data for the Control Group are presented in TABLE 2.

TABLE 2

Control Group: Relationship of urinary dopamine levels (group mean) to arterial blood pressure (group mean) in male albino rats.

Week	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of Animals
1	0.5 $\pm$ 0.1	83.4 $\pm$ 18.3	15.2 $\pm$ 3.0	108.5 $\pm$ 2.1	14
2	0.4 $\pm$ 0.2	55.3 $\pm$ 20.2	10.6 $\pm$ 3.9	109.6 $\pm$ 1.8	14
3	0.3 $\pm$ 0.2	19.7 $\pm$ 17.8	3.5 $\pm$ 3.2	110.1 $\pm$ 3.8	14
4	0.6 $\pm$ 0.2	96.0 $\pm$ 47.2	21.9 $\pm$ 11.4	112.3 $\pm$ 3.9	14
5	0.4 $\pm$ 0.1	57.6 $\pm$ 38.6	10.6 $\pm$ 2.3	108.9 $\pm$ 3.2	14
6	0.3 $\pm$ 0.2	83.1 $\pm$ 23.2	12.0 $\pm$ 8.0	110.5 $\pm$ 3.6	14
7	0.4 $\pm$ 0.5	96.7 $\pm$ 53.2	18.9 $\pm$ 4.4	108.4 $\pm$ 4.4	14
8	0.8 $\pm$ 0.3	297.2 $\pm$ 90.9	22.3 $\pm$ 11.5	109.0 $\pm$ 5.6	14
9	1.7 $\pm$ 0.3	143.6 $\pm$ 114.4	67.4 $\pm$ 19.3	104.2 $\pm$ 9.7	14
10	1.3 $\pm$ 0.6	39.9 $\pm$ 28.6	37.8 $\pm$ 31.1	104.4 $\pm$ 4.4	14
11	0.4 $\pm$ 0.2	55.7 $\pm$ 26.2	8.3 $\pm$ 5.3	104.2 $\pm$ 4.2	14
12	0.3 $\pm$ 0.1	29.7 $\pm$ 17.0	13.9 $\pm$ 6.2	106.5 $\pm$ 2.7	14
13	0.2 $\pm$ 0.1	28.6 $\pm$ 5.2	7.0 $\pm$ 3.8	108.5 $\pm$ 3.8	14

2. Data for the Individual Test Groups 1-6 are presented in Tables 3-8.

TABLE 3

Relationship between urinary dopamine level and blood pressure in unilaterally nephrectomized male albino rats receiving Parnate 3.3 mg/kg.

Unilateral and Parnate	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
1	0.4 $\pm$ 0.1	55.0 $\pm$ 27.1	12.3 $\pm$ 6.1	122.1 $\pm$ 9.0	12
2	0.2 $\pm$ 0.1	31.9 $\pm$ 12.3	5.0 $\pm$ 1.7	123.9 $\pm$ 10.6	12
3	0.2 $\pm$ 0.1	23.0 $\pm$ 10.8	4.9 $\pm$ 2.3	116.8 $\pm$ 7.5	12
4	0.3 $\pm$ 0.1	27.0 $\pm$ 15.7	5.8 $\pm$ 3.3	114.0 $\pm$ 10.8	12
5	0.3 $\pm$ 0.2	48.0 $\pm$ 15.6	4.2 $\pm$ 3.4	109.5 $\pm$ 6.4	12
6	0.4 $\pm$ 0.1	23.9 $\pm$ 14.1	9.5 $\pm$ 3.3	125.1 $\pm$ 10.0	12
7	0.2 $\pm$ 0.1	15.1 $\pm$ 15.6	5.1 $\pm$ 3.0	131.8 $\pm$ 6.6	12
8	0.2 $\pm$ 0.1	20.4 $\pm$ 10.0	3.2 $\pm$ 3.3	128.1 $\pm$ 6.3	12
9	0.2 $\pm$ 0.1	17.7 $\pm$ 15.8	4.3 $\pm$ 2.1	118.7 $\pm$ 6.6	12
10	0.3 $\pm$ 0.2	14.5 $\pm$ 3.2	4.0 $\pm$ 3.4	107.8 $\pm$ 8.3	12
11	0.2 $\pm$ 0.1	14.6 $\pm$ 6.4	3.2 $\pm$ 0.8	116.4 $\pm$ 11.4	12
12	0.2 $\pm$ 0.1	38.9 $\pm$ 35.6	9.1 $\pm$ 8.6	115.0 $\pm$ 6.1	12

TABLE 4

Relationship between urinary dopamine level and blood pressure in unilaterally nephrectomized male albino rats receiving Pargyline 25 mg/kg.

Unilateral and Pargyline	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
1	0.2 $\pm$ 0.2	21.3 $\pm$ 22.3	4.6 $\pm$ 4.8	116.2 $\pm$ 11.0	16
2	0.3 $\pm$ 0.1	32.6 $\pm$ 18.6	6.5 $\pm$ 3.7	129.1 $\pm$ 8.9	16
3	0.7 $\pm$ 0.4	52.4 $\pm$ 35.2	11.1 $\pm$ 7.6	123.3 $\pm$ 10.0	16
4	0.4 $\pm$ 0.2	27.2 $\pm$ 17.9	5.9 $\pm$ 4.1	124.7 $\pm$ 15.4	15
5	0.2 $\pm$ 0.1	59.1 $\pm$ 41.9	2.6 $\pm$ 1.1	103.9 $\pm$ 10.7	16
6	0.7 $\pm$ 0.2	21.2 $\pm$ 8.9	11.5 $\pm$ 7.5	104.5 $\pm$ 12.7	16
7	0.3 $\pm$ 0.1	13.3 $\pm$ 6.5	4.6 $\pm$ 1.9	105.1 $\pm$ 17.0	15
8	0.2 $\pm$ 0.1	3.8 $\pm$ 6.1	2.7 $\pm$ 1.3	98.0 $\pm$ 13.1	15
9	0.2 $\pm$ 0.1	61.8 $\pm$ 43.6	0.9 $\pm$ 1.3	92.8 $\pm$ 10.2	15
10	0.7 $\pm$ 0.3	34.1 $\pm$ 27.9	16.2 $\pm$ 9.8	91.2 $\pm$ 6.2	15
11	0.4 $\pm$ 0.3	29.3 $\pm$ 21.9	7.5 $\pm$ 6.0	106.8 $\pm$ 9.0	15
12	0.4 $\pm$ 0.2	7.4 $\pm$ 14.9	6.2 $\pm$ 4.8	99.6 $\pm$ 13.0	13

TABLE 5

Relationship between urinary dopamine level and blood pressure in unilaterally nephrectomized male albino rats receiving sham injection.

Unilateral	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
1	0.2 $\pm$ 0.1	33.7 $\pm$ 10.3	6.1 $\pm$ 3.6	119.1 $\pm$ 11.7	10
2	0.5 $\pm$ 0.1	29.2 $\pm$ 17.3	16.3 $\pm$ 6.3	123.5 $\pm$ 9.8	10
3	0.6 $\pm$ 0.4	75.8 $\pm$ 31.9	7.3 $\pm$ 5.0	124.6 $\pm$ 8.7	10
4	0.6 $\pm$ 0.1	29.9 $\pm$ 20.0	18.2 $\pm$ 3.7	122.2 $\pm$ 10.5	10
5	0.6 $\pm$ 0.1	72.7 $\pm$ 11.2	9.1 $\pm$ 2.5	122.2 $\pm$ 4.5	10
6	0.8 $\pm$ 0.3	86.6 $\pm$ 53.4	21.0 $\pm$ 12.8	125.5 $\pm$ 4.9	10
7	0.7 $\pm$ 0.4	43.5 $\pm$ 27.0	11.0 $\pm$ 6.7	120.9 $\pm$ 6.4	10
8	0.3 $\pm$ 0.1	20.8 $\pm$ 11.2	5.1 $\pm$ 2.8	111.5 $\pm$ 7.0	10
9	0.2 $\pm$ 0.1	8.1 $\pm$ 7.1	2.0 $\pm$ 1.7	106.5 $\pm$ 4.5	10
10	0.2 $\pm$ 0.1	12.6 $\pm$ 7.8	3.5 $\pm$ 2.2	106.1 $\pm$ 3.8	10
11	0.2 $\pm$ 0.1	27.0 $\pm$ 31.2	6.9 $\pm$ 8.0	111.4 $\pm$ 13.1	10
12	0.4 $\pm$ 0.1	34.3 $\pm$ 13.3	8.7 $\pm$ 3.3	110.9 $\pm$ 6.4	10

TABLE 6

Relationship between urinary dopamine level and blood pressure in sham operated male albino rats receiving Parnate 3.3 mg/kg.

Sham and Parnate	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
1	0.3 $\pm$ 0.2	30.7 $\pm$ 21.6	6.3 $\pm$ 4.2	123.1 $\pm$ 7.9	14
2	0.2 $\pm$ 0.1	28.9 $\pm$ 11.3	5.2 $\pm$ 1.9	125.0 $\pm$ 7.0	14
3	0.6 $\pm$ 0.5	54.2 $\pm$ 43.7	11.4 $\pm$ 9.5	133.2 $\pm$ 7.3	14
4	0.6 $\pm$ 0.2	59.7 $\pm$ 29.1	13.1 $\pm$ 6.3	104.4 $\pm$ 8.2	14
5	0.3 $\pm$ 0.2	53.3 $\pm$ 24.7	6.6 $\pm$ 1.9	136.4 $\pm$ 6.1	14
6	0.5 $\pm$ 0.3	32.4 $\pm$ 27.6	11.2 $\pm$ 5.4	110.5 $\pm$ 8.9	14
7	0.4 $\pm$ 0.3	58.6 $\pm$ 59.5	7.2 $\pm$ 6.1	106.7 $\pm$ 7.4	14
8	0.7 $\pm$ 0.6	40.4 $\pm$ 27.4	12.1 $\pm$ 12.1	104.7 $\pm$ 3.1	14
9	0.5 $\pm$ 0.2	26.2 $\pm$ 16.7	8.9 $\pm$ 6.2	105.0 $\pm$ 4.0	14
10	0.6 $\pm$ 0.4	30.5 $\pm$ 9.5	6.9 $\pm$ 4.7	103.1 $\pm$ 9.8	14
11	0.3 $\pm$ 0.1	24.6 $\pm$ 13.6	7.2 $\pm$ 2.2	104.4 $\pm$ 8.6	14
12	0.2 $\pm$ 0.1	95.9 $\pm$ 45.5	5.4 $\pm$ 2.9	106.4 $\pm$ 6.0	14

TABLE 7

Relationship between urinary dopamine level and blood pressure in sham operated male albino rats receiving Pargyline 25 mg/kg.

Sham and Pargyline	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
1	0.5 $\pm$ 0.1	37.6 $\pm$ 18.0	8.6 $\pm$ 4.2	117.5 $\pm$ 7.0	12
2	0.4 $\pm$ 0.2	61.1 $\pm$ 40.6	12.0 $\pm$ 8.3	110.0 $\pm$ 6.4	12
3	0.5 $\pm$ 0.1	72.2 $\pm$ 31.1	15.7 $\pm$ 6.3	105.5 $\pm$ 6.2	12
4	0.9 $\pm$ 0.5	136.4 $\pm$ 81.6	30.9 $\pm$ 18.3	99.5 $\pm$ 5.7	12
5	0.8 $\pm$ 0.1	141.8 $\pm$ 34.4	14.6 $\pm$ 2.0	100.4 $\pm$ 6.2	12
6	1.1 $\pm$ 0.2	53.5 $\pm$ 35.1	32.9 $\pm$ 7.4	98.8 $\pm$ 4.6	12
7	0.5 $\pm$ 0.3	53.7 $\pm$ 28.0	12.7 $\pm$ 8.1	96.5 $\pm$ 4.9	12
8	0.6 $\pm$ 0.2	138.6 $\pm$ 56.0	12.1 $\pm$ 6.4	95.0 $\pm$ 5.8	12
9	1.1 $\pm$ 0.6	144.9 $\pm$ 88.8	30.4 $\pm$ 12.3	96.4 $\pm$ 9.7	12
10	1.6 $\pm$ 0.8	80.1 $\pm$ 22.6	35.7 $\pm$ 23.5	102.2 $\pm$ 9.6	12
11	1.1 $\pm$ 0.3	32.7 $\pm$ 21.7	19.1 $\pm$ 5.5	83.5 $\pm$ 10.1	12
12	0.3 $\pm$ 0.2	101.2 $\pm$ 49.4	7.2 $\pm$ 4.9	72.8 $\pm$ 7.7	12

TABLE 8

Relationship between urinary dopamine level and blood pressure in sham operated male albino rats receiving Solvent.

Sham and Solvent	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
1	0.5 $\pm$ 0.1	83.4 $\pm$ 18.3	15.2 $\pm$ 3.0	108.5 $\pm$ 2.1	10
2	0.4 $\pm$ 0.2	55.3 $\pm$ 20.2	10.6 $\pm$ 3.9	109.6 $\pm$ 1.8	10
3	0.3 $\pm$ 0.2	19.7 $\pm$ 17.8	3.5 $\pm$ 3.2	110.1 $\pm$ 3.8	10
4	0.6 $\pm$ 0.2	96.0 $\pm$ 47.2	21.9 $\pm$ 11.4	112.3 $\pm$ 3.9	10
5	0.4 $\pm$ 0.1	57.6 $\pm$ 38.6	10.6 $\pm$ 2.3	108.9 $\pm$ 3.2	10
6	0.3 $\pm$ 0.2	83.1 $\pm$ 23.2	12.0 $\pm$ 8.0	110.5 $\pm$ 3.6	10
7	0.6 $\pm$ 0.5	96.7 $\pm$ 53.2	18.9 $\pm$ 4.6	108.4 $\pm$ 4.4	9
8	0.8 $\pm$ 0.3	287.2 $\pm$ 90.9	22.3 $\pm$ 11.5	109.0 $\pm$ 5.6	9
9	1.7 $\pm$ 0.3	143.6 $\pm$ 28.6	67.4 $\pm$ 19.3	104.2 $\pm$ 9.7	9
10	1.3 $\pm$ 0.6	55.7 $\pm$ 26.2	37.8 $\pm$ 31.1	107.4 $\pm$ 4.6	9
11	0.4 $\pm$ 0.2	29.7 $\pm$ 17.0	8.3 $\pm$ 5.3	104.2 $\pm$ 4.2	9
12	0.3 $\pm$ 0.1	28.6 $\pm$ 5.2	13.9 $\pm$ 6.2	106.5 $\pm$ 2.7	9

3. Pooled Data from all Individual Test Groups are presented in
TABLE 9 and FIGURES 4-18.

TABLE 9

Master Table: Relationship between urinary dopamine levels and arterial blood pressure of individual groups.

Group	Weeks	Dopamine ug/ml/24hrs	Dopamine ug/kg	Total Dopamine ug/24hr sample	Blood Pressure mm Hg	Number of animals
Sham + Solvent	12	0.6 $\pm$ 0.4	86.2 $\pm$ 63.8	19.2 $\pm$ 16.4	108.3 $\pm$ 2.4	14
Unilateral + Sham Injection	12	0.4 $\pm$ 0.2	36.7 $\pm$ 27.5	8.9 $\pm$ 5.9	115.2 $\pm$ 7.2	10
Unilateral + Parnate	12	0.3 $\pm$ 0.1	26.4 $\pm$ 10.6	5.7 $\pm$ 2.7	104.5 $\pm$ 14.6	11
Unilateral + Pargyline	12	0.3 $\pm$ 0.2	28.3 $\pm$ 18.3	6.2 $\pm$ 4.2	111.9 $\pm$ 12.9	11
Sham + Parnate	12	0.5 $\pm$ 0.2	44.6 $\pm$ 21.9	9.5 $\pm$ 4.5	110.0 $\pm$ 11.4	14
Sham + Pargyline	12	0.8 $\pm$ 0.3	84.8 $\pm$ 42.4	19.7 $\pm$ 10.0	97.7 $\pm$ 11.8	12

All values represent means with standard deviations of six groups of male albino rats for the length of time specified in column two.

Fig. 4. Effect of Parnate or Pargyline on the mean systolic blood pressure of unilaterally nephrectomized male albino rats.

All values represent means of at least nine animals at the times specified on the abscissa.

Fig. 5. Effect of Parnate 3.3 mg/kg on the mean systolic blood pressure of unilaterally nephrectomized or sham operated male albino rats.

All values represent means of at least eleven animals at the times specified on the abscissa.

Fig. 6. Effect of unilateral nephrectomy or sham operation on the mean systolic blood pressure of male albino rats receiving sham injection or solvent, respectively.

All values represent means of at least nine animals at the times specified on the abscissa.

Fig. 7. Effect of Pargyline 25 mm/kg on the mean systolic blood pressure of unilaterally nephrectomized or sham operated male albino rats

All values represent means of twelve animals at the times specified on the abscissa.

Fig. 8. Effect of Parnate or sham injection on the mean systolic blood pressure of unilaterally nephrectomized male albino rats.

All values represent means of ten animals at the times specified on the abscissa.

Fig. 9. Effect of Pargyline or sham injection on the mean systolic blood pressure of unilaterally nephrectomized male albino rats.

All values represent means of at least ten animals at the times specified on the abscissa.

Fig. 10. Effect of Solvent or Pargyline on the mean systolic blood pressure of sham operated male albino rats.

All values represent means of at least nine animals at the times specified on the abscissa.

Fig. 11. Effect of Solvent or Parnate on the mean systolic blood pressure of sham operated male albino rats.

All values represent means of at least nine animals at the times specified on the abscissa.

Fig. 12. Master Graph. Relationships between urinary dopamine levels, arterial blood pressure and weights of unilaterally nephrectomized male albino rats receiving Parnate 3.3 mg/kg.

Fig. 13. Relationship between urinary dopamine levels and weights of unilaterally nephrectomized male albino rats receiving Parnate 3.3 mg/kg.

All values represent means of twelve animals at the times specified on the abscissa.

Fig. 14. Relationship between urinary dopamine levels and weight of sham operated male albino rats receiving Pargyline 25 mg/kg.

All values represent means of twelve animals at the times specified on the abscissa.

Fig. 15. Relationship between urinary dopamine levels and weights of unilaterally nephrectomized male albino rats receiving sham injection.

All values represent means of at least ten animals at the times specified on the abscissa.

Fig. 16. Relationship between urinary dopamine levels and weights of sham operated male albino rats receiving Parnate 3.3 mg/kg.

All values represent means of at least fourteen animals at the times specified on the abscissa.

Fig. 17. Relationship between urinary dopamine levels and weights of unilaterally nephrectomized male albino rats receiving Pargyline 25 mg/kg.

All values represent means of at least fourteen animals at the times specified on the abscissa.

Fig. 18. Relationship between urinary dopamine levels and weights of sham operated male albino rats receiving Solvent.

All values represent means of nine animals at the times specified on the abscissa.

IV. DISCUSSION

Blood Pressure Studies.

The indirect tail-cuff method employed for determining arterial blood pressure of male albino rats was found to be satisfactory. The blood pressure of the experimental rats receiving tranylcypromine was elevated to 134 mm Hg during the sixth week but at the end of the twelfth week the blood pressure had dropped to 115 mm Hg for a net drop of approximately three percent. The blood pressure of the experimental group receiving pargyline dropped from a high of 128 mm Hg to 84 mm Hg for a net drop of approximately thirty-four percent. The mean blood pressure of the control group receiving a sham operation and the solvent was 108 mm Hg and remained at that level to the end of the investigation. At no time during the study did the blood pressure of this group deviate more than 9 mm Hg during any week.

In comparing the various groups by using the Student's t-test for a test of significance at the 95 percent level, it was found that any change in arterial blood pressure was the result of the drug, either tranylcypromine or pargyline. It was noted that after twelve weeks of treatment, the experimental rat group receiving pargyline could not tolerate the slightest stress such as pre-heating in the incubator prior to determining the blood pressure. This stress caused some of them to succumb to suffocation. This unusual sensitivity to stress can probably be explained on the basis of pargyline induced hyperthermia. The experimental rats receiving tranylcypromine were also susceptible to this stress but seemed to

tolerate it better than the experimental rats receiving pargyline. Although tranylcypromine also can induce hyperthermia, the hyperthermic effect at a dose of 3.3 mg/kg was not sufficient to cause rapid suffocation and death.

Dopamine Studies.

A modification of the Carlsson & Waldeck (1958) method for determining urinary dopamine was found to be satisfactory. Dopamine concentrations of 2 ug, 4 ug, and 6 ug/ml were added to samples and extracted on chromatographic columns containing 700 mg of acid washed alumina. Percent recoveries of dopamine added to samples was seventy-nine percent. A linear relationship was found to exist between dopamine concentrations of 0.1 ug to 0.4 ug/ml as reported by Carlsson et al. (1958). Dopamine concentrations were reported in ug/ml/24 hour urine sample, total dopamine in ug/24 hour urine sample and in ug/kg of body weight. In all instances urinary dopamine was assayed from paired rat urine samples. The dopamine, ug/kg of body weight of the sham operated rat group receiving the solvent and the sham operated group receiving pargyline was nearly the same, however, the blood pressure of the group receiving pargyline was approximately 13 mm Hg lower. This was expected since pargyline has been reported to lower blood pressure experimentally in rats, DeFanti & DeFeo (1962).

The two enzymes responsible for the metabolic transformation of dopamine in the body are catechol-O-methyltransferase (COMT), Axelrod, J., (1957) and monoamine oxidase

(MAO), Blaschko, H., (1957). Dopamine may be methylated directly by the enzyme COMT or deaminated by the enzyme MAO. The preferential route for the metabolic transformation seems to be oxidative deamination via the enzyme MAO; however, the administration of dopamine to rats results in excretion of 3-methoxytyramine both free and conjugated, Axelrod, J., et al., (1958). In contrast to epinephrine and norepinephrine, the methoxylation of dopamine occurred only to a minor extent, and considerable quantities of homovanillic acid, a deaminated metabolite of 3-methoxytyramine were found in the rat urine. This might lead one to search for other metabolites of dopamine in the urine instead of a free or conjugated form.

An attempt to compare the two variables, using a simple regression method proved unsatisfactory. According to the data obtained from this study no linearity exists between arterial blood pressure and urinary dopamine.

Results from this investigation indicate that the blood pressure in experimental male albino rats receiving the MAO inhibitors tranylcypromine or pargyline is not elevated, nor is the urinary dopamine level increased. Further investigation into the alternate pathways of the metabolic transformation of dopamine and assaying for metabolites of dopamine must be conducted before one can fully conclude whether there is a correlation between arterial blood pressure and urinary dopamine.

V. SUMMARY AND CONCLUSIONS

This study investigated the interrelationship between arterial blood pressure and urinary dopamine levels in male albino rats receiving a monoamine oxidase inhibitor and a unilateral nephrectomy.

1. The arterial blood pressure of the experimental rats receiving a monoamine oxidase inhibitor and a unilateral nephrectomy was not elevated. The blood pressure of the groups receiving a MAO inhibitor and a unilateral nephrectomy reached a high of approximately 135 mm Hg during the sixth week but gradually dropped to approximately 85 mm Hg.

A modification of the dopamine bioassay of Carlsson & Waldeck, (1958) was used to determine dopamine quantitatively and proved satisfactory. Dopamine was extracted from paired urine samples by chromatographic alumina columns and measured by spectrofluorimetric methods. This method proved satisfactory with average recovery for dopamine being seventy-nine percent.

2. The urinary dopamine levels of the experimental groups were based upon ug of dopamine/kg of body weight and were found to be lower than the control group.

3. Based upon the method and design of this investigation and applying the data obtained, no linear correlation exists between the two variables blood pressure and dopamine. Applying the data to test the goodness of fit it was found that no linearity existed between blood pressure and urinary dopamine.

4. No definite relationship between blood pressure and urinary dopamine was established. Dopamine levels of the experimental rats did not rise nor was the blood pressure elevated. Further investigation into the relationship between arterial blood pressure and dopamine is needed before a clear relationship can be established between arterial blood pressure and urinary dopamine.

VI. REFERENCES

- Allwood, M.J., Cobbold, A.F., and Ginsburg, J.: Peripheral vascular effects of noradrenaline, isopropylnoradrenaline and dopamine. *British Medical Bulletin*. 19:132-136, 1963.
- Allwood, M.J. and Ginsburg, J.: In: XXII International Congress of Physiological Sciences (under the auspices of the International Union of Physiological Sciences). Leiden, September 10-17, Abstract No. 185, (International Congress Series No. 47) Excerpta Medica Foundation, Amsterdam. 2: 1962.
- Bing, R.J.: The formation of hydroxytyramine by extracts of renal cortex and by perfused kidneys. *Amer. J. Physiol.* 132: 497, 1941.
- Burn, J.H. and Rand, M.J.: The depressor action of dopamine and adrenaline. *British J. Pharmacol.* 13:473, 1958.
- Blaschko, H., Holton, P. and Stanley, G.: Enzymatic formation of pressor amines. *J. Physiol.* 108:427, 1949.
- Brest, A.N. and Moyer, J.H.: Hypertension recent advances. The 2nd Hahnemann symposium on hypertensive disease. Lea and Febiger, Philadelphia, 1961.
- Carlsson, A., Falck, B. and Hillarp, N.A.: Cellular localization of brain monoamines. *Acta Physiol. Scand.* 56:1-27, 1962.
- Demis, D.J., Blaschko, H. and Welch, A.D.: The conversion of dihydroxyphenylalanine to $t^0 - 2C^{14}$ (DOPA) to norepinephrine by bovine adrenal homogenates. *J. Pharmacol. exp. Therap.* 117:208, 1956.
- Drujan, B.D., Soirkes, T.L., Lagne, D.S. and Murphy, G.F.: The differential determination of catecholamines in urine. *Can. J. Biochem. Physiol.* 37:1153-1160, 1959.
- Defanti, D.R., and DeFeo, J.J.: Urinary dopamine levels and kidney MAO activity in hypertensive rats. *Biochemical Pharmacology*. 12:173-177, 1963.
- Ellison, T. and Scott, G.C.: Effects on the rat of chronic dietary supplementation with a MAO inhibitor, tranlycypromine. *J. Toxicology and Applied Pharmacology*. 6:411-420, 1964.
- Falck, B. and Owman, C.: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. *Acta Universitatis Lundensis Section 11*. 7, 1965.
- Giordano, C., Samily, A.H., Bloom, J. Haynes, F.W. and Merrill, J.R.: Studies on experimental hypertension in rabbits. *Fed. Proc.* 19:100, 1960.

- Goldberg, L.I. and Sjoerdsma, A.: Effects of several monoamine oxidase inhibitors on the cardiovascular actions of naturally occurring amines in the dog. *J. Pharmacol. Exp. Ther.* 127:212, 1959.
- Goldblatt, H., Lynch, J., Hanzal, R.F. and Summerville, W.W.: Studies on experimental hypertension. *J. Exp. Med.* 59:347-379, 1934.
- Ginsberg, J. and Duff, R.S.: Inhibition of Levarterenol by intra-arterial phenoxybenzamine in man. *Circulat. Res.* 6:751, 1958.
- Goodman, L.S. and Gilman, A.: *The Pharmacological Basis of Therapeutics*. 3rd Edit. The Macmillan Company, New York, 1965.
- Goldstein, M., Friedhoff, A.J., Pomerantz, S. and Simmons, C.: The characterization of a new metabolite of dopamine. *Acta Biochem. Biophys.* 39:189-191, 1960.
- Holtz, P. and Credner, K.: *Arch. Exp. Path. Pharmacol.* 200:356, 1942. (Cited by Allwood, M.J., Cobbold, A.F., and Ginsburg, J., 1963).
- Levin, E.Y., Levenberg, B. and Kaufman, S., The enzymatic conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. *J. Biol. Chem.* 235:2080-2085, 1960.
- McDonald, R.H., and Goldberg, L.I., The cardiovascular effects of dopamine in the dog. *Fed. Proc.* 21:127, 1962.
- McNay, U.L., and Goldberg, L.I.: Comparison of the effects of dopamine, isoproterenol, norepinephrine and bradykinin on canine renal and femoral blood flow. *J. Pharmacol. Exp. Therap.* 151:23-31, 1965.
- Schroeder, H.: The effect of a preparation of amine oxidase on experimental hypertension. *Science.* 95:306, 1942.
- Weil-Malherbe, H. and Bone, A.D.: The estimation of catecholamines in the urine by a chemical method. *J. Clin. Path.* 10:138, 1957.
- Zirkle, C.L., Kaiser, C., Tedeschi, D.H., Tedeschi, R.E. and Burger, A.: 2-substituted cyclopropylamines. *J. of Medicinal and Pharmaceutical Chemistry.* 5:1265-1284, 1962.