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Investigation of the Role of Dopamine in Maintenance of Arterial Hypertension

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INVESTIGATION OF THE ROLE OF DOPAMINE IN MAINTENANCE OF ARTERIAL HYPERTENSION

BY

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ABSTRACT

The relationship between urinary dopamine levels and arterial blood pressure was studied in normal, unilaterally nephrectomized, and renal hypertensive male albino rats.

Experimental hypertension was produced by a unilateral nephrectomy followed by compression of the contralateral renal artery. The two operations were spaced three weeks apart to allow for recovery.

Nine weeks after the second operation, mean systolic blood pressure of the renal hypertensive control group increased to 170 mm Hg. Mean systolic blood pressure of the diethyldithiocarbamate (DDC)-treated renal hypertensive group decreased abruptly after two weeks of treatment and remained at 105 mm Hg throughout the nine week period. Mean systolic blood pressures of the normal and unilaterally nephrectomized group of animals remained between 100-125 mm Hg throughout the study. However, in comparisons with control groups, the systolic blood pressures of the DDC-treated unilaterally nephrectomized and normal groups of animals were significantly lower after four or seven weeks of treatment respectively.

Urinary dopamine was extracted by adsorption onto alumina, converted to its trihydroxyindole fluorophore and measured spectrofluorimetrically.

In comparisons with control groups, urinary dopamine levels (μg/24 hr, μg/kg/24 hr) did not differ significantly in either the normal or DDC-treated renal hypertensive group of animals. Mean dopamine levels remained relatively constant throughout the nine week period and varied from 15 to 27 μg/24 hr and 13 to 21 μg/24 hr in the normal and renal hypertensive group of animals respectively. Mean dopamine levels varied from 35 to 45 μg/kg/24 hr and 23 to 26 μg/kg/24 hr in the normal and renal hypertensive groups respectively.

After seven weeks of treatment, the mean urinary dopamine levels (μg/24 hr) of the DDC-treated unilaterally nephrectomized group of animals were significantly higher than controls. Mean dopamine levels of the DDC-treated group increased from 11 μg/24 hr to 23 μg/24 hr after the seventh week

of treatment. The control group remained relatively constant (15 μg/24 hr) within the same period.

Mean body weights of the DDC-treated animals were significantly lower than controls after six, four, and two weeks of treatment in the normal, unilaterally nephrectomized, and renal hypertensive groups respectively.

In all groups of drug-treated animals there was a correlation between decreased body weight, decreased amount of functional kidney tissue, and decreased arterial blood pressure. No correlation was found between arterial blood pressure and urinary dopamine levels.

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I. INTRODUCTION

In the treatment of disease, a knowledge of the physiological and metabolic derangements of the disease must first be considered. Only then can the treatment be effectively employed.

Within the last decade many attempts have been made to show a relationship between erroneous catecholamine metabolism and the hypertensive disease state. Because of their prominent pressor effects on the cardiovascular system, norepinephrine and epinephrine have been the focus of this attention. However, their precursor, dopamine, also possesses pressor effects. This led to a study by DeFanti and DeFeo (1963) of the role of dopamine in the etiology of experimental renal hypertension in male albino rats. These investigators reported evidence indicating a positive correlation between arterial blood pressure and urinary dopamine concentration in renal hypertensive rats.

To further investigate the role of dopamine in experimental hypertension, the present investigation was designed to inhibit the enzyme dopamine-β-hydroxylase, the enzyme responsible for the conversion of dopamine to norepinephrine, with diethyldithiocarbamate. Evaluation was based on the effect of this drug on systolic blood pressure, weight gains, and urinary dopamine levels in renal hypertensive, unilaterally nephrectomized, and normal male albino rats.

II. REVIEW OF THE LITERATURE

In recent years much attention has been focused on the role of catecholamines in the pathogenesis of arterial hypertension. Suspected aberrations of catecholamine metabolism have led investigators to study the role of circulating catecholamines and their metabolites during hypertension. Interference with the metabolism of these pressor substances could lead to an accumulation of one or more of these compounds and conceivably maintain a hypertensive state.

Figure 1 represents the main pathway, first proposed by Blaschko (1939), in the formation of the catecholamines. The last three compounds in this sequence represent the pressor catecholamines.

Evidence in support of this pathway was reported by Gurin and Delluva (1947) who observed that ^{14}C- and ^{3}H-phenylalanine labeled in the side chain and ring respectively was incorporated into adrenal epinephrine (E). Later, Langemann (1951) measured the enzyme Dopa decarboxylase in the adrenal medulla in rats. Leeper and Udenfriend (1956) administered ^{14}C-3, 4-dihydroxyphenylalanine (Dopa) to rats and measured ^{14}C-norepinephrine (NE) in the adrenals. It wasn't until 1957 that Goodall and Kirshner, using isotope dilution techniques, demonstrated unequivocally that Blaschko's proposed pathway was correct. Incubation of ^{14}C-tyrosine or ^{14}C-Dopa with bovine adrenal slices resulted in the label appearing in dopamine (DA), followed by NE and subsequently E. Nagatsu et al. (1964) confirmed the synthetic pathway with the isolation and characterization of tyrosine hydroxylase.

Udenfriend (1966) observed tyrosine hydroxylase to be the rate - limiting step in NE synthesis, because the amount of enzyme was limiting. When tyrosine, Dopa, or DA was incubated with tyrosine hydroxylase, saturation could only be achieved with tyrosine. The V_{max} for Dopa decarboxylase or dopamine-β-hydroxylase (DBH) [3,4-dihydroxyphenylethylamine, ascorbate: oxygen, oxidoreductase, E.C. 1.14.2.1] was reported as two or three orders of magnitude higher than that for tyrosine hydroxylase.

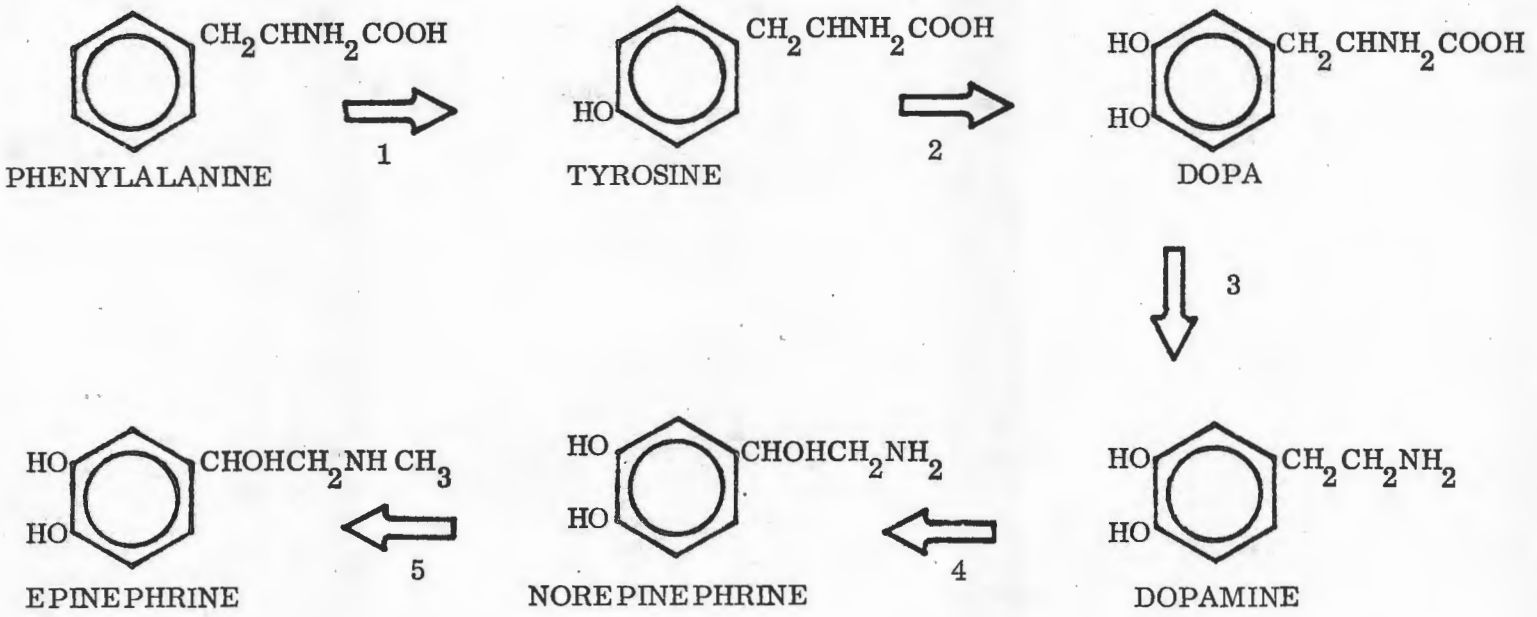

Fig. 1. Main pathway in the biosynthesis of the active catecholamines: 1) phenylalanine hydroxylase, 2) tyrosine hydroxylase, 3) DOPA decarboxylase, 4) dopamine-β-hydroxylase, 5) phenylethanolamine-N-methyl transferase.

Reports that DBH may be the rate-limiting step are conflicting (Musacchio et al., 1964; Nikodijevic et al., 1963; Udenfriend et al., 1966).

With the introduction of more sensitive qualitative and quantitative analyses, the identification of the physiological mechanisms of inactivation of the catecholamines was confirmed. A short summary of the catabolism of the catecholamines appears in Figure 2.

Evidence that monoamine oxidase [monoamine: oxygen, oxidoreductase, E.C. 1.4.3.4] (MAO) oxidizes DA at a more rapid rate than either NE or E was presented by Blaschko (1952). This preferential attack appears to be due to the lack of the β-hydroxyl group on the catecholamine moiety. This report suggested that because of the tremendous affinity of MAO for DA, this catecholamine might be the primary physiological substrate for the enzyme. Blaschko further speculated that MAO may serve a regulatory role by limiting the amount of DA available for metabolism to NE and E.

Axelrod et al. (1957) demonstrated a predominant in vitro O-methylation of the catecholamines E and NE. However, after intraperitoneal injection of DA in the rat only 3% of the administered dose was excreted as its O-methylated derivative, 3-methoxytyramine. Pretreatment of the animals with iproniazid, an MAO inhibitor, resulted in a five-fold increase of excreted 3-methoxytyramine. These findings suggested that, although E and NE were largely O-methylated, DA was metabolized predominantly by MAO.

This was further substantiated by Goldstein et al. (1959) who administered 3-hydroxy-1-^{14}C-tyramine to rats and reported 60% of the administered radioactivity excreted as 3-methoxy-4-hydroxyphenylacetic acid and 2-3% excreted as 3-methoxytyramine. After pretreatment with iproniazid 31% of administered radioactivity was excreted as 3-methoxytyramine and 10% as 3-hydroxytyramine.

Additional support that MAO should be the first enzyme involved in the catabolism of DA was provided by Carlsson and Waldeck (1964) who reported that both the enzyme and DA are concentrated within sympathetic nerve endings.

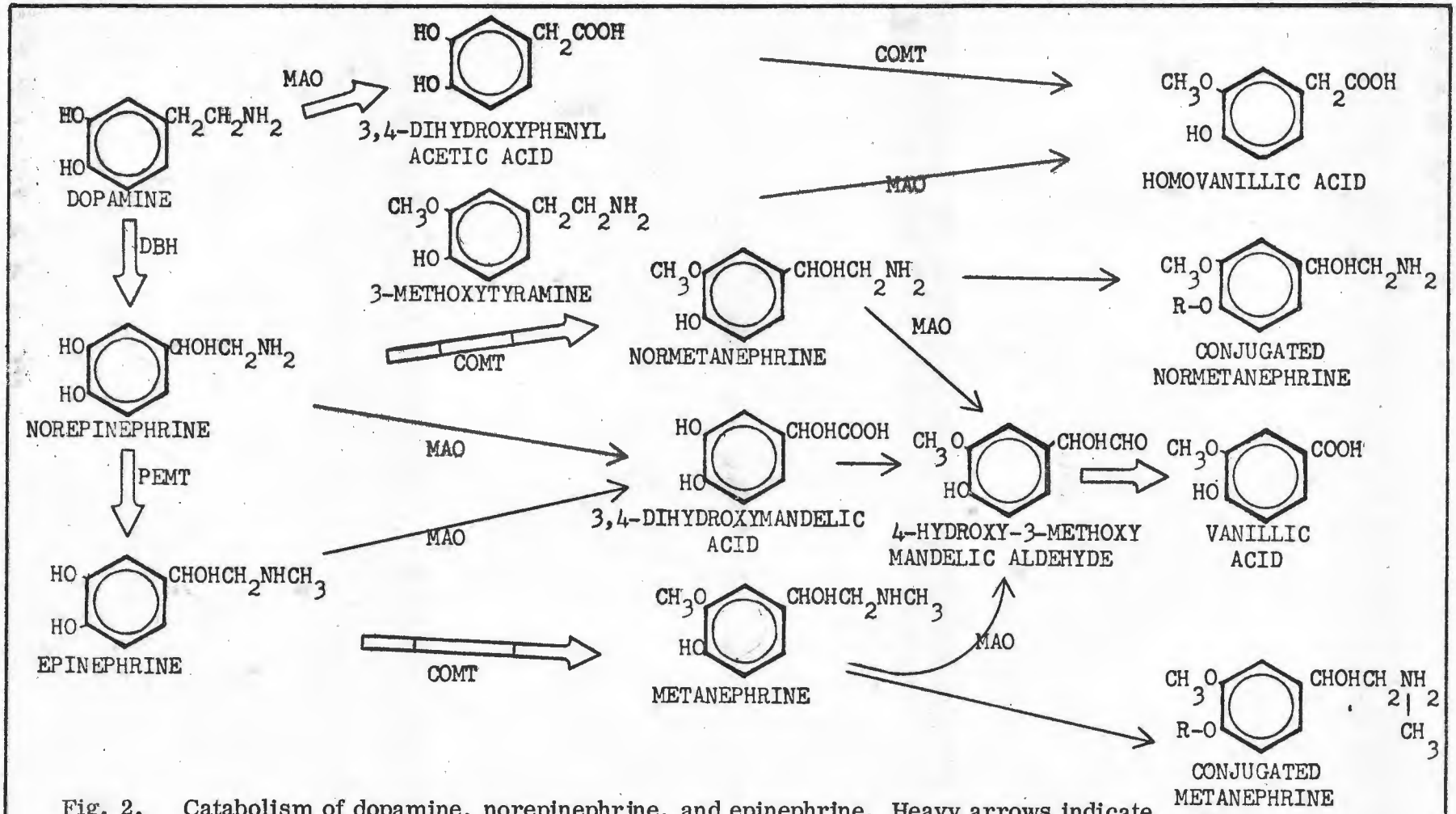

Fig. 2. Catabolism of dopamine, norepinephrine, and epinephrine. Heavy arrows indicate main catabolic pathway, other arrows indicate alternate pathway of catecholamine destruction. Catechol-O-methyl transferase (COMT), monoamine oxidase (MAO), dopamine-β-hydroxylase (DBH), phenylethanolamine N-methyl transferase (PEMT).

Reports relating catecholamine levels and arterial hypertension have been sparse and equivocal. Von Euler (1956) reported no significant difference in NE levels when hypertensive subjects were compared to normals. Alternatively, a two-fold increase in NE and E and a five-fold increase in DA was observed in hypertensive patients by Weil-Malherbe and Bone (1957). These authors also observed a greater variation in daily DA excretion than with either NE or E.

Peart (1966) observed that there is hardly any evidence of credit which associates increased circulating catecholamines with hypertension. This suggests, therefore, that, if a relationship does exist, some change in synthesis, degradation, storage, release, or sensitivity of the vasculature to NE and E may exist. However, this author also suggests that sympathetic tone is normal in hypertension.

Studies into the possiblity of an altered turnover rate of any of the catecholamines during the hypertensive disease have been sporadic (Gitlow et al., 1964; Sjoerdsma et al., 1961; Mendlowitz et al., 1959).

Although DA has been shown to possess vasoactive properties, this catecholamine has been largely ignored as a possible participant in hypertension. Dependent on the route of administration, DA has been reported as a pressor agent in the cat (Holtz et al., 1962), dog (Holmes and Fowler, 1962), man (Horwitz et al., 1962) and in the rat (Holtz et al., 1962; Pogrund et al., 1961). In most cases the pressor response was a result of increased cardiac output with little or no change in peripheral resistance observed.

Reports of the distribution of DA in mammalian tissues have been conflicting. Schumann (1959) reported a wide distribution of DA in various tissues in the rat, whereas Wegmann (1963) could detect the presence of DA only in the spleen and kidney of the dog. DA could not be found in the plasma of humans, dogs, or rabbits (Anton and Sayre, 1964; Carlsson, 1959).

Bing (1941) reported evidence for the production of a pressor substance, presumably DA, from the decarboxylation of Dopa in extracts of guinea pig

kidneys under anerobic conditions. He observed a similar reaction in vivo in cat's ischemic kidney perfused with blood containing Dopa. This report suggests that deamination but not decarboxylation of amino acids is incomplete in anoxic kidneys. This possibility was verified by Giordano et al. (1959), who observed increased kidney Dopa decarboxylase and decreased kidney MAO after prolonged hypertension in rabbits with renal ischemia. These altered enzyme levels could conceivably lead to an accumulation of DA, NE, and/or E in ischemic tissue.

DeFanti and DeFeo (1963) reported evidence indicating a positive correlation between arterial blood pressure and urinary DA concentration in renal hypertensive rats.

These studies suggested that decreased MAO activity in the ischemic kidney results in an excess of DA in the tissues. The established pressor activity of DA in man and animal and the reported increase in urinary DA in the hypertensive rat suggested a role for DA in hypertensive disease.

If, under these conditions of decreased MAO activity, the enzyme responsible for the conversion of DA to NE were inhibited by the use of drugs, the excess DA present might result in an increased arterial pressure.

DBH, the enzyme responsible for the conversion of DA to NE, has been reported to be inhibited by two classes of drugs. Analogs of the natural substrate DA, with the α-carbon atom substituted with either N or O, proved to be very potent competitive inhibitors of the active site on the enzyme (Creveling et al., 1962). Although effective in vitro, the compounds in vivo were hydrolyzed at a slow rate resulting in false transmitters with little or no DBH inhibiting activity.

DBH has been shown to require copper as a cofactor in a concentration of 0.65 to 1.0 μg per mg of enzyme (Friedman and Kaufman, 1965). Binding of the copper by drugs could inactivate this enzyme.

Disulfiram and one of its reduced metabolites, diethyldithiocarbamate (DDC), belong to this second class of DBH inhibitors and have been reported

to inhibit this enzyme in vivo (Goldstein and Anagnoste et al., 1964; Goldstein and Lauber et al., 1964). Goldstein and Anagnoste et al. (1964) have observed a 100% inhibition of DBH with 10^{-5}M DDC in vitro. Musacchio et al. (1966) reported that DDC prevented the conversion of DA-H^3, tyramine-H^3 and other phenylethylamines to their β-hydroxylated analogs. Increased DA levels with a simultaneous decrease in NE in rat and rabbit small intestine have been demonstrated by Collins (1965). Carlsson et al. (1966) could not verify this change in ileum tissue but did observe a significant change in the adrenals and brain stem of the rat.

The findings of DeFanti and DeFeo (1963), the reported pressor activity of DA in man and animal, and the availability of a suitable DBH inhibitor suggest the need for further study into the possible role of DA in renal hypertension.

III. INVESTIGATION

A. Objectives

The possibility that 3-hydroxytyramine (dopamine) was implicated in renal hypertension was suggested by DeFanti and DeFeo (1963). They reported a positive correlation between the increased arterial blood pressure and urinary dopamine concentration in renal hypertensive rats. The objectives of this study were to determine if there was an increase in the urinary excretion of dopamine in rats rendered hypertensive by renal ischemia and if the increased arterial blood pressure was significantly dependent upon an increase in dopamine.

B. Materials and Methods

1. General Considerations and Daily Protocol

Adult male albino rats of the Sprague-Dawley strain[1] weighing 125-150 g were divided into three groups: surgically intact, unilaterally nephrectomized, and renal hypertensive. Each group was further divided into two sub-groups: animals receiving sodium diethyldithiocarbamate[2] (66.5 mg/kg) and animals receiving the vehicle, 0.1 M phosphate buffer (pH 7.4) 1 ml/kg. All animals were injected on alternate days and housed in a room lighted from 7:00 a.m. to 7:00 p.m. and maintained at 22 $\pm$ 0.5^{o} C. Purina[3] rat chow and water were provided ad libitum. Student's "t" test (Snedecor, 1956) was used for all comparisons reported.

2. Production of Experimental Hypertension

Experimental renal hypertension was induced by the method of Goldblatt et al. (1934) as modified by Drury (1938). The method consisted of a unilateral nephrectomy followed by compression of the right renal artery. The two operations were spaced three weeks apart to allow for the recovery of the animals.

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1. Charles River Breeding Farms, North Wilmington, Massachusetts.
2. J. T. Baker Chemical Company, Phillipsburg, New Jersey.
3. Ralston Purina, St. Louis, Missouri.

The unilateral nephrectomy was performed on the right kidney by making a dorsolateral incision an inch in length parallel to the spinal cord. The kidney was palpated and located retroperitoneally to the abdominal cavity and laterally to the incision. The organ was forced through the incision and exposed for ligation with nylon thread. The artery, vein and ureter were ligated; the organ was then excised and frozen in liquid nitrogen for later analysis. The adrenal gland remained intact. The incision through the muscle was closed with 3-0 silk suture and the epidermal incision closed with stainless steel wound clips.

Approximately three weeks after the first operation the left renal artery was located by making a one inch ventrolateral incision in the lower abdominal area. The artery was separated from the renal vein, and a stylet approximately one third the size of the artery was placed parallel to the vessel. A nylon suture was looped around both stylet and vessel and secured. The stylet was withdrawn allowing the artery to expand to one-third of its normal size. The incision was closed in the manner previously mentioned. All animals were fasted for twenty-four hours prior to both operations to facilitate operative procedures.

3. Blood Pressure Measurement

The systolic blood pressure of all animals was monitored on a bi-weekly basis using the tail cuff method (Coates, 1968). The method utilizes an inflatable tail cuff placed about one centimeter from the base of the tail and connected to a Physiograph[1] manometer system. The tail was passed through a holder to bring it into contact with a Beckman[2] microphone transducer which was connected to an Infraton[2] signal divider adjusted for maximum pulse. This lead was then connected to an HP oscilloscope[3]. As the cuff was inflated, circulation stopped, and no pulses were transferred to the oscilloscope. Conversely, as the cuff was deflated, the circulation was restored, and, at the point where pulses reappeared, the impulses were observed on the oscilloscope

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1. E & M Instruments, Houston, Texas.
2. Beckman Co., Palo Alto, California.
3. Model 130 B., Hewlett-Packard Co., Palo Alto, California.

and the pressure read on the manometer. Each value was the mean of three separate determinations spaced a minute apart.

4. Weight Gain Measurement

The effects of treatment with drug and vehicle on the individual body weights of the animals were determined. All animals were weighed to the nearest gram at the time of injection on an Ohaus small animal balance[1]. The individual weights of the drug-treated animals were compared with the weights of the control group on a bi-weekly basis.

5. Collection of Urine

The animals from each group were selectively paired in an effort to house animals of similar weights. Urine samples passed through stainless steel funnels were collected over 0.5 ml of concentrated sulfuric acid. The acid hydrolyzed any conjugated dopamine and maintained an acid pH to avoid auto-oxidation to 6-OH dopamine. The samples were stored in test tubes at -40°C for later analysis.

6. Extraction of Urine

The urine samples were centrifuged for twenty minutes, and 10 ml of the supernatant was decanted into a clean test tube. Two ml of 0.2 M disodium ethylenediamine tetraacetate was added to the sample with two drops of concentrated hydrochloric acid. The samples were boiled for twenty minutes and cooled. The samples were then adjusted to pH 8.4 with 1 N NaOH, and 1 ml of an antioxidant (sodium metabisulfite 10 mg/ml) was added. The samples were passed through a column prepared by the addition of aluminum oxide[2] suspended in 10 ml of 0.1 M ammonium acetate buffer, pH 8, and 0.28 ml 0.1 N NaOH. The alumina used was a chromatographic quality. The column had an inside diameter of 0.6 cm and a length of 7.5 cm. The flow rate was adjusted to

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1. Ohaus Scale Corporation, Union, New Jersey.

2. British Drug Houses, LTD; exclusive United States Distributors Gallard-Schlesinger Manufacturing Corp., Long Island, New York.

2 ml/min. by a Hoffman clamp. Bertler et al. (1958) have demonstrated that Dopa will readily pass through the Na^+ form of the alumina. Since Dopa has the same fluorescence characteristics as dopamine, it was desirable to pass a 5 ml portion of sodium acetate 0.1 M through the column, prior to elution to eliminate any contaminating Dopa. The samples were eluted by two 5 ml portions of 0.2 N acetic acid.

7. Development of Sample

Development procedure was carried out at room temperature according to the method of Carlsson and Waldeck (1958) as modified by Coates (1968). A 3 ml aliquot of the eluate was adjusted to pH 6.5 with the addition of 1 ml of 0.3 M potassium carbonate. The samples were treated with 1 ml of 0.1 M phosphate buffer pH 6.5 and the volumes diluted to 7 ml with glass distilled water. Two drops of a 0.25 M iodine solution were added to each sample. Three minutes after the addition of iodine, 1 ml of alkaline sulfite reagent (500 mg sodium sulfite. 7 H_2O per ml water diluted to 10 ml with 5 N NaOH) were added to the samples. After four minutes, 1 ml of 6 N acetic acid was added to all samples. The samples were diluted to 10 ml with glass distilled water, boiled for 10 minutes and the supernatant decanted to quartz cuvettes. The cuvettes were irradiated at 253 mμ in ultraviolet light[1] for ten minutes to sustain fluorescence. The sample fluorescence was measured at 370 mμ on an Aminco-Bowman spectrophotofluorometer[2]. Activation wavelength was 325 mμ (uncorrected instrument values). A tissue blank which contained a 3 ml eluate aliquot was prepared and assayed as above, except that 4.5 N NaOH was substituted for the alkaline sulfite reagent. Two drops of a sodium sulfite solution (500 mg/ml) were added prior to centrifugation to remove the remaining iodine color to avoid quenching. A standard curve of known dopamine concentration was obtained with each set of samples. A linear relationship between fluorescence and dopamine concentration as reported by Carlsson and Waldeck (1958), DeFanti (1961), and

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1. Chromato-Vue, Black Light Eastern Corp., New York, New York.

2. American Instrument Co., Inc., Silver Springs, Maryland. Slit Arrangement number 3. Fused quartz cells were used.

Coates (1968) was found to exist up to 0.5 μg/ml of dopamine (Figure 3).

An estimate of the dopamine content was made by dividing the corrected fluorescent intensities of the unknowns by a value calcualted to represent the fluorescent intensity of a solution of dopamine containing 0.1 μg/ml. The fluorescent intensity contributed by the reagents was subtracted from the fluorescent intensity of the standard solutions. The corrected values were adjusted and averaged to obtain a single value which represented the fluorescent intensity of a 0.1 μg/ml solution of dopamine. Coates (1968) summarizes the mechanics as follows:

$$I_s = (F_s - R.B. \times \frac{0.1}{C}) / n$$

Where I_s = fluorescent intensity of a 0.1 μg/ml solution of dopamine

F_s = uncorrected fluorescent intensity of the developed solutions at concentration C

R.B. = fluorescent intensity of the reagent blank

C = final concentrations of dopamine in μg/ml

n = the number of determinations

and finally:

$$C_u = (F_u - B_u) / I_s$$

Where C_u = the concentration of dopamine (μg/ml) in the developed solution of the unknown

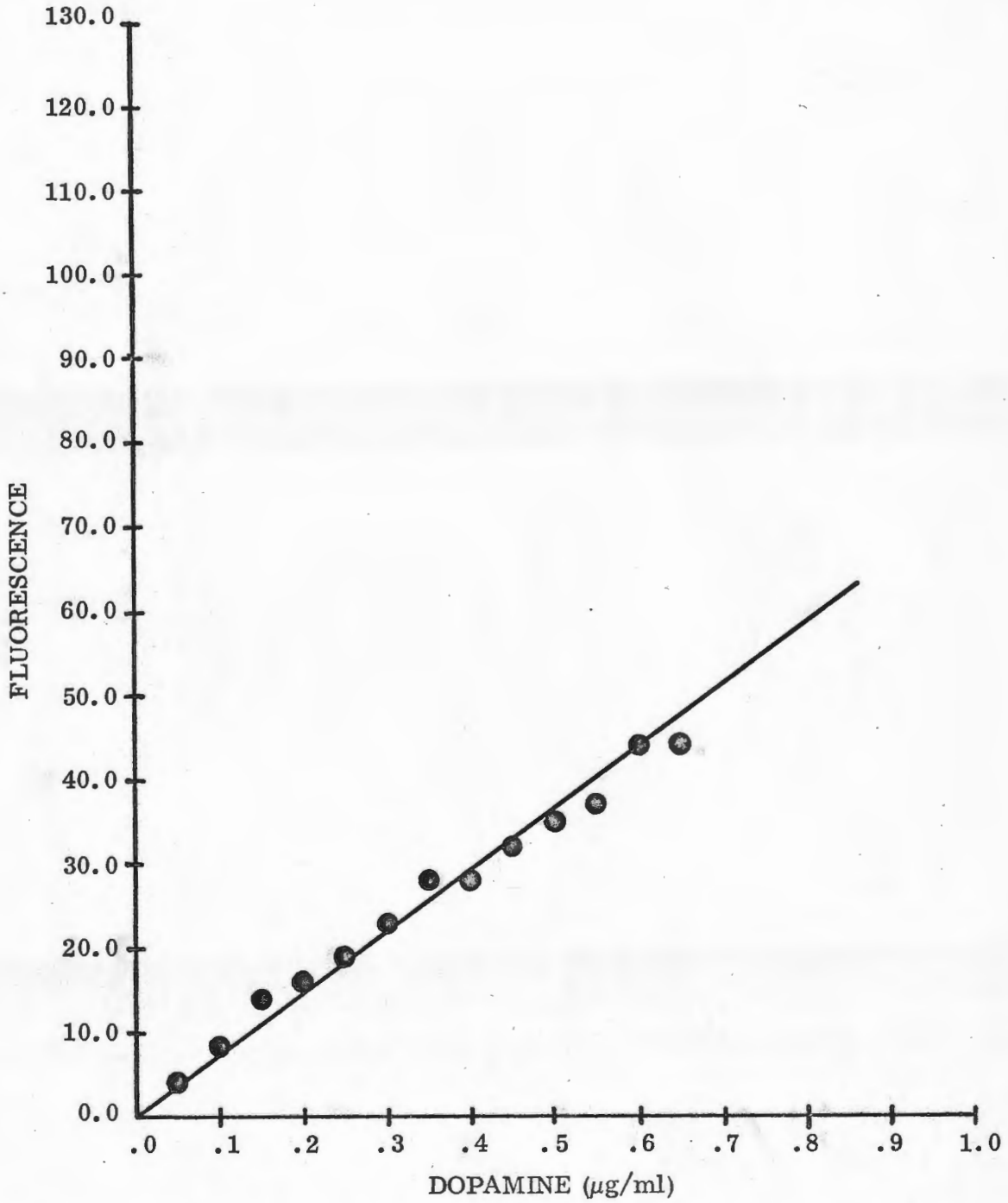
F_u = the uncorrected fluorescence intensity of the developed solution of the unknown

B_u = the fluorescent intensity of corresponding blank of the unknown

I_s = the fluorescent intensity of the standard as determined above

Since the final volume of the developed solutions was 10 ml, the total dopamine content of a 3 ml column aliquot was equivalent to 10 times the concen-

Fig. 3. Fluorescence intensity at varying concentrations of dopamine.



Fluorescence is given in arbitrary units (meter multiplier value x % transmission x 100). Activating wavelength 325 mμ; fluorescent wavelength 370 mμ.

tration in μg/ml (10 C_u) and the total in a 10 ml eluate to 100 C_u/3. The total amount of dopamine in a sample was then calculated by the use of the following formula:

$$D_t = \frac{V_1}{V_2} \cdot \frac{100C_u}{3}$$

Where D_t = the total quantity (μg) of dopamine in a sample

V_1 = the volume (ml) of the sample extracted for dopamine content

V_2 = the total volume (ml) of the sample

C_u = the concentration of dopamine (μg/ml) in the developed solution of the unknown

This method of dopamine determination provided accurate recoveries. The average recovery obtained for known concentrations of dopamine from 0.2 N acetic acid was 88.2 $\pm$ 6.5%, (Table I).

Throughout the experimental period internal standards (known amounts of dopamine added to the urine prior to processing) were determined at selected intervals. The mean recovery was 84.3 $\pm$ 8.7% (Table II).

Table I

Percent recovery of dopamine from 0.2 N acetic acid

Dopamine added (μg)	Dopamine found (μg)	% Recovery
2.00	1.68	84.0
2.00	1.85	93.0
2.00	1.80	90.0
2.00	1.83	91.3
2.00	1.38	69.0
2.00	1.85	92.5
4.00	3.56	89.0
4.00	3.74	93.5
4.00	3.90	97.5
4.00	3.51	87.8
4.00	3.61	86.3
4.00	3.69	88.1
6.00	5.45	90.8
6.00	5.71	95.1
6.00	5.49	91.5
6.00	4.76	79.2
6.00	5.47	87.1
6.00	4.95	83.0

Mean recovery $\pm$ standard deviation = 88.2 $\pm$ 6.5%

Table II

Percent recovery of dopamine added to urine samples

Endogenous dopamine (μg)	Added dopamine (μg)	Total dopamine (μg)		% Recovery
		Calculated	Found	
2.29	2.00	4.29	3.65	88
1.33	2.00	3.33	2.33	70
2.30	2.00	4.30	3.78	86
1.90	2.00	3.90	2.97	76
2.50	2.00	4.50	4.20	93
0.93	2.00	2.93	2.53	86
3.17	2.00	5.17	4.23	81
1.86	2.00	3.86	3.90	101
1.43	3.00	4.43	3.60	81
1.09	4.00	5.09	4.09	80

Mean recovery $\pm$ standard deviation = 84.3 $\pm$ 8.7%

IV. RESULTS

The effect of DDC on mean body weight in normal male albino rats is illustrated in Fig. 4. The mean body weight of the vehicle-treated animals increased over a ten week period from an initial 180 g. to a final weight of 400 g. At the end of ten weeks of treatment the mean body weight of the DDC-treated animals had increased to 350 g. The drug-treated animals had a significantly lower mean body weight than vehicle-treated animals after six weeks of treatment with DDC.

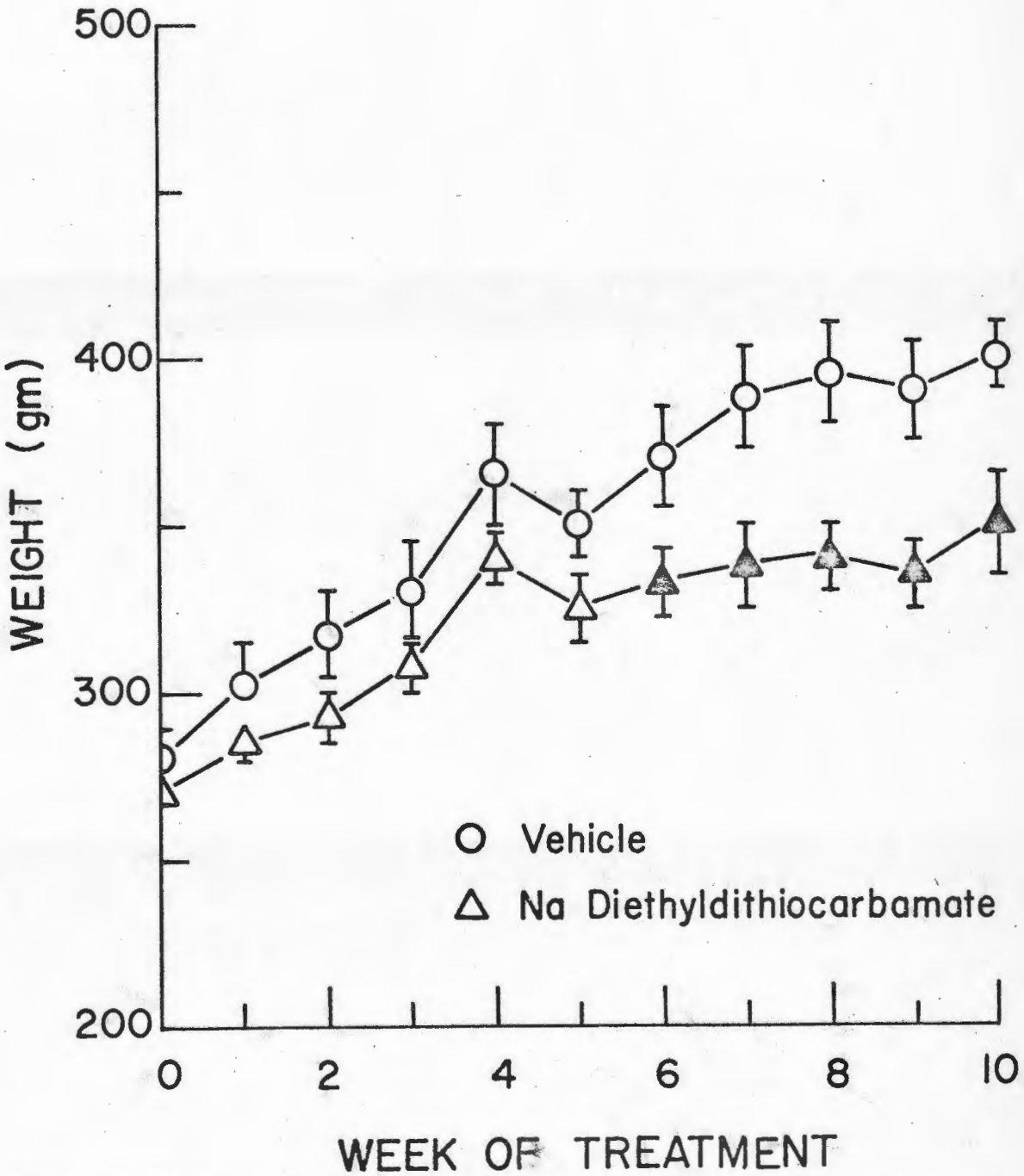
Fig. 5 represents the effect of DDC treatment on body weight in unilaterally nephrectomized male albino rats. The mean body weight of vehicle-treated animals increased from 240 g. to 350 g. during the nine week study. The mean body weight of the DDC-treated animals increased from 230 to 300 g. during the same period. There was a significant difference in body weight observed after four weeks of treatment when DDC-treated animals were compared to controls.

The mean body weight of vehicle-treated versus DDC-treated renal hypertensive male albino rats appears in Fig. 6. The mean body weight of the control animals increased to 450 g., whereas, mean body weight of the DDC-treated animals increased to 375 g. after ten weeks of treatment. The difference from control values became significant after two weeks of treatment with DDC.

The method used to initiate renal hypertension proved successful. After nine weeks of treatment the mean systolic blood pressure of the vehicle-treated animals increased to 165 mm Hg (Fig. 9). The mean pressure of the surgically intact and unilaterally nephrectomized groups of vehicle-treated animals remained relatively constant throughout the same period (Fig. 7, 8).

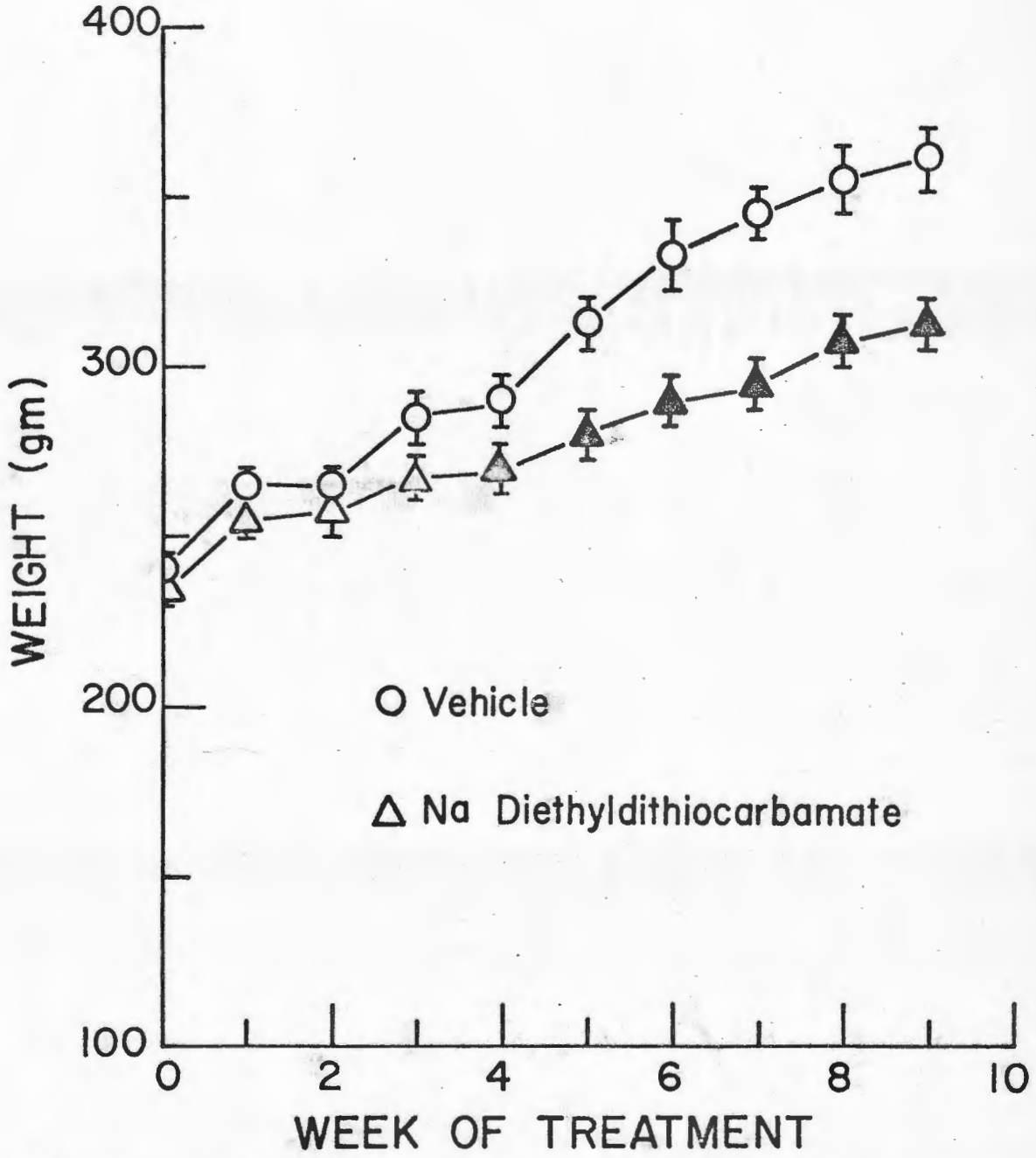
The effect of DDC treatment on systolic blood pressure in normal male albino rats appears in Fig. 7. On the seventh week of treatment with DDC the drug-treated animals had a significantly lower mean systolic blood pressure when compared to vehicle-treated controls. At the end of eight weeks of treatment the pressure of the vehicle-treated animals had increased from 115 to 125 mm Hg whereas that of the DDC-treated animals decreased from 115 to [illegible] mm Hg.

FIG. 4 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON BODY WEIGHT IN MALE ALBINO RATS.



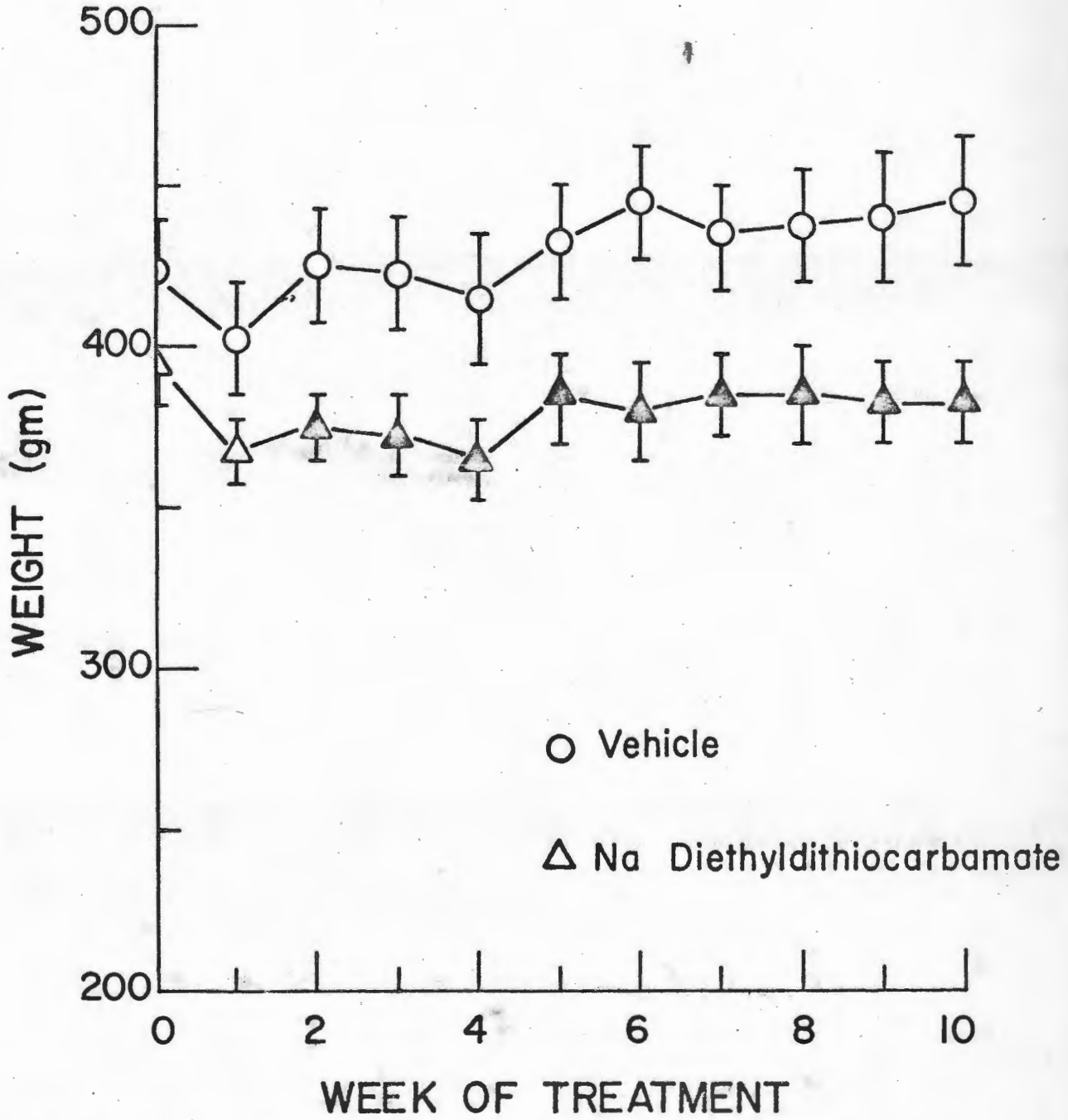
Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean ± s.e. of six determinations.

FIG. 5 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON BODY WEIGHT IN UNILATERAL NEPHRECTOMIZED MALE ALBINO RATS.



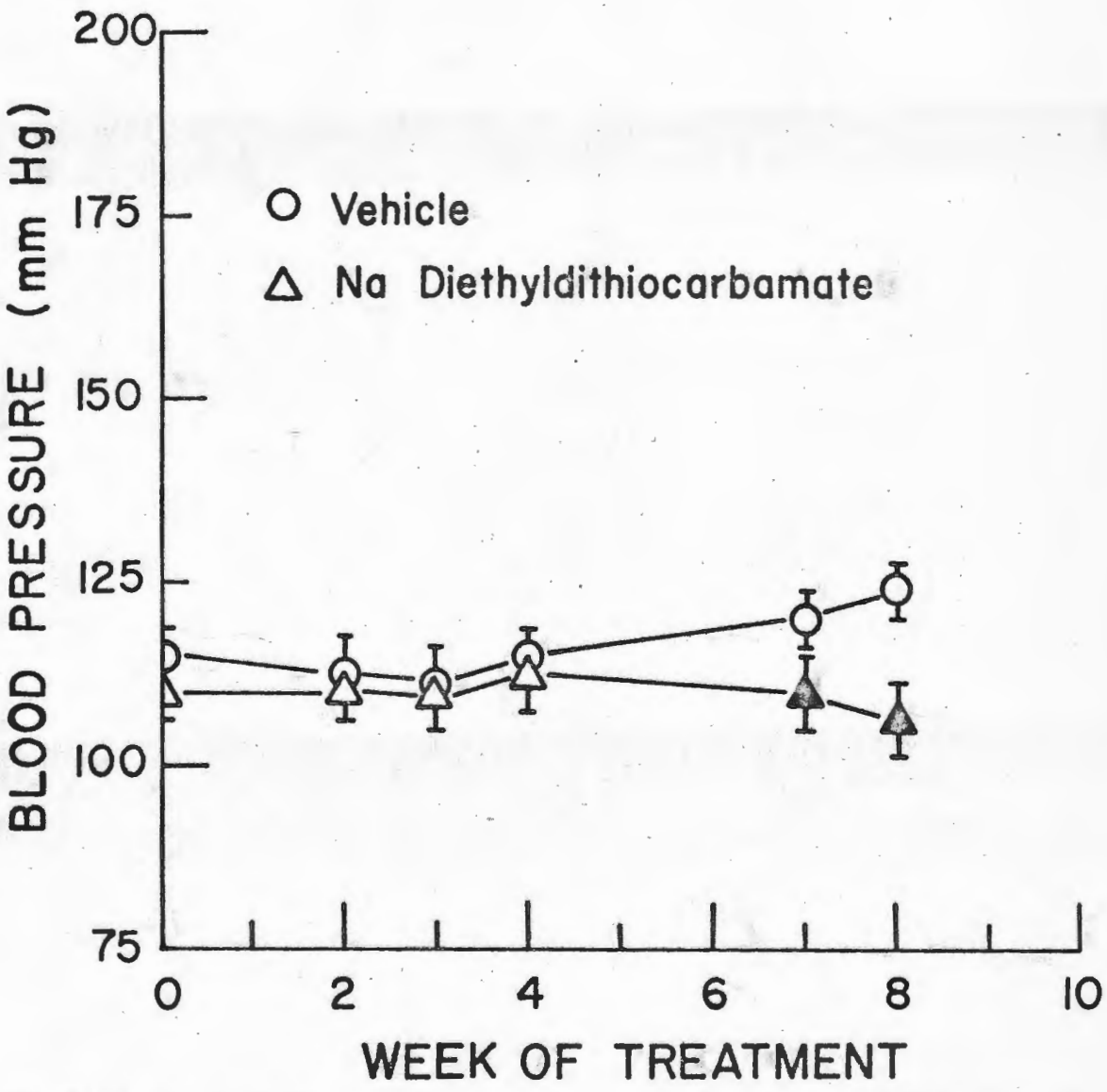
Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean $\pm$ s.e. of six determinations.

FIG. 6 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON BODY WEIGHT IN RENAL HYPERTENSIVE MALE ALBINO RATS.



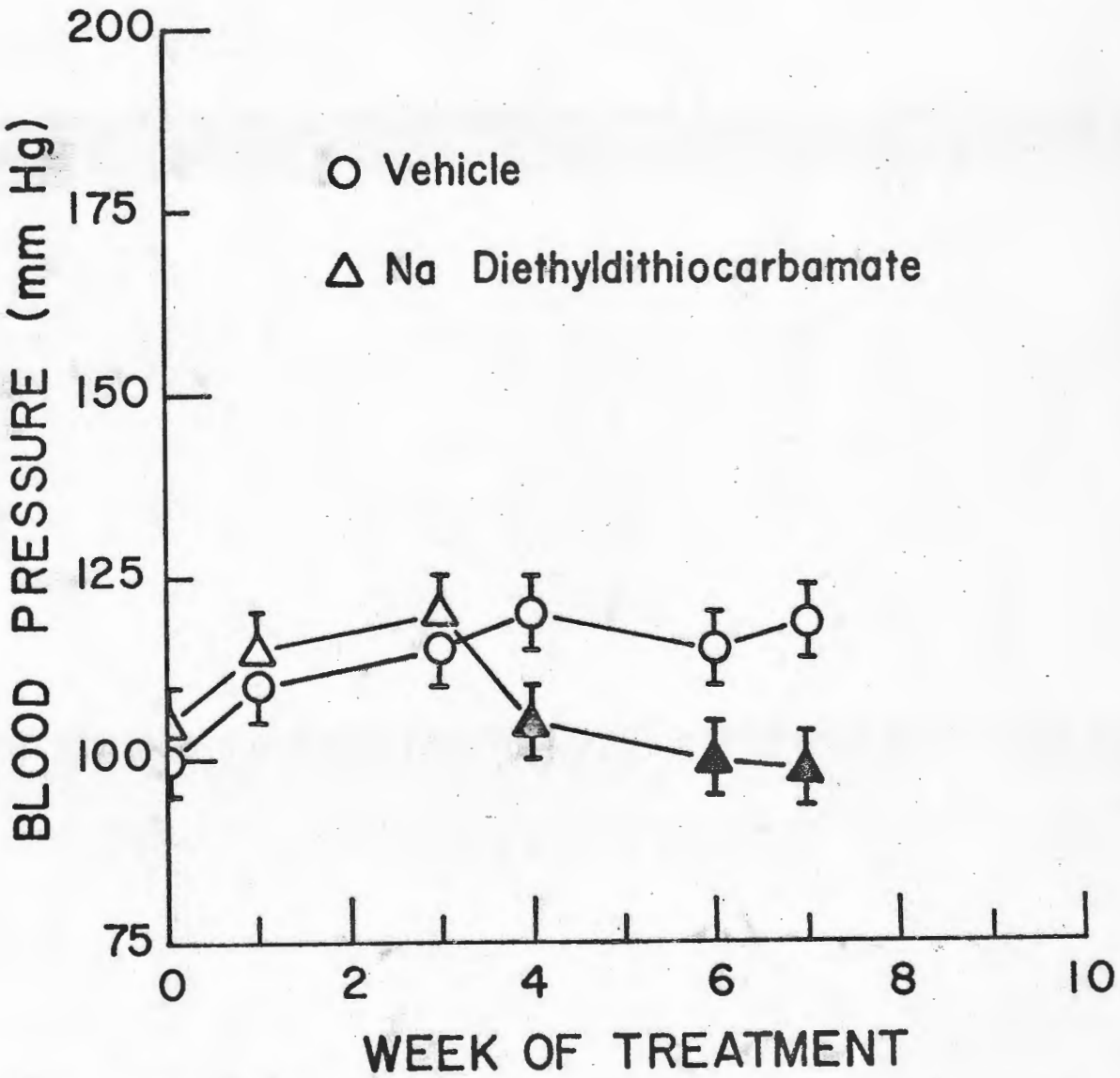
Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean ± s.e. of six determinations.

FIG. 7 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON SYSTOLIC BLOOD PRESSURE IN MALE ALBINO RATS.



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean $\pm$ s.e. of six determinations.

FIG. 8 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON SYSTOLIC BLOOD PRESSURE IN UNILATERAL NEPHRECTOMIZED MALE ALBINO RATS.



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean ± s.e. of six determinations.

The systolic blood pressure of the unilaterally nephrectomized vehicle-treated male albino rats increased from a mean of 100 mm Hg after seven weeks of treatment (Fig. 8). A decrease in systolic blood pressure was observed in the drug-treated unilaterally nephrectomized animals ranging from an initial mean value of 110 mm Hg to a mean of 98 mm Hg after seven weeks of treatment with DDC. The decrease in systolic blood pressure became significant after the fourth week of treatment.

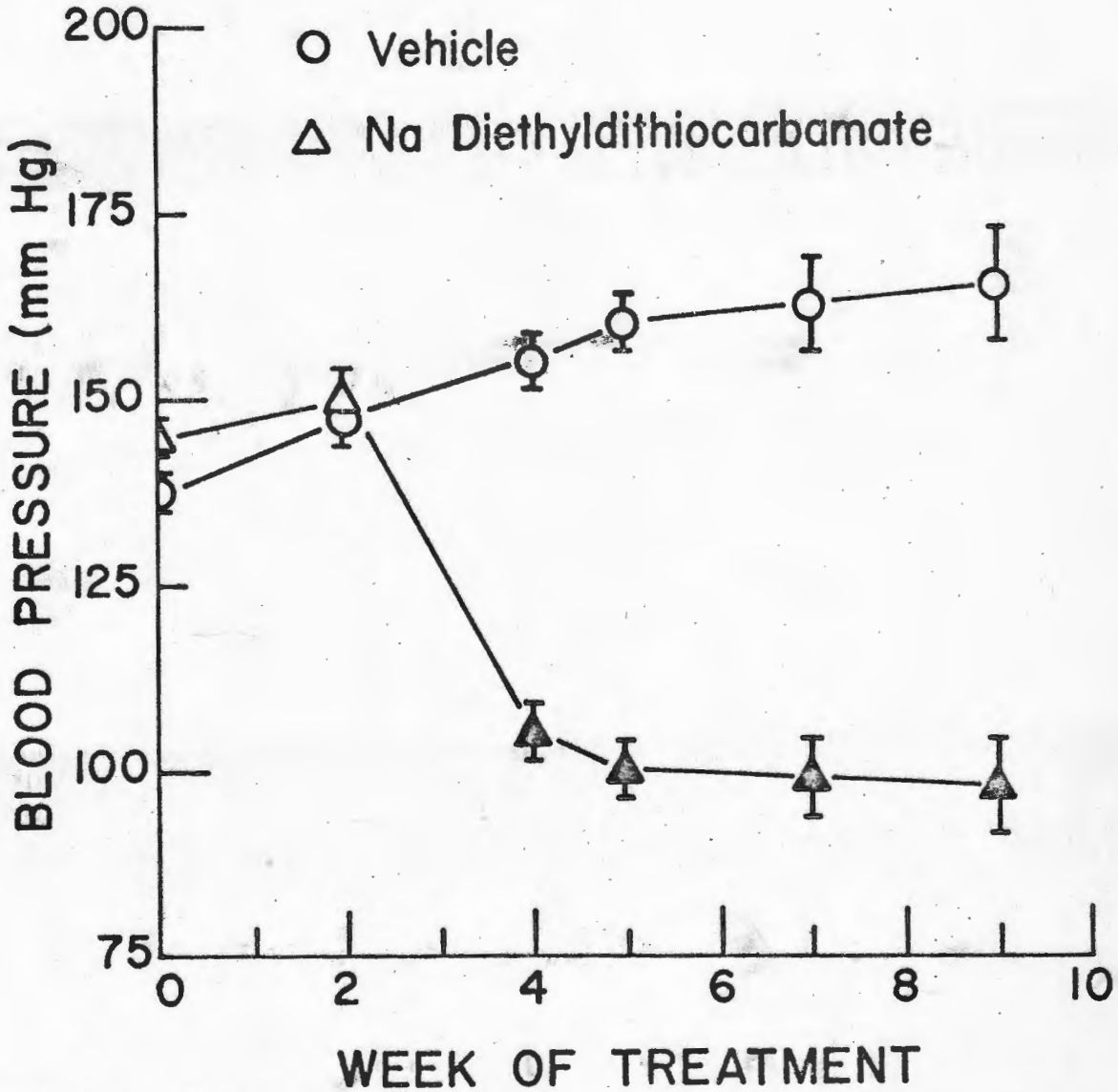
The hypotensive effect of DDC was more dramatic in renal hypertensive animals (Fig. 9). The mean systolic blood pressure of the vehicle-treated renal hypertensive male albino rats increased from an initial value of 135 mm Hg to a final value of 165 mm Hg. However, the mean systolic blood pressure of the DDC-treated animals decreased from a mean of 140 mm Hg initially to 100 mm Hg after nine weeks of treatment. A significant difference from controls was observed after four weeks of treatment with DDC in the renal hypertensive animals.

The effect of DDC treatment on urinary dopamine levels in normal male albino rats is represented in Fig. 10. There was no significant difference observed between control and DDC-treated animals at any time during treatment. Urinary dopamine levels of the vehicle-treated animals ranged from an initial value of 22 μg/24 hr to a value of 20 μg/24 hr after nine weeks of treatment. Similarly, urine dopamine levels of the DDC-treated animals ranged from a low of 20 μg/24 hr after two weeks of treatment to a high of 28 μg/24 hr after six weeks of treatment, returning to normal after nine weeks of treatment.

Fig. 11 represents the effect of DDC on urinary dopamine in unilaterally nephrectomized male albino rats. Urinary dopamine levels of the DDC-treated animals increased from 18 μg/24 hr to 22 μg/24 hr after seven weeks of treatment. The mean urine dopamine levels of the controls decreased from a normal of 15 μg/24 hr to 13 μg/24 hr after the same length of treatment. There was a significant difference between drug-treated and control animals observed after seven weeks treatment with DDC.

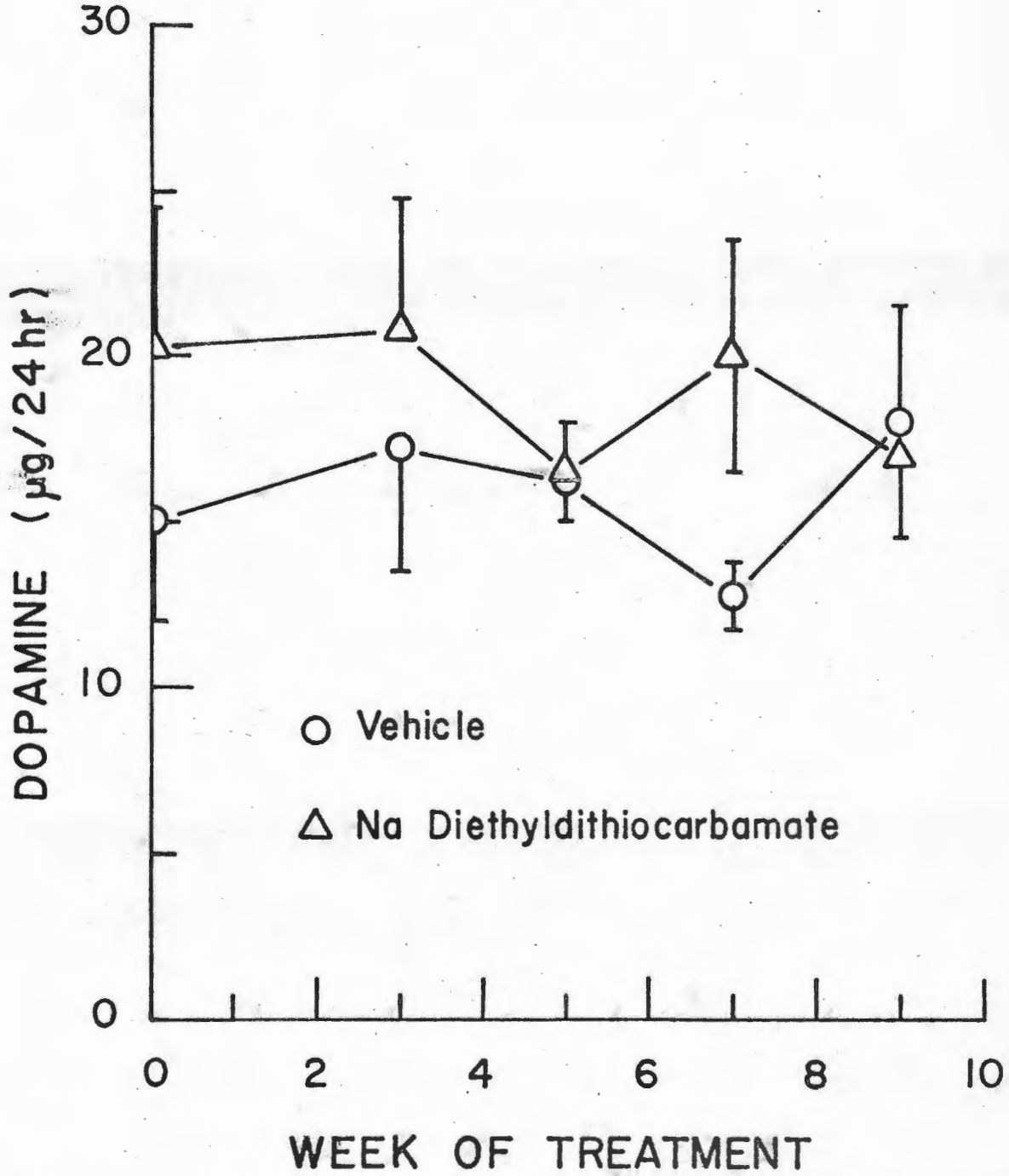
The effect of DDC on urinary dopamine levels in renal hypertensive male albino rats appears in Fig. 12. Mean urine dopamine levels of the controls increased from 15 μg/24 hr to a high of 18 μg/24 hr after nine weeks of treat-

FIG. 9 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON SYSTOLIC BLOOD PRESSURE IN RENAL HYPERTENSIVE MALE ALBINO RATS.



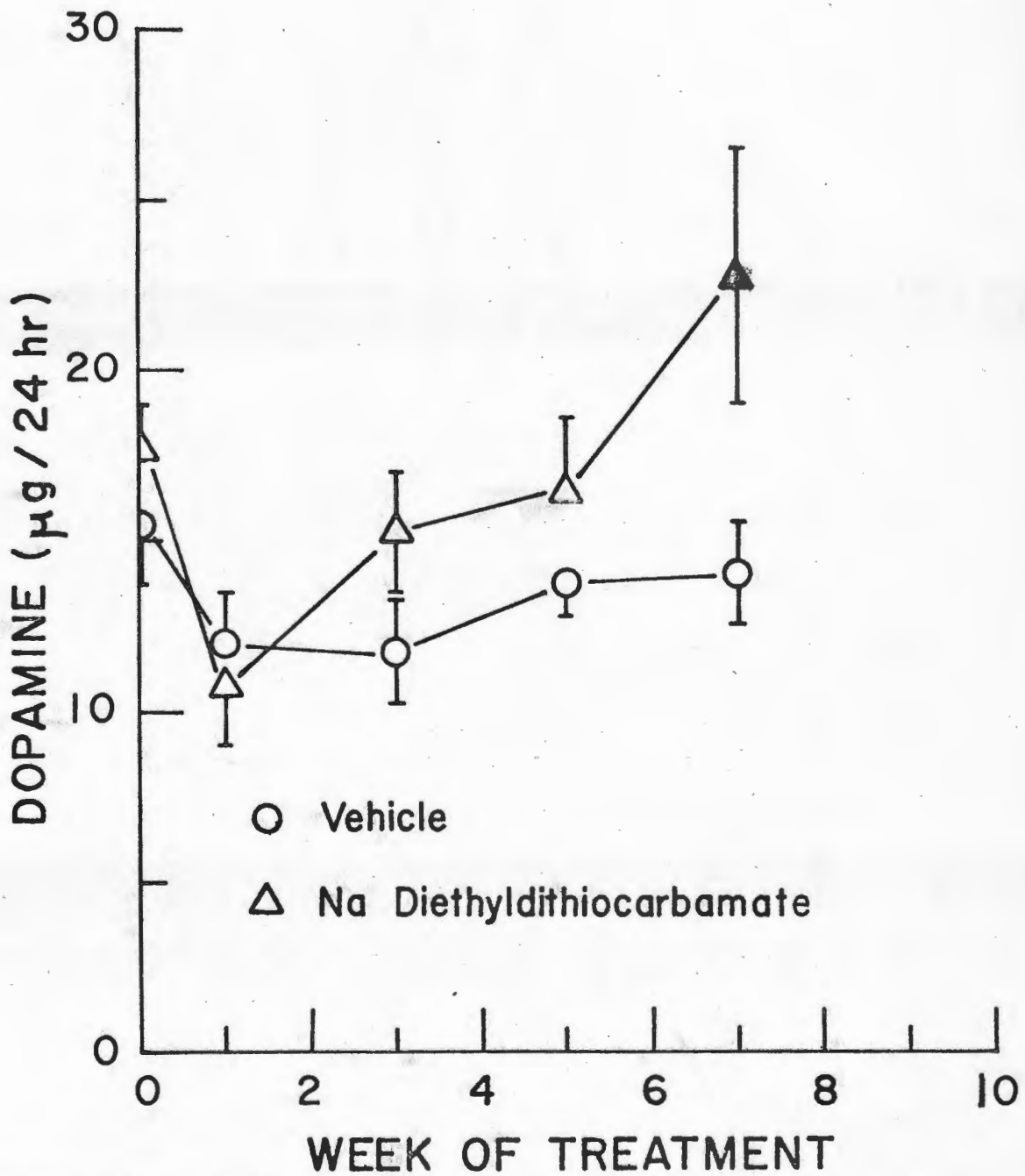
Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean $\pm$ s.e. of six determinations.

FIG. 10 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON URINARY DOPAMINE (μg/24 hr) IN RENAL HYPERTENSIVE MALE ALBINO RATS.



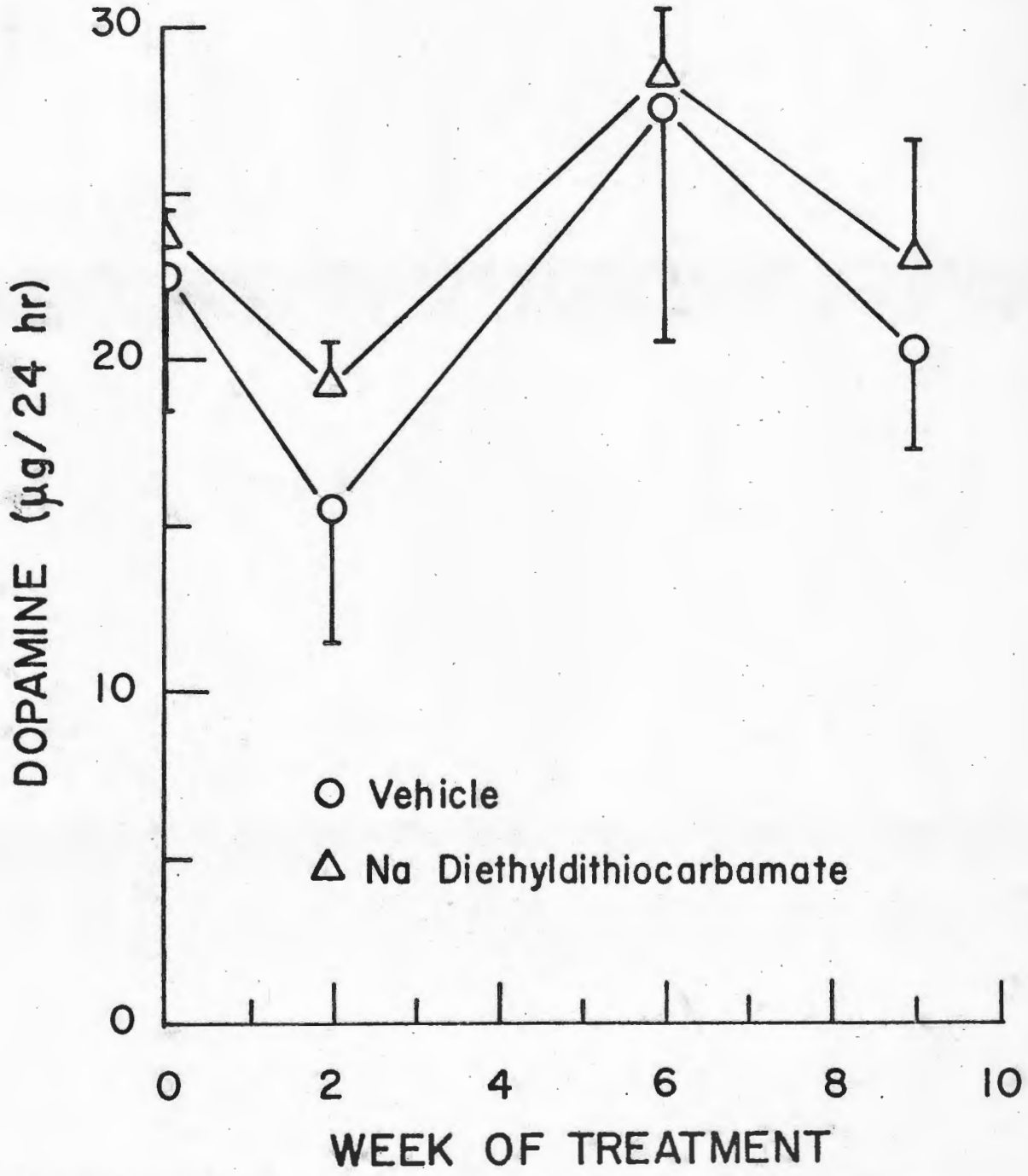
No significant difference ($P < 0.05$) observed between vehicle- and drug-treated groups. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean $\pm$ s.e. of six determinations.

FIG. 11 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON URINARY DOPAMINE (μg/24 hr) IN UNILATERAL NEPHRECTOMIZED MALE ALBINO RATS.



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean $\pm$ s.e. of six determinations.

FIG. 12 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON URINARY DOPAMINE (μg/24 hr) IN MALE ALBINO RATS.



No significant difference ($P < 0.05$) observed between vehicle- and drug-treated groups. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, .1M phosphite buffer pH 7.4, (1 ml/kg). Each point represents the mean ± s.e. of six determinations.

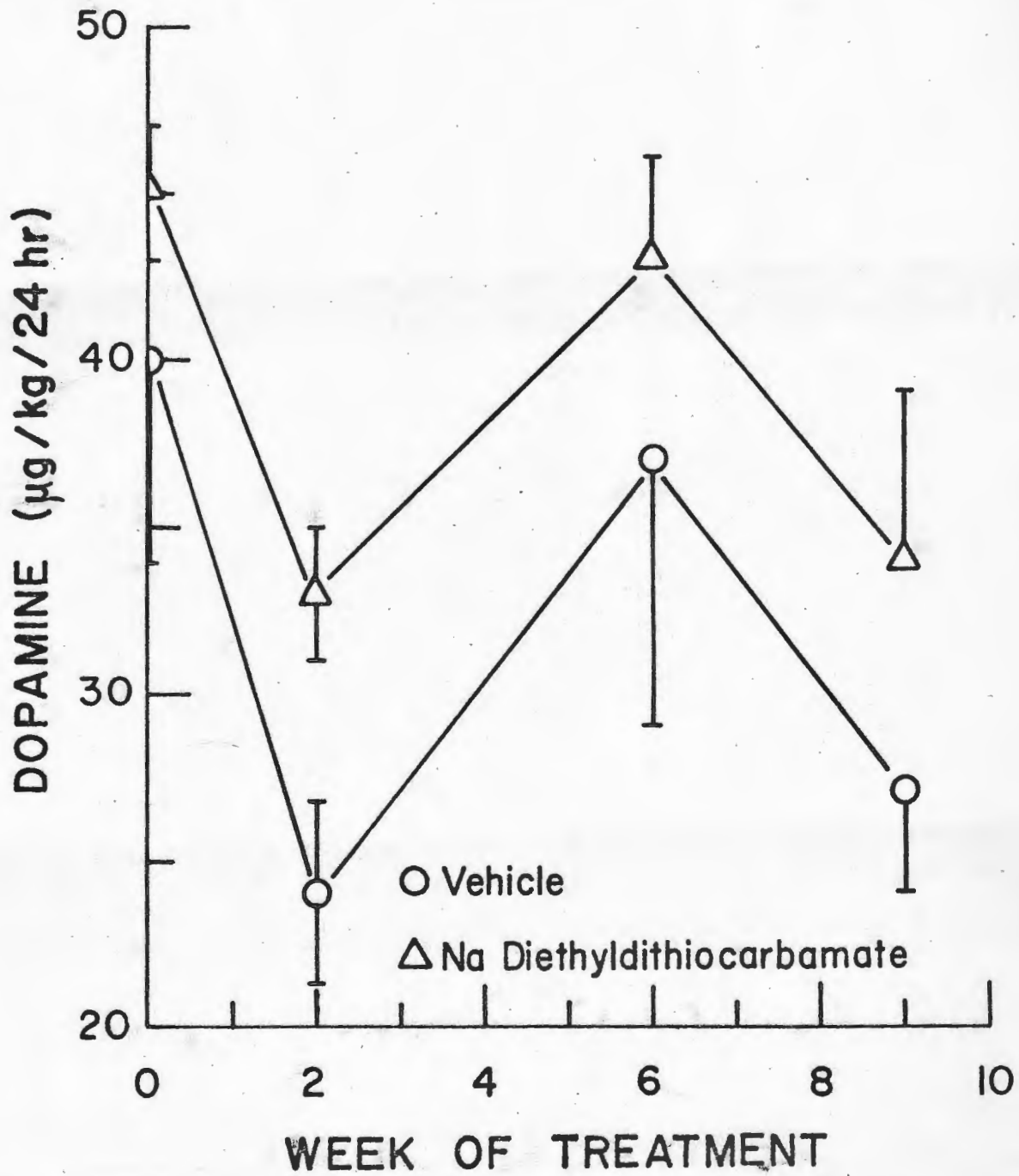
ment, whereas the total urinary dopamine of the DDC-treated animals ranged from 20 μg/24 hr to a low of 17 μg/24 hr after nine weeks of treatment with DDC. There was no significant difference from controls observed at any time during treatment.

Urinary dopamine levels expressed as micrograms per kilogram of body weight per 24 hours in normal animals are represented in Fig. 13. Dopamine levels (μg/kg/24 hr) varied widely in the DDC and vehicle-treated normal animals. There was a tendency of the dopamine concentration of all animals to decrease with decreasing weight, however, this trend proved to be statistically insignificant.

Urine dopamine levels (μg/kg/24 hr) in the unilaterally nephrectomized group of animals decreased from 33 to 22 μg/kg/24 hr in the vehicle-treated group. After seven weeks of treatment with DDC, the urine dopamine levels of the drug-treated group of animals decreased from 38 to 34 μg/kg/24 hr becoming statistically significant from controls after the seventh week.

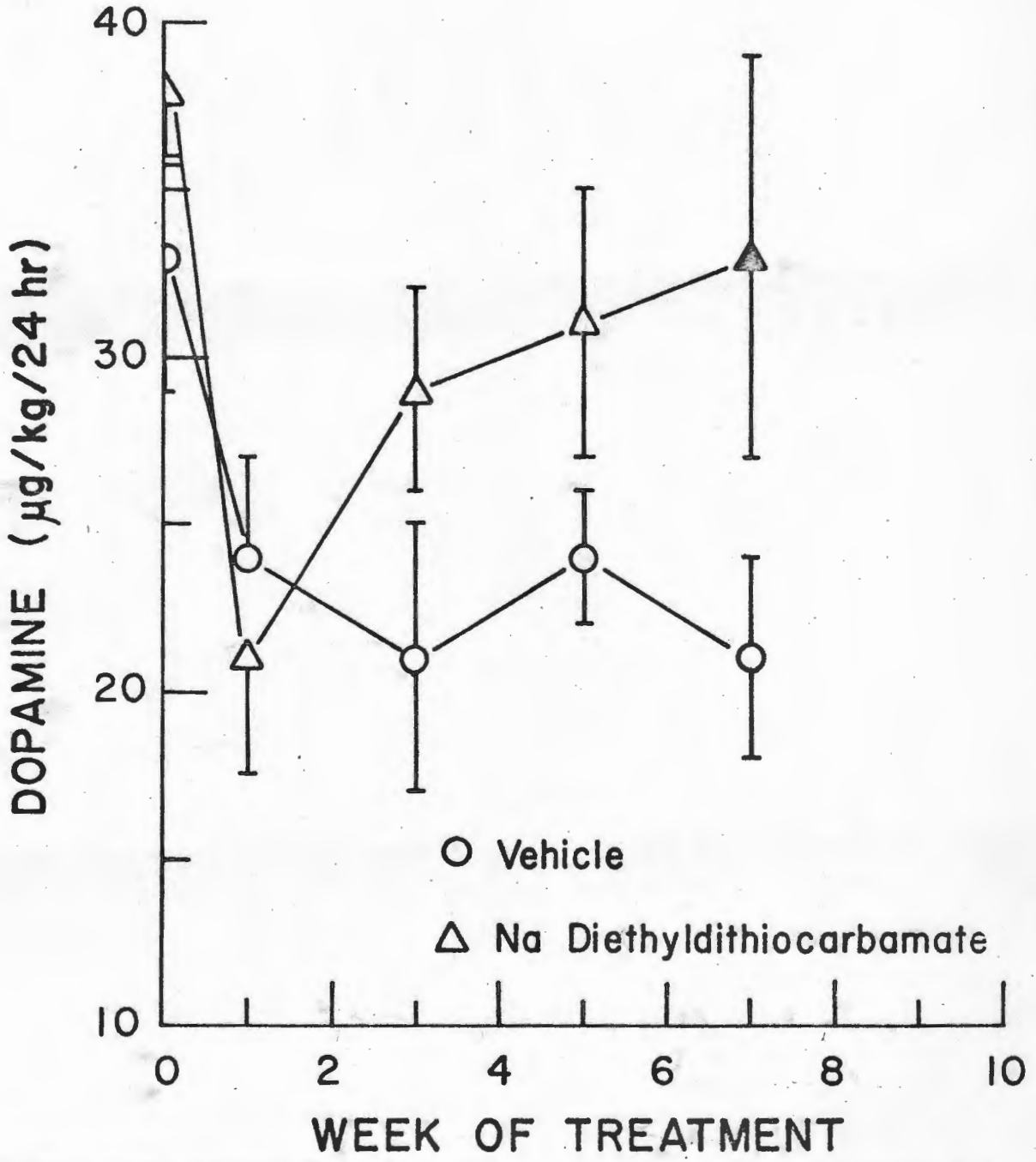
The urinary excretion of dopamine per kg per 24 hr did not differ significantly when renal hypertensive drug-treated animals were compared with vehicle-treated renal hypertensive animals. The dopamine levels of the latter group increased from 21 μg/kg/24 hr to 24 μg/kg/24 hr whereas the urinary dopamine excretion of the DDC-treated group decreased from 26 μg/kg/24 hr to 23 μg/kg/24 hr. (Fig. 15)

FIG. 13 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON URINARY DOPAMINE IN MALE ALBINO RATS. (μg/kg/24 hr)



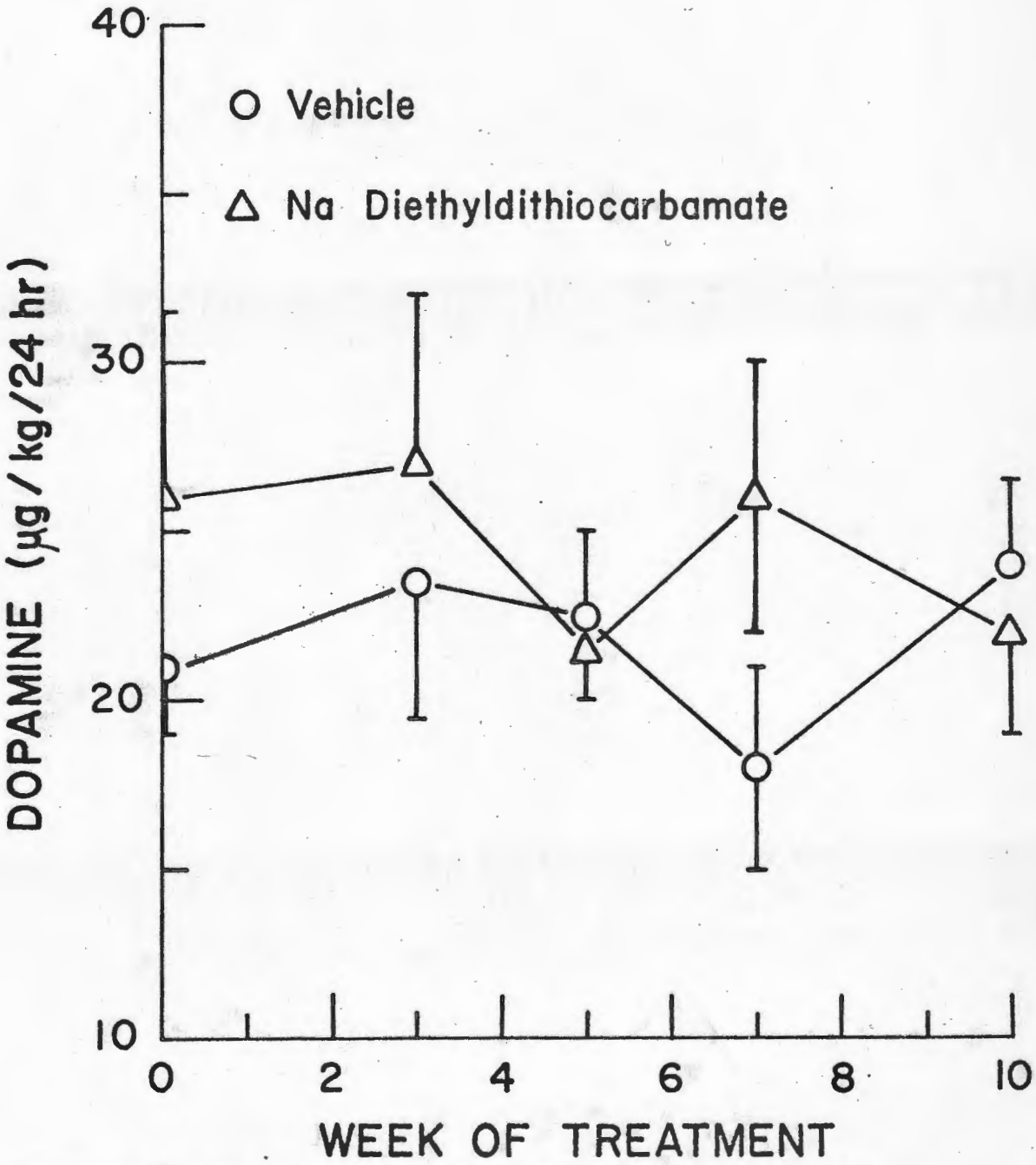
No significant difference ($P < 0.05$) observed between vehicle- and drug-treated groups. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean ± s.e. of six determinations.

FIG. 14 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON URINARY DOPAMINE IN UNILATERAL NEPHRECTOMIZED MALE ALBINO RATS. (μg/kg/24 hr)



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean $\pm$ s.e. of six determinations.

FIG. 15 THE EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE ON URINARY DOPAMINE IN RENAL HYPERTENSIVE MALE ALBINO RATS. (μg/kg/24 hr)



No significant difference ($P < 0.05$) observed between vehicle- and drug-treated groups. Drug doses, (i.p.) on alternate days: Sodium diethyldithiocarbamate (66.5 mg/kg), vehicle, 0.1M phosphate buffer pH 7.4, (1 ml/kg). Each point represents the mean ± s.e. of six determinations.

V. DISCUSSION

The time interval subsequent to initiation of treatment at which the body weight of the DDC-treated animals became significantly different from that of the controls appeared to reflect the amount of functional kidney tissue in the three groups of animals studied. This correlation was based on the assumption that a smaller number of nephrons were functional in renal hypertensive and unilaterally nephrectomized than in the normal animals. The unilaterally nephrectomized and renal hypertensive DDC-treated animals showed a significantly lower body weight than that of the control animals after four and two weeks of treatment, respectively. The normal or surgically intact animals showed this difference after six weeks of treatment with DDC. This effect may be due to a deficiency in detoxication or excretion as a result of decreased functional kidney tissue thereby causing a cumulative effect.

The significant hypotensive effect observed in the three groups treated with DDC agrees with the observations of Wohl et al. (1965). These authors using desoxycorticosterone acetate-hypertensive rats noted a marked and sustained hypotensive effect when 100 mg/kg of disulfiram was administered intraperitoneally. An explanation of the hypotensive effect on the basis of increased circulating or tissue dopamine levels appears incongruous to the findings of Pogrund et al. (1961), who demonstrated a pressor response to dopamine in the rat. However, Thoenen et al. (1967) observed that after inhibition of dopamine-β-hydroxylase by disulfiram, dopamine incompletely replaced the missing norepinephrine suggesting the affinity of dopamine for storage sites is weaker than that of norepinephrine. This view appears to be in agreement with the observations of Burn and Rand (1958). These authors in an effort to explain the depressor effect of dopamine in guinea pig hypothesized that dopamine, when present in larger quantities than norepinephrine, occupied some of the vacated norepinephrine receptor sites. But, being a much feebler constrictor agent, dopamine's occupation of the sites results in a loss of vascular tone. This supports the belief that the hypotensive effect observed in

the DDC-treated animals in this study may be a consequence of decreased norepinephrine levels.

An inability to demonstrate increased urinary dopamine levels with the DDC-treated animals appears contradictory to the explanation of the hypotensive effect. However, dopamine has been reported to be the natural substrate for MAO (Blaschko, 1952). Since norepinephrine levels are depressed in DDC-treated animals, excess MAO would be available to metabolize circulating or tissue dopamine. Although dopamine and norepinephrine concentrations were not measured in the kidney tissues, it would appear that the dose of DDC administered was not sufficient to cause a large enough increase in circulating dopamine to overcome the available MAO. As a result a significant increase in urinary dopamine could not be detected.

DeFanti and DeFeo (1963) reported finding a positive correlation between increased excretion of dopamine (μg/kg/24 hr) and body weight (g.) for renal hypertensive rats. However, a decrease in dopamine excretion was observed in normotensive rats.

The decreased urinary dopamine (μg/kg/24 hr) observed in normotensive rats in the present study agrees with the findings of DeFanti and DeFeo (1963) and Leduc (1961). In normotensive DDC-treated animals however, there was a tendency of the dopamine levels (μg/kg/24 hr) to increase to a greater degree than that of the vehicle-treated animals, thus providing some indication that the dopamine levels were increased as a result of the DDC treatment. The slight increase noted in the urinary dopamine levels (μg/kg/24 hr) of the vehicle-treated group of renal hypertensive animals is in agreement with the previously mentioned findings of DeFanti. However, this slight increasing trend is not enough evidence to warrant implication of dopamine in arterial hypertension. Coates (1968) also reported finding no correlation between dopamine levels and arterial blood pressure. A possible explanation for the discrepancy between the findings in the present study and the observations of DeFanti may be found in the assay methods used in the two studies. As mentioned previously, alumina was used in the present study for the extraction of dopamine from urine, where-

as DeFanti used Dowex Sodium 50W-X8. Coates (1968) reported finding that Dowex resin was extracting a substance with native fluorescence from the urine of rats and that the blank (Carlsson and Waldeck, 1958) by quenching this fluorescence was resulting in erroneously high urinary dopamine levels. Subsequently, by using alumina and altering the blank, this difficulty was circumvented in the present study and allowed a more valid evaluation of dopamine excretion in the renal hypertensive animals.

An apparent discrepancy to this explanation was the significant increase in urinary dopamine observed in the unilaterally nephrectomized DDC-treated group after seven weeks of treatment. An increase in urine volume per 24 hr in the drug-treated group might explain the increased excretion of total dopamine, however no significant difference was observed in the volume of urine excreted from either group. The large increase in dopamine excreted in the DDC-treated group may be explained on the basis of an inhibition of dopamine-β-hydroxylase, however no such increase was observed in the DDC-treated renal hypertensive group of rats, nor in the normal animals. Further investigation into the meaning of and explanation for the increased excretion of dopamine in DDC-treated unilaterally nephrectomized rats appears necessary.

VI. SUMMARY AND CONCLUSIONS

Normal, unilaterally nephrectomized, and renal hypertensive male albino rats were treated with diethyldithiocarbamate, a dopamine-β-hydroxylase inhibitor. Weight gain, systolic blood pressure, and urinary dopamine levels were measured in an effort to further investigate the role of dopamine in experimental hypertension. A significant hypotensive effect was observed in the three groups treated with diethyldithiocarbamate when compared to vehicle-treated control animals. Mean body weights of the drug-treated rats were significantly lower than controls after six, four, and two weeks of treatment in the normal, unilaterally nephrectomized, and renal hypertensive groups respectively. There appeared to be a positive correlation between decreasing body weight, decreased amount of functional kidney tissue, and decreased arterial blood pressure. There was no significant difference in urinary dopamine observed between drug-treated and control animals in either the normal or renal hypertensive groups. After seven weeks of treatment with diethyldithiocarbamate, a significant increase in urinary dopamine was observed in the unilaterally nephrectomized drug-treated group when compared to controls. No further explanation was offered, however, the results of this study do not indicate any correlation between arterial blood pressure and urinary dopamine levels.

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VIII. VITA

Harold Llewellyn Crossley was born to Mr. and Mrs. Harold L. Crossley on April 28, 1941 in Providence, Rhode Island. Mr. Crossley obtained his elementary and secondary education in Warwick, Rhode Island. In 1959, Mr. Crossley enrolled at the University of Rhode Island and received the Bachelor of Science degree in Pharmacy in June, 1964. Mr. Crossley began full-time graduate studies at the University of Rhode Island in September, 1965 where he completed the requirements for the Master of Science degree in Pharmacology in December, 1969.

Mr. Crossley is married to the former Peggy Anne Peirce of East Greenwich, Rhode Island. He is a member of the Lambda Chi Alpha and Kappa Sci fraternities and has been elected to membership in the Phi Sigma and Rho Chi honor societies.

Mr. Crossley will continue at the graduate school of the University of Rhode Island for the Doctor of Philosophy degree in Pharmacology.