Effect of Sodium Diethyldithiocarbamate (DDC) on Renal Hypertension in Rats

H. L. CROSSLEY, J. J. DEFEO, and D. R. DEFANTI

Abstract □ The effect of DDC on renal hypertension was studied in male albino rats. Evaluation was based on the relationship of urinary dopamine levels to arterial blood pressure in surgically intact, unilaterally nephrectomized and modified Goldblatt hypertensive rats for a 9-week period. Following treatment with DDC, a dopamine hydroxylase inhibitor, a significant increase in urinary dopamine was observed in the unilaterally nephrectomized group of animals. There was no significant increase observed in the other groups. A significant hypotensive effect was observed in all groups of drug-treated animals. In all groups of drug-treated animals there was a correlation between decreasing cumulative weight gains, decreased amount of functional kidney tissue, and decreased arterial blood pressure. No correlation was found to exist between arterial blood pressure and urinary dopamine levels.

Keyphrases □ Na diethyldithiocarbamate—renal hypertension, rats □ Hypotensive activity—Na diethyldithiocarbamate □ Dopamine, urinary levels—Na diethyldithiocarbamate effect

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 levels of circulating catecholamines and their metabolites in hypertension.

Bing (1) reported evidence for the production of a pressor substance, presumably dopamine (DA),[1] from the decarboxylation of dihydroxyphenylalanine (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 also suggested that deamination but not decarboxylation of amino acids is incomplete in anoxic kidneys. This possibility was verified by Giordano *et al.* (2), 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 epinephrine in ischemic tissue.

DeFanti and DeFeo (3) reported evidence indicating a degree of positive correlation between arterial blood pressure and urinary DA concentration in renal hypertensive rats. To further investigate the role of DA in experimental hypertension, the present investigation was designed to inhibit the enzyme DBH, the enzyme responsible for the conversion of DA to its B-hydroxylated analog NE, with DDC. DDC has been reported to inhibit DBH *in vivo* (4, 5) resulting in reduced NE levels with a concomitant increase in DA in rat and rabbit small intestine (6).

[1] DA, dopamine; NE, norepinephrine; DOPA, decarboxylase (3,4-dihydroxy-1-phenylalanine carboxylase E.C. 4.1.1.26); MAO, monoamine oxidase (monoamine: oxygen, oxidoreductase, E.C. 1.4.3.4); DBH, (dopamine-B-hydroxylase, 3,4-dihydroxyphenylethylamine, ascorbate: oxygen oxidoreductase E.C. 1.14.2.1.).

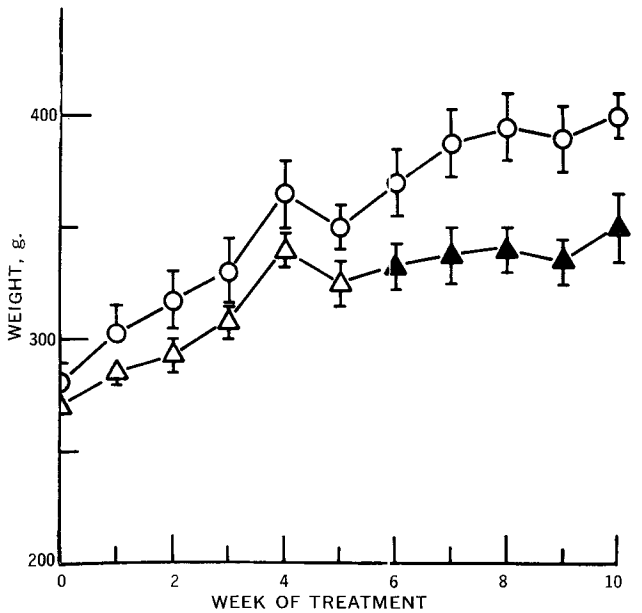

Figure 1—*The effect of Na diethyldithiocarbamate on body weight in male albino rats (average of six rats/group). Key:* ○, *vehicle;* △, *Na diethyldithiocarbamate;* ▲[3].

Evaluation of the present investigation 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.

EXPERIMENTAL

Male albino rats of the Sprague-Dawley strain weighing 125 to 150 g. were divided into three groups: surgically intact, unilaterally nephrectomized, and renal hypertensive. Each group was further divided into two subgroups: animals receiving Na diethyldithiocarbamate 66.5 mg./kg. and animals receiving vehicle, 0.1 *M* phosphate buffer (pH 7.4), 1 ml./kg. of body weight. Injections were made every other day. Animals were fed 20 g. Purina rat chow[2] per animal per day and water was provided *ad libitum*.

Renal hypertension was produced by the method of Goldblatt *et al.* (7) as modified by Drury (8) and consisted of two separate operations. The first involved right unilateral nephrectomy followed in 2 weeks by compression of the contralateral renal artery.

Indirect blood pressure was measured on a biweekly basis using the tail cuff method (9).

Determination of urinary DA was carried out according to the method of Carlsson and Waldeck (10) as modified by Coates (9).

Student's *t* test was used to measure level of significance at $p < 0.05$ for all comparisons reported (11).

RESULTS AND DISCUSSION

The effect of DDC on body weight in normal male albino rats is illustrated in Fig. 1. The mean body weight of the vehicle-treated

[2] Ralston Purina Co., St. Louis, Mo.

[3] The solid triangles ▲ designates significant differences.

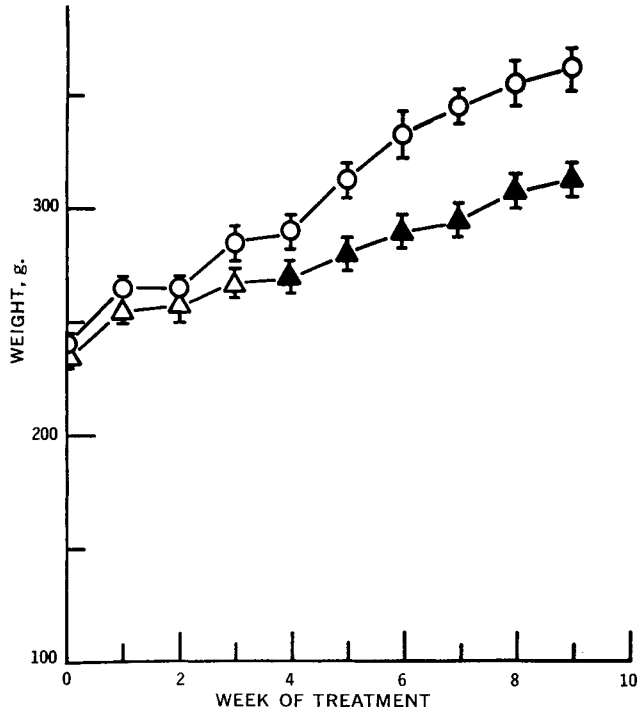

Figure 2—*Effect of Na diethyldithiocarbamate on body weight in unilaterally nephrectomized male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate; ▲[3].*

animals increased from an initial value of 180 g. to a final weight of 400 g. The mean body weight of the DDC-treated animals increased from an initial value of 175 g. to 350 g. during 10 weeks of treatment. The drug-treated animals had a significantly lower mean body weight than vehicle-treated animals after 6 weeks of treatment.

Figure 2 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 an initial value of

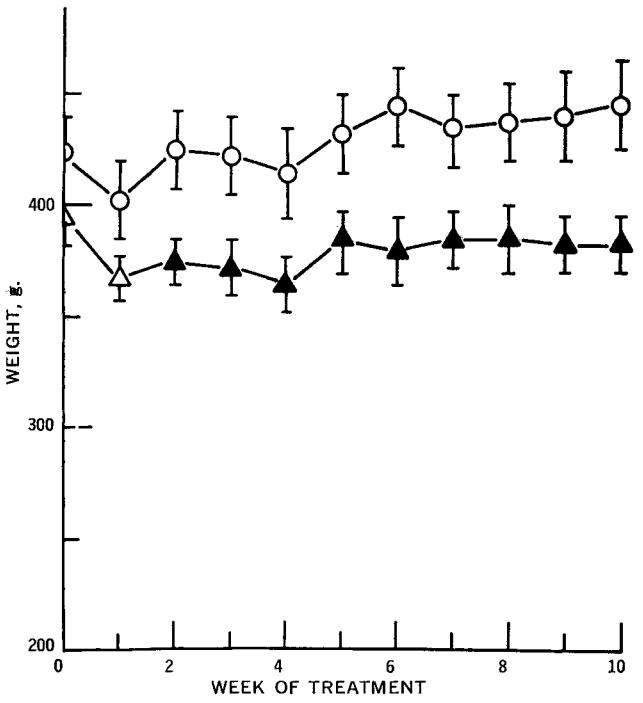

Figure 3—*Effect of Na diethyldithiocarbamate on body weight in renal hypertensive male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate; ▲[3].*

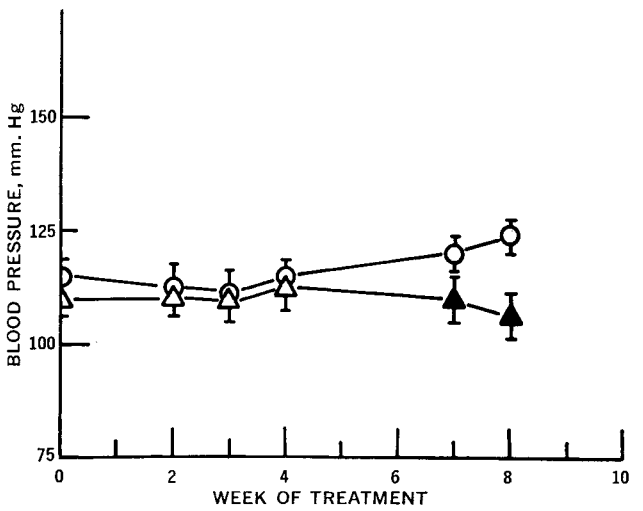

Figure 4—*Effect of Na diethyldithiocarbamate on systolic blood pressure in male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate; ▲[3].*

240 g. to 350 g. during the ninth week of the study, whereas the mean body weight of the DDC-treated animals increased from 235 g. to 300 g. during the same period. The DDC-treated animals had a significantly lower mean body weight than that of the control animals after 4 weeks of treatment.

The mean body weight of vehicle-treated *versus* DDC-treated renal hypertensive male albino rats is represented in Fig. 3. The mean body weight of the control animals increased from 425 g. to 450 g.; however, mean body weight of the DDC-treated animals decreased from 395 g. initially to 375 g. after 10 weeks of treatment. The difference from control values became significant after 2 weeks of treatment with DDC.

The time interval subsequent to initiation of treatment at which the 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 studied. The unilaterally nephrectomized and renal hypertensive DDC-treated animals showed a significantly lower body weight than that of the control animals after 4 and 2 weeks of treatment, respectively. The normal or surgically intact animals showed this difference after 6 weeks of treatment with DDC. This effect may be nothing more than an inability to excrete an entire single dose resulting in a cumulative effect.

The effect of DDC treatment on systolic blood pressure in male albino rats appears in Fig. 4. The mean systolic blood pressure of

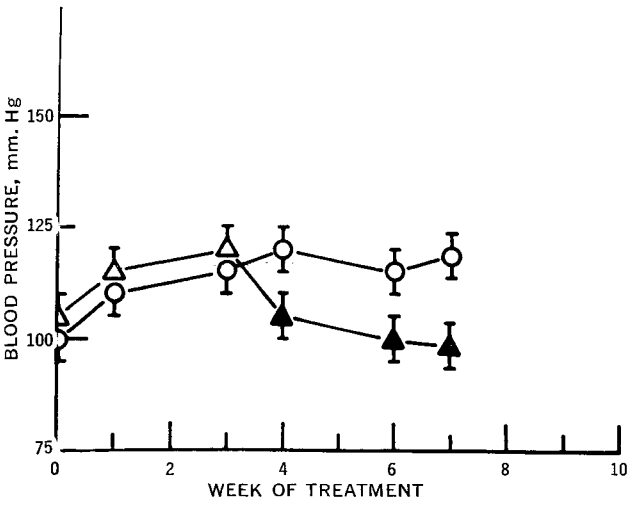

Figure 5—*Effect of Na diethyldithiocarbamate on systolic blood pressure in unilaterally nephrectomized male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate; ▲[3].*

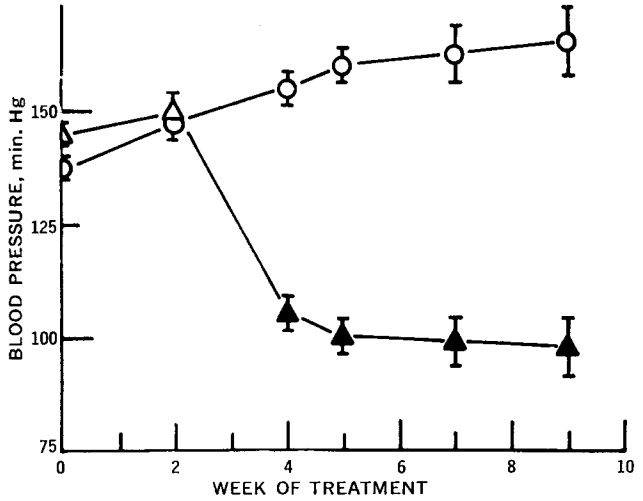

Figure 6—*Effect of Na diethyldithiocarbamate on systolic blood pressure in renal hypertensive male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate; ▲[3].*

the vehicle-treated animals increased from an initial value of 115 mm. Hg to a final value of 125 mm. Hg. The blood pressure of the DDC-treated animals decreased from 110 mm. Hg to 105 mm. Hg. However, there was a significant difference from control after 7 weeks of treatment with the DDC.

The systolic blood pressure of the unilaterally nephrectomized vehicle-treated male albino rats increased from a mean of 100 mm. Hg to a mean of 120 mm. Hg after 7 weeks of treatment (Fig. 5). 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 7 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. 6). 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 a mean of 100 mm. Hg after 9 weeks of treatment. A significant difference from controls was observed after 4 weeks of treatment with DDC in the renal hypertensive animals.

The significant hypotensive effect observed in the three groups treated with DDC agrees with the observations of Wohl *et al.* (12). These authors using DOPA hypertensive rats noted a marked and sustained hypotensive effect when 100 mg./kg. of disulfiram was administered intraperitoneally. The explanation of the hypotensive effect on the basis of increased circulating or tissue DA levels appears incongruous to the findings of Pogrund *et al.* (13), who demonstrated a pressor response to DA in the rat. However, Thoenen *et al.* (14) observed that after inhibition of DBH by disulfiram, DA incompletely replaced the missing NE suggesting the affinity of DA for storage sites is weaker than that of NE. This view appears to be in agreement with the observations of Burn and Rand (15). These authors in an effort to explain the depressor effect of DA in guinea pig hypothesized that DA when present in larger quantities than NE occupied some of the vacated NE receptor sites. But, being a much feebler constrictor agent, DA's occupation of the sites results in loss of vascular tone. Therefore, the hypotensive effect observed in the DDC-treated animals in this study may be a consequence of decreased NE levels.

The effect of DDC treatment on urinary DA levels in normal male albino rats is represented in Fig. 7. There was no significant difference observed between control and DDC-treated animals at any time during treatment. Urinary DA levels of the vehicle-treated animals ranged from an initial value of 22 mcg./24 hr. to a value of 20 mcg./24 hr. after 9 weeks of treatment. Similarly, urine DA levels of the DDC-treated animals ranged from a low of 20 mcg./24 hr. after 2 weeks of treatment to a high of 28 mcg./24 hr. after 6 weeks of treatment, finally returning to normal after 9 weeks of treatment.

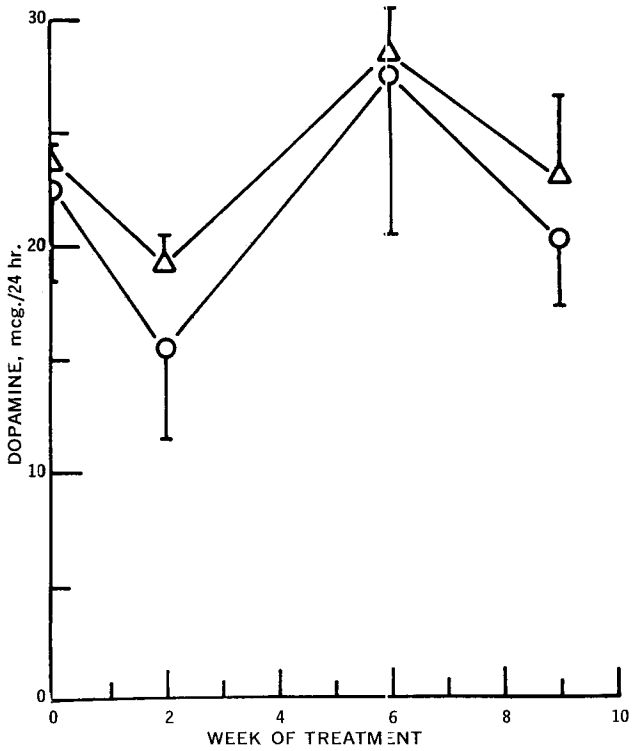

Figure 7—*Effect of Na diethyldithiocarbamate on urinary dopamine in male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate.*

Figure 8 represents the effect of DDC on urinary DA in unilaterally nephrectomized male albino rats. Urinary DA levels of the DDC-treated animals increased from a normal value of 18 mcg./24 hr. to 22 mcg./24 hr. after 7 weeks of treatment. The mean urine DA levels of the controls decreased from a normal of 15 mcg./24 hr. to 13 mcg./24 hr. after 7 weeks of treatment. There was a significant

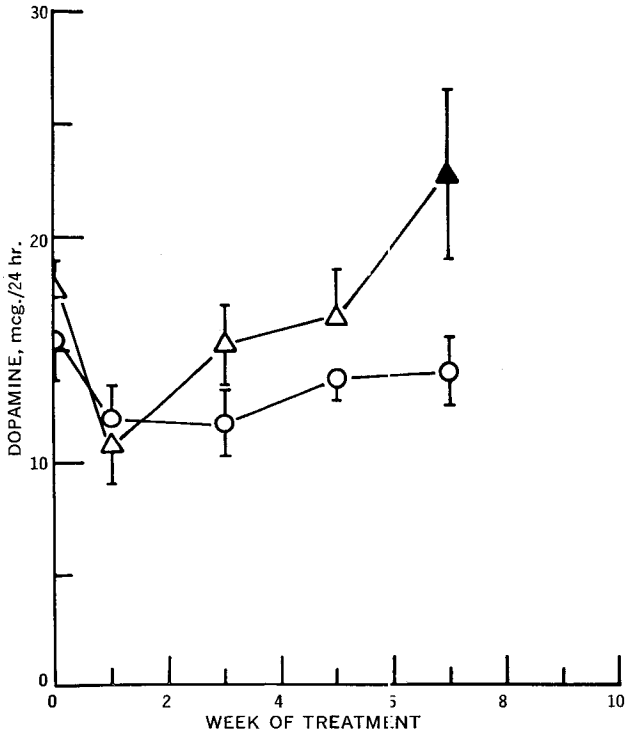

Figure 8—*Effect of Na diethyldithiocarbamate on urinary dopamine in unilaterally nephrectomized male albino rats (average of six rats/group). Key: O, vehicle; △, Na diethyldithiocarbamate; ▲[3].*

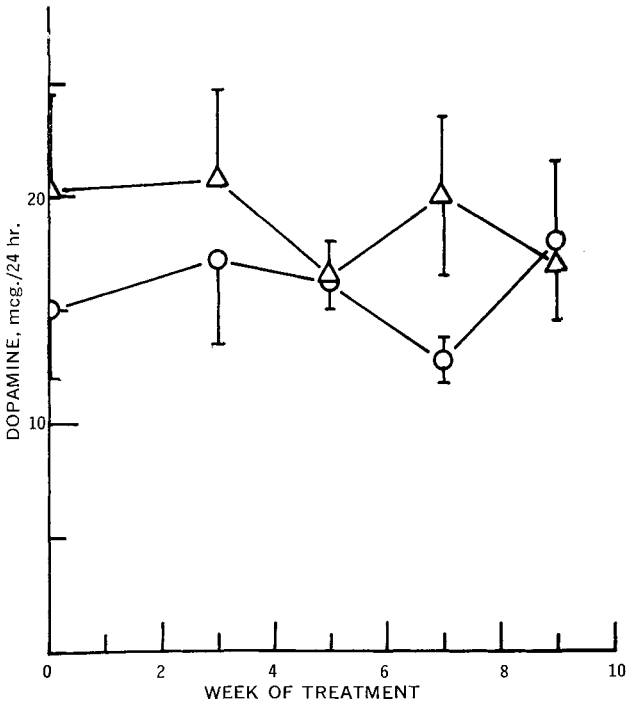

Figure 9—*Effect of Na diethyldithiocarbamate on urinary dopamine in renal hypertensive male albino rats (average of six rats/group). Key: ○, vehicle; △, Na diethyldithiocarbamate.*

difference between drug-treated and control animals observed after 7-weeks treatment with DDC.

The effect of DDC on urinary DA levels in renal hypertensive male albino rats appears in Fig. 9. Mean urine DA levels of the controls increased from 15 mcg./24 hr. to a high of 18 mcg./24 hr. after 9 weeks of treatment, whereas the total urinary DA of the DDC-treated animals ranged from 20 mcg./24 hr. to a low of 17 mcg./24 hr. after 9 weeks of treatment with DDC. There was no significant difference from controls at any point during treatment.

The inability to demonstrate increased urinary DA levels with the DDC-treated animals does not appear contradictory to the explanation of the hypotensive effect. Although circulating or tissue DA levels may be slightly increased as a result of enzyme inhibition, MAO has been reported to have a greater affinity for DA than for NE. As a result and at this dose of DDC a significant increase in urinary DA would not be detected. The latter hypothesis appears to agree with the results in this study.

SUMMARY

Normal, unilaterally nephrectomized, and renal hypertensive male albino rats were treated with DDC, a DBH inhibitor. Weight gain, systolic blood pressure, and urinary DA levels were measured in an effort to further investigate the role of DA in experimental hypertension. A significant hypotensive effect was observed in the three groups treated with DDC. A significant decrease in body weight was also observed in the DDC-treated animals in the three groups studied. The significant hypotensive effect and decreased body weight appeared to correlate with amount of functional kidney tissue present. There was no significant difference in urinary DA excretion between drug-treated and control animals in either the normal or renal hypertensive groups. A significant increase in urinary DA was observed in the unilaterally nephrectomized drug-treated group after 7 weeks of treatment with DDC. This increase cannot be explained at the present time. The results of this study do not indicate any correlation between arterial blood pressure and urinary DA levels.

REFERENCES

(1) R. J. Bing, *Am. J. Physiol.*, **132,** 497(1941).

(2) C. Giordano, A. H. Samily, J. Bloom, F. W. Haynes, and J. R. Merrill, *Federation, Proc* , **19,** 100(1960).

(3) D. R. DeFanti and J. J. DeFeo, *Biochem. Pharmacol.*, **12,** 173(1963).

(4) M. Goldstein, B. Anagoste, E. Lauber, and M. R. McKereghan, *Life Sci.*, **3,** 763(1964).

(5) A. Carlsson, M. Lindqvist, K. Fuxe, and T. Hokfelt, *J. Pharm. Pharmacol.*, **18,** 60(1966).

(6) C. G. S. Collins, *ibid.*, **17,** 526(1965).

(7) H. Goldblatt, J. Lynch, R. F. Hanzel, and W. W. Summerville, *J. Exptl. Med.*, **59,** 334(1934).

(8) D. R. Drury, *ibid.*, **68,** 695(1938).

(9) D. W Coates, Ph.D. thesis, University of R. I., 1968.

(10) A. Carlsson and B. Waldeck, *Acta Physiol. Scand*, **44,** 295(1958).

(11) G. W. Sedecor, "Statistical Methods," Iowa State University Press, Ames, Iowa, 1956, p. 45.

(12) A. J. Wohl, M. Nemeth, and C. Korduba, *Federation Proc.*, **24,** 389(1965).

(13) R. S. Pogrund, W. Drell, and W. G. Clark, *J. Pharmacol. Exptl. Therap.*, **131,** 294(1961).

(14) H. Thoenen, W. Haefely, K. F. Gey, and A. Huerlimann, *ibid.*, **156,** 246(1967).

(15) J. H. Burn and M. J. Rand, *Brit. J. Pharmacol.*, **13,** 471(1958)

ACKNOWLEDGMENTS AND ADDRESSES

Received July 28, 1969 from the *Department of Pharmacology, College of Pharmacy, University of Rhode Island, Kingston, RI 02881*

Accepted for publication August 13, 1969.

Abstracted in part from the thesis submitted by H. L. Crossley to the School of Graduate Studies, University of Rhode Island, in partial fulfillment of Master of Science degree requirements.

This investigation was supported in part by Public Health Service grant HE-09292.