

# Circulating Catecholamine Levels in Human and Experimental Hypertension

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**SUMMARY** The radiometric enzymatic technique of Coyle and Henry (*J. Neurochem.* 21: 61-67, 1973) was adapted to the measurement of serum catecholamines. This technique requires less time than other enzymatic techniques and is sensitive to quantities as small as 25 pg. In normotensive subjects lying supine for 20 minutes serum catecholamine levels averaged 0.218 ng/ml, with no obvious sex or age difference. Under these standardized conditions, the circulating catecholamine levels for a given individual are highly reproducible on different days over a period of several months. In 22 patients with essential hypertension, circulating levels were significantly higher, with an average of 0.370 ng/ml. More than 50% of the

hypertensive patients had values greater than the highest value measured in normotensives. Systolic blood pressure and heart rate were significantly higher in the hypertensive group with elevated levels of circulating catecholamines than in the hypertensive group with normal levels. In one model of experimental hypertension, produced in the rat by administration of deoxycorticosterone acetate (DOCA) and saline for 4-8 weeks, serum catecholamines were significantly elevated. These findings suggest that the sympathetic system may play an important role in maintaining an elevated blood pressure in experimental hypertension and in a significant proportion of patients with essential hypertension.

BECAUSE OF the poor sensitivity of available fluorometric techniques and the low concentrations of circulating catecholamines, fluorometric measurements of these amines have yielded variable results.<sup>1</sup> In recent years the introduction of a highly sensitive enzymatic double-isotope technique for the determination of plasma catecholamines has made possible a more accurate estimation of these amines.<sup>2</sup> More recently, Coyle and Henry<sup>3</sup> have reported a method for the differential determination of catecholamines and dopamine in tissues. Their technique, based on the same principle as that of Engelman and Portnoy,<sup>2</sup> is more sensitive and considerably less time-consuming than the latter. Although this technique is applicable to small pieces of tissue, it cannot be used on plasma or serum because of the presence of an inhibitor of the activity of catechol *O*-methyl transferase (COMT), which is required to convert the catecholamines into tritiated *O*-methylated compounds in presence of <sup>3</sup>H-S-adenosine methionine. In our study we modified the technique of Coyle and Henry to prevent the inhibition of COMT. This modified technique can be used to measure, with a high degree of sensitivity, catecholamines in small quantities of serum.

Using this technique, we studied serum catecholamines in an experimental model of hypertension as well as in patients with essential hypertension. Our results suggest that circulating catecholamines may play an important role in the development and maintenance of hypertension induced by deoxycorticosterone acetate (DOCA) and sodium. Moreover, it appears that a significant proportion of hypertensive patients have elevated circulating catecholamine levels,

suggesting a state of hyperactivity of the sympathetic system in these patients.

## Methods

### EXPERIMENTAL MODEL OF HYPERTENSION

The right kidney and adrenal gland were removed from male Sprague-Dawley rats. One group of animals was made hypertensive by weekly subcutaneous injections of deoxycorticosterone acetate, 10 mg (Ciba-Geigy), and 1% saline to drink ad libitum. We determined blood pressure in unanesthetized warmed animals, using a pulse transducer applied to the tail (E and M Instrument, Houston). Circulating catecholamines in the serum of these animals were determined 1, 4, 6, and 8 weeks after the beginning of DOCA treatment. In animals under sodium pentobarbital anesthesia (60 mg/kg), we took 2 ml of blood from the aorta immediately after clamping the adrenal pedicle to prevent the liberation of adrenal catecholamines into the circulation during blood sampling.

### HUMAN INVESTIGATION

Circulating catecholamines were determined in the serum of 15 normotensive subjects and 22 patients with essential hypertension after they had rested for 20 minutes in the supine position. In some patients circulating catecholamines were determined also after 20 minutes of standing. Blood samples were taken between 9 and 10 in the morning following a 9- to 10-hour fast. Hypertensive patients were diagnosed as being essentially hypertensive if there was no sign of associated diseases or complications. None of the patients had received antihypertensive medication for at least 1 month prior to blood sampling.

### SERUM CATECHOLAMINE DETERMINATION

For the determination of serum catecholamine levels, we used a modification of the radiometric enzymatic microtech-

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nique developed by Coyle and Henry<sup>3</sup> for catecholamine and dopamine in small pieces of brain tissue. This technique is based on the conversion of endogenous catecholamines into their tritiated metabolites during an incubation with catechol *O*-methyl transferase (COMT) purified from rat liver<sup>4</sup> in the presence of tritiated *S*-adenosine methionine (New England Nuclear Corp., Boston). The methylated metabolites of epinephrine, norepinephrine, and dopamine then are purified, a series of organic extractions being used for this procedure. With this technique it is also possible to separate completely the combined methylated derivatives of epinephrine and norepinephrine from the methylated derivatives of dopamine after cleavage of the side chain of the  $\beta$ -hydroxylated metabolites during an incubation with periodate.

Standard curves were prepared by diluting various concentrations of norepinephrine and dopamine in 0.2 N perchloric acid. These curves confirmed that the production of tritiated metabolites was proportional to the concentration of substrate up to 10 ng (Fig. 1). In our study the linearity of the curve for both norepinephrine and dopamine was lost with levels higher than 10 ng. The method was sensitive to quantities as low as 25 pg; this quantity gave

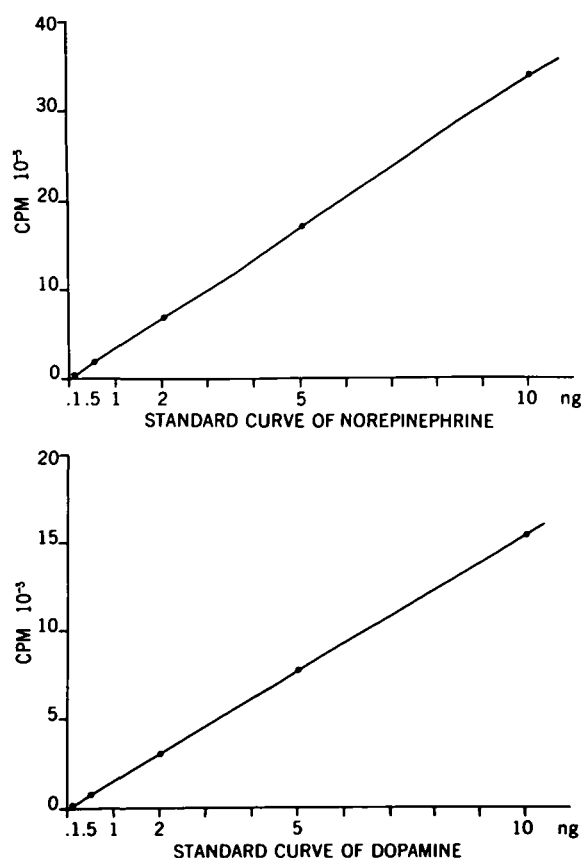


FIGURE 1 Standard curves of formation of tritiated methylated metabolites with various concentrations of nonradioactive norepinephrine and dopamine added to a perchloric acid (0.1 N) solution after incubation with catechol *O*-methyl transferase (COMT) and <sup>3</sup>H-*S*-adenosine methionine. With concentration varying from 0.1 to 10 ng the formation of tritiated metabolites was proportional to the substrate concentration for both compounds. Each point is the mean of 10 individual values.

TABLE 1 Separation of Catecholamine from Dopamine

Substrate	<sup>3</sup> H-Vanillin (catecholamine fraction) (cpm)	<sup>3</sup> H-Methoxytyramine (dopamine fraction) (cpm)
Ringer's solution	109 ± 31	246 ± 16
Norepinephrine (0.5 ng)	1846 ± 121	7 ± 4
Dopamine (0.5 ng)	42 ± 7	852 ± 34

The values are the average of 10 determinations ± SEM.

twice the counts obtained for the blanks. The separation of norepinephrine metabolites from dopamine metabolites was nearly complete (Table 1). The counts derived from the norepinephrine standards were found exclusively in the catecholamine fraction (after incubation with periodate) and did not contaminate the dopamine fraction. On the other hand, nearly all of the counts derived from the dopamine standard were found in the dopamine fraction. A slight and constant contamination equivalent to 4.8% of the dopamine counts was found in the catecholamine fraction. Whenever dopamine was detected in the serum, the catecholamine values were corrected for the contamination.

In tissue homogenates there was no sign of inhibition of the activity of COMT.<sup>3</sup> In contrast, when deproteinized serum or plasma samples were used with the same technique almost no tritiated *O*-methylated metabolites were formed even with the addition of exogenous norepinephrine (0.5 ng) to the samples (Table 2). Compared to the external standard value, which gave 1,936 cpm for 0.5 ng of norepinephrine, the same amount added to the serum gave only 151 cpm, thus suggesting the presence of a powerful inhibitor of COMT in the serum or plasma. To make the technique usable for the determination of circulating catecholamines with the same accuracy and sensitivity as in tissue samples, we made several attempts to identify and eliminate the inhibitory factor present in the serum. Calcium was found to be the major factor responsible for this inhibition. The chelation of calcium with ethylenediaminetetraacetic acid (EDTA) partially eliminated the inhibition at an optimum concentration of 10 mg/ml. The use of ethylenedis(oxyethylenetri)l(tetraacetic acid (EGTA), which chelates calcium more specifically, improved the activity of the enzyme more than did EDTA; nevertheless, an inhibition of

TABLE 2 Effect of EDTA, EGTA, and MgCl<sub>2</sub> on the Inhibition of COMT Activity by the Serum

	Norepinephrine* (cpm above sample)	% inhibition
Ringer's solution	1936	0
Serum (S)	151	92
S + EDTA (10 mg/ml)	660	65.9
S + EGTA (10 mg/ml)	900	53.5
S + EGTA + MgCl <sub>2</sub> (0.75 μM)	1095	43.6
S + EGTA + MgCl <sub>2</sub> (0.1 μM)	1295	33.6
S + EGTA + MgCl <sub>2</sub> (2.0 μM)	1830	5.5

EDTA = ethylenediaminetetraacetic acid; EGTA = ethylenedis(oxyethylenetri)l(tetraacetic acid; COMT = catechol *O*-methyl transferase.

\* Norepinephrine (0.5 ng) was added to each sample and was considered as the internal standard. Each value is the mean of at least four determinations.

more than 50% persisted. These findings are consistent with those reported by Axelrod and Tomchick, showing that  $\text{CaCl}_2$  is a potent inhibitor of COMT. EGTA has also the property of chelating magnesium, which has been found to be an activator of COMT. In our study, the addition of a proper amount of  $\text{MgCl}_2$  restored about 95% of the COMT activity (Table 2).

The following modifications were adopted for the measurement of circulating catecholamines. Blood was sampled without anticoagulant and kept at  $0^\circ\text{C}$ . After centrifugation at 12,000 g for 20 minutes in a refrigerated centrifuge, the serum was mixed with a 2 N solution of perchloric acid containing  $\text{MgCl}_2$  (7.0 mmol/100 ml) and 1% EGTA in a proportion of 9 parts of serum to 1 part of the perchloric acid solution. After it was mixed on a Vortex for 20 seconds, the mixture was centrifuged for 10 minutes at 2,000 rpm. For each determination a sample of the supernatant fluid (300  $\mu\text{l}$ ) was used for the catecholamine assay according to the procedure described by Coyle and Henry.<sup>3</sup> The determinations were run in duplicate. In two other samples norepinephrine (0.5 ng) or dopamine (0.5 ng) were added as internal standards. Blanks consisted of Ringer's solution mixed with the perchloric acid solution containing  $\text{MgCl}_2$  and EGTA in the same proportion as that used for the serum. The concentration was calculated from the number of counts given by the internal standard according to the following formula:

$$\text{Norepinephrine or dopamine (ng/ml serum)} = \frac{(\text{cpm, sample}) - (\text{cpm, blank})}{(\text{cpm, internal standard}) - (\text{cpm, sample})} \times \frac{1000 \mu\text{l}}{270 \mu\text{l}} \times 0.5 \text{ ng.}$$

In serum, the lower limit of sensitivity of the technique was approximately 80 pg/ml. Statistical analysis of the data was made with Student's *t*-test.

### Results

#### BASAL SERUM CATECHOLAMINE LEVELS IN NORMOTENSIVE SUBJECTS

Basal serum catecholamine levels were 0.218 ng/ml in 15 normotensive subjects from whom blood samples were taken in the morning after 20 minutes in the supine position (Table 3). When the group was divided according to sex, there appeared to be no significant difference between male and female subjects, although the average value was slightly

TABLE 3 Circulating Catecholamine Levels in Supine Normotensive Subjects

Groups	No. of subjects	Serum catecholamines (ng/ml)
Normotensive subjects	15	$0.218 \pm 0.014$
Female	7	$0.227 \pm 0.030$
Male	8	$0.210 \pm 0.023$
20–27 years old	10	$0.215 \pm 0.022$
33–56 years old	5	$0.223 \pm 0.032$

Values are the mean  $\pm$  SEM.

higher in the females. Nor did age appear to be an important factor in determining circulating catecholamine levels; when normotensive subjects were separated into two age groups, no significant difference in the average catecholamine levels was observed (Table 3).

The catecholamine levels did not vary significantly in a given subject when sampled under the same basal conditions on different days, indicating that the sampling of blood after 20 minutes in the supine position may be a reliable indicator of basal circulating levels in a given subject. To eliminate the influence of climatic variations, serum catecholamine levels of six normotensive subjects were measured during the summer (July) and during the winter (January). No significant difference could be observed (summer,  $0.234 \pm 0.032$  ng/ml; winter,  $0.213 \pm 0.043$  ng/ml).

#### BASAL SERUM CATECHOLAMINE LEVELS IN ESSENTIAL HYPERTENSION

The average serum catecholamine levels in 22 patients with essential hypertension, free of any other associated diseases or any complications related to their disease, were significantly higher than the levels found in normotensive subjects ( $0.370 \pm 0.032$  ng/ml vs.  $0.218 \pm 0.014$  ng/ml,  $P < 0.01$ ). In this series more than 50% of the hypertensive patients were found to have circulating catecholamine levels

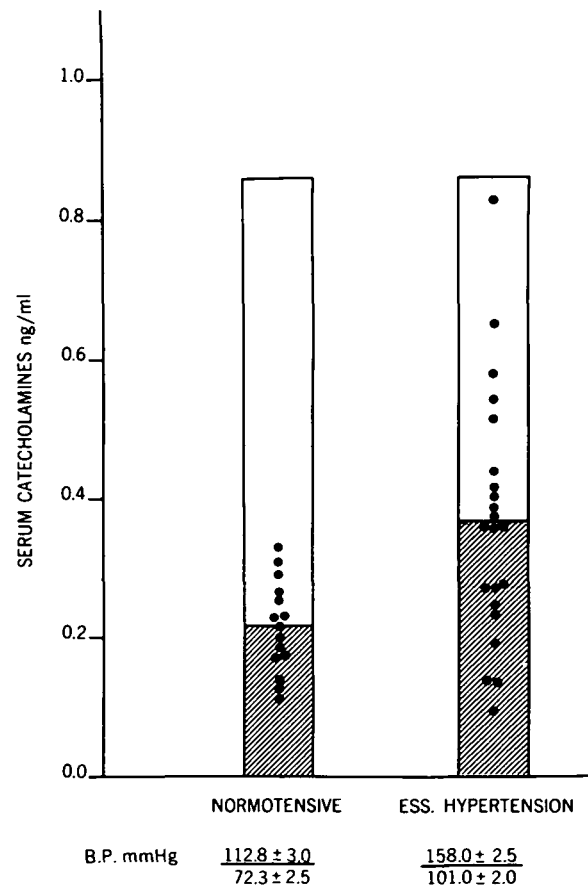


FIGURE 2 Serum catecholamine levels in normotensive subjects and in patients diagnosed as essential hypertension. The shaded area at the bottom of each rectangle represents the average level for all values in each group. BP = average blood pressure  $\pm$  SEM.

higher than the maximum level detected in normotensive subjects (Fig. 2). When the patients were separated into two groups, a normoadrenergic group (nine patients with circulating catecholamine levels within the normotensive range, averaging  $0.206 \pm 0.020$  ng/ml) and a hyperadrenergic group (13 patients with levels above normal range, averaging  $0.484 \pm 0.037$  ng/ml), it was found that the two groups had different cardiovascular characteristics (Table 4). The normoadrenergic group had significantly slower heart rates ( $P < 0.05$ ) and lower systolic blood pressures ( $P < 0.01$ ) than the group of patients with elevated circulating catecholamine levels. The diastolic blood pressures also were lower, although not significantly, in the normoadrenergic group of patients. The heart rate of the normoadrenergic group did not differ from the heart rate ( $76.6 \pm 2.5$  beats/min) in normotensive subjects.

#### EFFECTS OF STANDING ERECT ON CIRCULATING CATECHOLAMINE LEVELS IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS

Blood samples were taken from 10 normotensive and 14 hypertensive subjects while they were in the supine position; they were then asked to stand for a period of 20 minutes, at the end of which blood samples were again taken. This maneuver significantly and markedly increased circulating catecholamine levels in normotensive and hypertensive subjects. In normotensive subjects the average levels increased from  $0.228 \pm 0.024$  ng/ml in supine position to  $0.510 \pm 0.042$  ng/ml in the standing position, the average increase being 0.282 ng above basal levels (Fig. 3). In hypertensive subjects the basal levels were significantly higher than for normotensives ( $0.431 \pm 0.050$  ng/ml,  $P < 0.01$ ) and the change to the upright position was accompanied by a marked increase in circulating catecholamine levels to  $0.832 \pm 0.130$  ng/ml (average increase, 0.401 ng/ml). These findings suggest a slightly greater positional response in hypertensive subjects. Circulating dopamine was detected sporadically in only a few normotensive and hypertensive subjects. When dopamine was detectable in a given subject, the values were identical in supine and standing positions, suggesting that circulating dopamine levels are independent of the sympathetic activity.

#### CIRCULATING CATECHOLAMINE LEVELS IN RATS MADE HYPERTENSIVE BY DEOXYCORTICOSTERONE AND SODIUM

Circulating catecholamine levels were measured in a model of experimental hypertension in which the participa-

tion of the sympathetic system in the development and maintenance of hypertension has been demonstrated previously.<sup>5, 6</sup> To obtain more standardized basal catecholamine levels we anesthetized the rats with pentobarbital prior to blood sampling and, to minimize the adrenal medullary reflex secretion of catecholamines during blood sampling, we clamped the adrenal pedicles before withdrawing about 2 ml of blood through the aorta. The average circulating catecholamine level for 18 normotensive rats was  $0.344 \pm 0.025$  ng/ml. In 19 rats, hypertensive after being treated with DOCA and sodium for 4–8 weeks, the circulating catecholamine levels were significantly increased (mean,  $0.895 \pm 0.090$ ;  $P < 0.001$ ). When individual values were plotted a remarkable similarity was found in the pattern of distribution of values in hypertensive rats (Fig. 4) and in hypertensive humans (Fig. 2). Although the hypertensive condition was triggered by the same etiological factor in all rats, a wide spectrum of values was observed. As in patients with essential hypertension, some rats had levels within the range of values found in normotensive rats. Hypertensive animals treated for shorter periods with DOCA and sodium tended to have lower values than those treated for longer periods. Preliminary findings also suggested that circulating catecholamine levels started to increase before the development of hypertension in animals treated for only 1 week with DOCA and sodium, when blood pressure averaged 138 mm Hg. The blood pressure reached hypertensive levels during the 2nd week of treatment and continued to increase progressively until the 6th week of treatment.

#### Discussion

Evaluation of the neurogenic sympathetic component in man has always constituted a major problem; this is due mainly to the absence of accurate and sensitive means of determining adrenergic activity. The measurement of urinary catecholamines has been used extensively for that purpose;<sup>5</sup> however, urinary catecholamines represent the sum of events occurring in the body over a long period of time, and minute or transitory changes in sympathetic activity are hidden by the general pattern of excretion and cannot be detected or correlated directly with changes in cardiovascular function. Moreover, the role of the kidney in the metabolism of catecholamines is still unknown; therefore differences in urinary pattern may reflect only an alteration in kidney function.<sup>7</sup>

The measurement of circulating catecholamines probably is a better means of evaluation, because circulating levels are

TABLE 4 Heart Rate and Systolic and Diastolic Blood Pressure in Normoadrenergic and Hyperadrenergic Hypertensive Patients

	Normoadrenergic hypertension (9 patients)	Hyperadrenergic hypertension (13 patients)
Heart rate (beats/min)	$77.8 \pm 4.4$	$90.7 \pm 3.2^*$
Systolic BP (mm Hg)	$147.4 \pm 3.3$	$171.3 \pm 3.8^\dagger$
Diastolic BP (mm Hg)	$95.6 \pm 4.3$	$103 \pm 2.3$
Serum catecholamine (ng/ml)	$0.206 \pm 0.020$	$0.484 \pm 0.037^\dagger$

Values are the mean  $\pm$  SEM.

\*  $P < 0.05$  vs. normoadrenergic hypertensive group.

†  $P < 0.01$  vs. normoadrenergic hypertensive group.

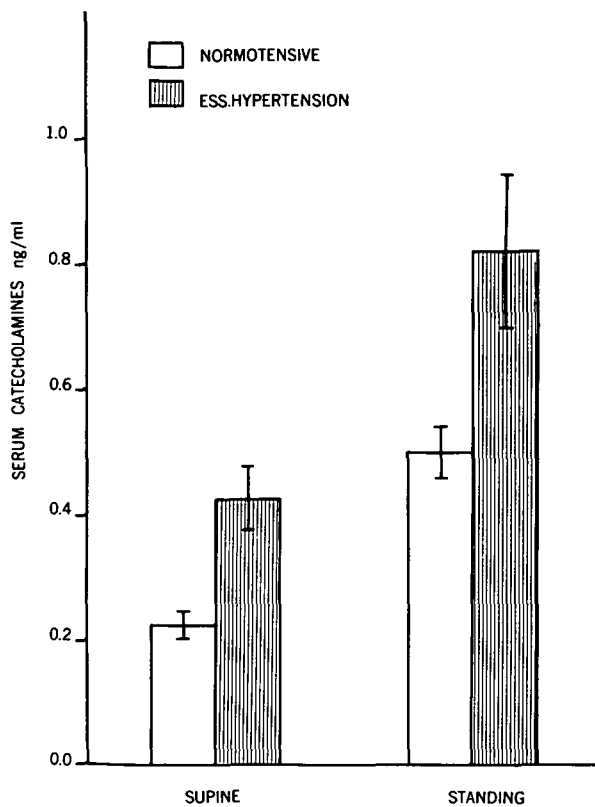


FIGURE 3 Serum catecholamine levels in normotensive and hypertensive subjects in response to 20 minutes in the standing position. The bars represent the mean  $\pm$  SE of 10 normotensive subjects and 14 hypertensive patients.

maintained mainly by catecholamines liberated in proportion to the degree of sympathetic activity. The determination of circulating catecholamines also offers the possibility of simultaneously correlating the changes in cardiovascular function and the sympathetic response after physiological stimuli. Because circulating catecholamine levels are very low, the major problem preventing their measurement has been the relative insensitivity of conventional fluorometric methods. Large quantities of blood are required to attain a detectable catecholamine concentration with these methods; yet with most fluorometric techniques catecholamine levels are still being measured at the lower, unreliable limit of sensitivity. This explains the wide range of average values obtained in prior studies.<sup>1</sup>

The development by Engelman and Portnoy<sup>2</sup> of a sensitive enzymatic double-isotope technique for the measurement of catecholamines has made possible the precise evaluation of circulating catecholamine levels as an index of sympathetic activity in man. Our adaptation of the method of Coyle and Henry<sup>3</sup> constitutes an improvement in several areas. The use of organic extraction procedures instead of chromatography considerably shortens the time required for purification and extraction of the metabolites. Moreover, the partial elimination of COMT inhibition through use of EGTA and  $MgCl_2$  has increased the level of sensitivity, so that it now is possible to measure accurately the catecholamine levels in 300  $\mu$ l of serum. Finally, the circulating catecholamine levels found in control subjects with this

modified technique are similar to those reported by several investigators<sup>8-12</sup> who used the technique of Engelman and Portnoy.<sup>2</sup> In our study no significant difference was observed between sexes; this is in agreement with Engelman and Portnoy,<sup>2</sup> but is counter to the observations reported by De Quattro and Chan.<sup>9</sup>

Our finding of increased catecholamine levels in some patients with essential hypertension is in accord with the conclusions reached in recent studies with enzymatic assays.<sup>8-11</sup> When the patients were separated into two groups, we found that the heart rate and systolic blood pressure were significantly higher in patients with elevated circulating catecholamine levels than in those with normal catecholamine levels; this suggests a causal relationship between circulating catecholamines and the hemodynamic state in essential hypertension. Louis and his co-workers<sup>11</sup> also have reported a highly significant correlation between diastolic blood pressure and circulating norepinephrine levels in patients with essential hypertension under basal conditions and after treatment with ganglionic blocking agents. These observations suggest that the sympathetic system may be hyperactive and directly responsible for the maintenance and severity of hypertension in a significant proportion of the population with essential hypertension. Since a diagnosis of essential hypertension is made by

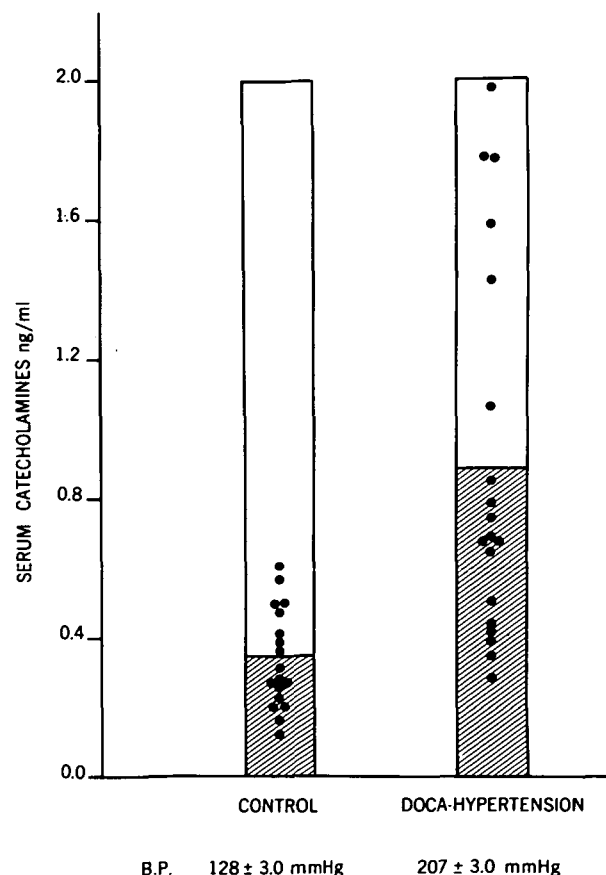


FIGURE 4 Circulating catecholamines in anesthetized normotensive rats and in rats made hypertensive by treatment with deoxycorticosterone acetate (DOCA) and saline for 4 to 8 weeks. The shaded area at the bottom of each rectangle represents the average level for all values in each group. BP = mean systolic blood pressure  $\pm$  SEM.

excluding all known causes of hypertension, it is likely that this population of patients is heterogeneous and that the sympathetic system would play a role in only a fraction of that population.

The metabolism of circulating catecholamines is rapid, and catecholamines liberated from the adrenal medulla and from sympathetic nerve fibers diffuse into the blood stream;<sup>13</sup> therefore, the level of circulating catecholamines at a given time may reflect the sympathetic tone under specific conditions. To be able to compare several individuals, it therefore is necessary to standardize the conditions under which blood is sampled. The maintenance of the supine position for 20 minutes was found to be adequate for estimating basal levels. The marked increase in circulating levels, in response to standing erect for 20 minutes, supports the hypothesis that circulating catecholamine levels may be a good index of sympathetic activity, because it is known that a change from the supine to the erect position is associated with sympathetic activation. Although basal levels were higher in hypertensive patients, the response to changes in position was slightly greater than in normotensive subjects, suggesting an increased sympathetic reactivity in that condition. These results differ from those reported by De Quattro and Chan,<sup>9</sup> who showed that the response to 5 minutes of standing erect was normal or lower in hypertensive patients. Differences in time of blood sampling could account for the discrepancy.

The finding of elevated circulating catecholamine levels in rats made hypertensive by administration of DOCA and sodium also supports the hypothesis that circulating catecholamines may be an accurate index of sympathetic activity. In this model of hypertension, hyperactivity of the sympathetic system has been demonstrated and confirmed by several investigators.<sup>5, 14, 16</sup> Although the etiological factors was similar for all animals, it is interesting to note that circulating levels are spread over a wide range in a pattern similar to that in essential hypertension. Moreover, since it also has been reported that sodium may be an important factor in the etiology of essential hypertension,<sup>17, 18</sup> these findings suggest that DOCA and sodium hypertension may constitute an excellent model for the study of essential hypertension. The large range of values in human and in experimental hypertension still remains unexplained. Preliminary studies in animals suggest that the highest levels may be linked to a longer duration and to a greater severity of the hypertensive disease. It therefore is possible that circulating catecholamine levels may play an important role

not only in the etiology and maintenance of human and experimental hypertension but also in the evolution of the disease and, since elevated catecholamine levels have been associated with myocardial infarct,<sup>19</sup> in the incidence of cardiovascular complications.

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