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An assessment of the role of the sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats

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Summary

1. The role of the sympathetic nervous system in experimental hypertension and associated changes in aortic sodium and potassium was studied using normal and immunosympathectomized Sprague-Dawley rats.

2. A single injection of antiserum to nerve-growth factor to rats at birth produced less intensive destruction of the peripheral sympathetic system than did two injections (one daily for 2 days). The former are referred to as "partial" immunosympathectomized and the latter as "total" immunosympathectomized rats.

3. Maintenance of rats on 1% sodium chloride after unilateral nephrectomy and implantation of 40 mg desoxycorticosterone acetate (DOCA) pellets resulted in sustained DOCA-NaCl hypertension in both normal and "partial" immunosympathectomized rats but not in "total" immunosympathectomized rats. Hypertension was associated with an increase in aortic sodium.

4. Constriction of one renal artery with contralateral nephrectomy caused sustained hypertension and an increase in aortic sodium in normal and "partial" immunosympathectomized rats. Renal hypertension in "total" immunosympathectomized rats was not sustained.

5. It is concluded that a certain minimum control of the cardiovascular system by the sympathetic system is essential for the production of experimental hypertension and associated electrolyte changes.

Introduction

It was reported from this laboratory that experimental hypertension could be produced in immunosympathectomized rats (Varma, 1967). However, in that study immunosympathectomy was produced by a single injection of antiserum to nerve-growth factor in rats on the first day after birth. We have since found that two successive daily injections of the antiserum in rats caused more extensive destruction of the peripheral sympathetic system than did a single injection. Similar findings have been reported by other workers (Iversen, Glowinski & Axelrod, 1966; Zaimis, Berk & Callingham, 1965).

The role of the sympathetic nervous system in hypertension is still not certain (Carlsson, 1966). Alpert, Alving & Grimson (1937), Freeman & Jeffers (1940) and

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Grimson (1940, 1941) used surgical sympathectomy and demonstrated the importance of the sympathetic nervous system in hypertension. Other workers (Freeman & Page, 1937; Goldblatt, Gross & Hanzal, 1937) failed to demonstrate such an importance of the sympathetic nervous system in hypertension. Recent observations have indicated alterations of sympathetic function in both essential hypertension (Gitlow, Mendlowitz, Wilk, Wilk, Wolf & Naftchi, 1964; Brunjes, 1964) and experimental hypertension (De Champlain, Krakoff & Axelrod, 1966, 1967, 1968; Krakoff, De Champlain & Axelrod, 1967; Dequattro, Nagatsu, Maronde & Alexander, 1969; Louis, Spector, Tabei & Sjoerdsma, 1969).

It was therefore of interest to study the role of the sympathetic nervous system in experimental hypertension using rats in which the sympathetic innervation to the cardiovascular system had been almost completely destroyed by immunosympathectomy. The relationship between the sympathetic nervous system, experimental hypertension and electrolyte changes in the arterial wall was also studied.

Methods

The experiments were performed on Sprague-Dawley rats of either sex. They were fed commercial rat diet and given tap water unless otherwise stated. The experimental procedure was similar to that described earlier (Varma, 1967).

Immunosympathectomy was produced by injecting newborn rats subcutaneously with 12,000 units of antiserum to nerve-growth factor (Cohen, 1960; Levi-Montalcini & Angeletti, 1962) for 1 day or once daily for 2 consecutive days immediately after birth. Rats injected once were referred to as "partial" immunosympathectomized rats and those injected twice as "total" immunosympathectomized rats. The treated rats were raised together with the untreated litter mates which served as their controls.

The uptake of ^{3}H-noradrenaline by various tissues of these rats was measured. The rats were anaesthetized with pentobarbitone sodium (30–40 mg/kg intraperitoneally) and 20 μCi of ($\pm$)-7-^{3}H-noradrenaline (specific activity 4·37 Ci/mmol, New England Nuclear Corporation) was injected into the femoral vein. The heart, aorta, spleen and oesophagus were removed 15 min after the injection of ^{3}H-noradrenaline; they were cleaned, blotted dry, weighed and dissolved in a strong base (quaternary ammonium hydroxide in toluene supplied as NCS by Nuclear Chicago) as described by Hansen & Bush (1967). The total radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb); each sample was counted for 5–10 min or a minimum of 1×10^4 counts/min. The efficiency of the counting system was approximately 20%.

DOCA-NaCl hypertension was produced in the rats when they were about 2 months old (200–250 g). For this purpose, rats were anaesthetized with pentobarbitone sodium; unilateral nephrectomy was performed and a 40 mg desoxycorticosterone acetate (DOCA) pellet in beeswax was subcutaneously implanted. In some rats adrenal demedullation was performed, by splitting both adrenal glands with an incision on the cortex and squeezing out the medulla. These demedullated rats were subsequently used for DOCA implantation. All the animals were maintained on 1% sodium chloride solution instead of drinking water.

Renal hypertension was produced by constricting one renal artery and removing the contralateral kidney (Goldblatt *et al.*, 1937); these rats were maintained on tap water.

Systolic blood pressure was determined indirectly by the tail cuff method using an Electrosphygmograph (E. & M. Instruments). It was not possible to determine the blood pressure of " total " immunosympathectomized rats by the method, so blood pressure was determined directly in these rats. A cannula attached to a Statham transducer was inserted into the femoral artery of anaesthetized rats, the animals being anaesthetized with ether or by an intraperitoneal injection of 50 mg/kg chloralose plus 350 mg/kg urethane. When ether was used, the blood pressure was recorded after recovery from anaesthesia. A Gilson polygraph was used for blood pressure recording. The rats with a systolic blood pressure greater than 160 mmHg (1 mmHg$\equiv$1·333 mbar) or mean arterial pressure greater than 150 mmHg were considered hypertensive. These animals were observed for 30–60 days after starting the experimental hypertension.

Some of the rats were killed by a blow on the head and the electrolytes of their aortae were extracted with 10% trichloroacetic acid after washing the tissues. Sodium and potassium levels of the tissue extracts, and of urine (24-h excretion), were determined on a Beckman Flame Photometer (Model 150) as described by Boling (1964). The differences between means were evaluated by using Student's *t* test for paired and unpaired data (Steel & Torrie, 1960), and were considered statistically significant when $P<0·05$.

Results

Immunosympathectomy

The uptake of ^{3}H-noradrenaline by the heart, aorta and spleen of " partially " immunosympathectomized rats which received a single injection of the antiserum was 24%, 58·4% and 71·9% of the controls, respectively (Fig. 1). The uptake by the heart, aorta and spleen of the animals treated twice (" total " immunosympathectomy) was 11·7%, 28·2% and 40% of the controls. It can be seen that the uptake of ^{3}H-noradrenaline by the tissues of " partial " immunosympathectomized rats was less than that by the tissues of the control rats but greater than that by the tissues of " total " immunosympathectomized rats. This indicates that one injection of the antiserum produced less intensive destruction of the peripheral sympathetic nervous system than did the two injections. There was no significant reduction in the uptake of ^{3}H-noradrenaline by the oesophagus of the " partial " immunosympathectomized rats. However, the uptake by the oesophagus of " total " immunosympathectomized rats was significantly less than that by the oesophagus of normal rats. By the indirect method, it was possible to determine the systolic pressure of the " partial " but not of the " total " immunosympathectomized rats. However, there was no clear difference in the degree of ptosis in the two groups of immunosympathectomized rats.

Hypertension

The time-course of DOCA-NaCl hypertension in normal and demedullated rats and of renal hypertension in normal rats in shown in Fig. 2. The development of hypertension in the demedullated rats was faster than in the unoperated normal rats given DOCA-NaCl. Eighteen of the thirty-two (56%) normal rats, fourteen out of fifteen (93%) demedullated rats and twelve out of twenty-one (58%) " partial " immunosympathectomized rats developed DOCA-NaCl hypertension (Fig. 3A).

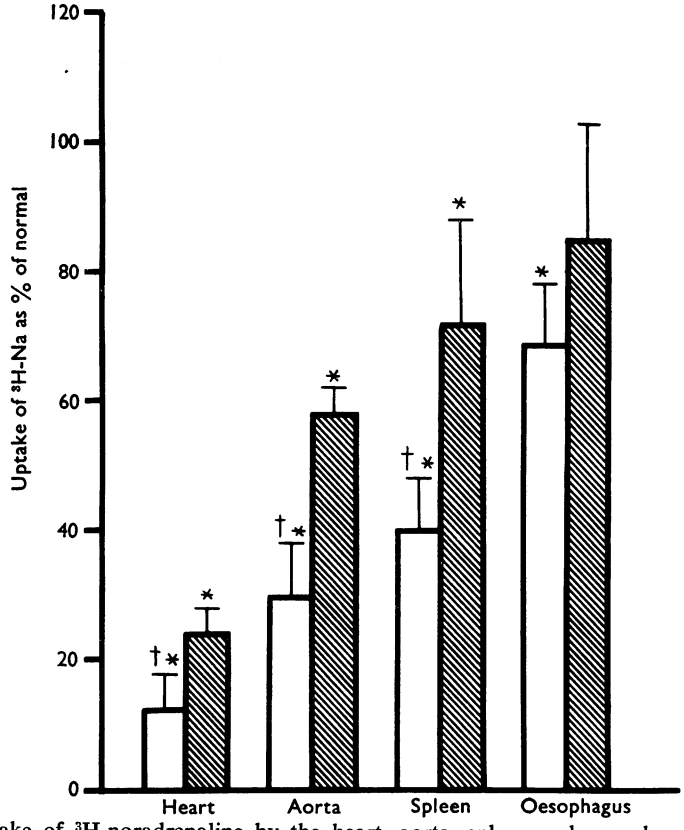

FIG. 1. Uptake of ^{3}H-noradrenaline by the heart, aorta, spleen and oesophagus of different groups of rats. Open columns, " total " immunosympathectomized ; hatched columns, " partial " immunosympathectomized. The values are expressed as percentage of the normal control value, which was taken as 100%. Vertical lines represent one half of the S.E. Asterisks (*) indicate those values which were significantly different ($P<0.05$) from normal. Crosses (†) indicate that the values were significantly different ($P<0.05$) from the values in " partial " immunosympathectomized rats. There were five rats in each group.

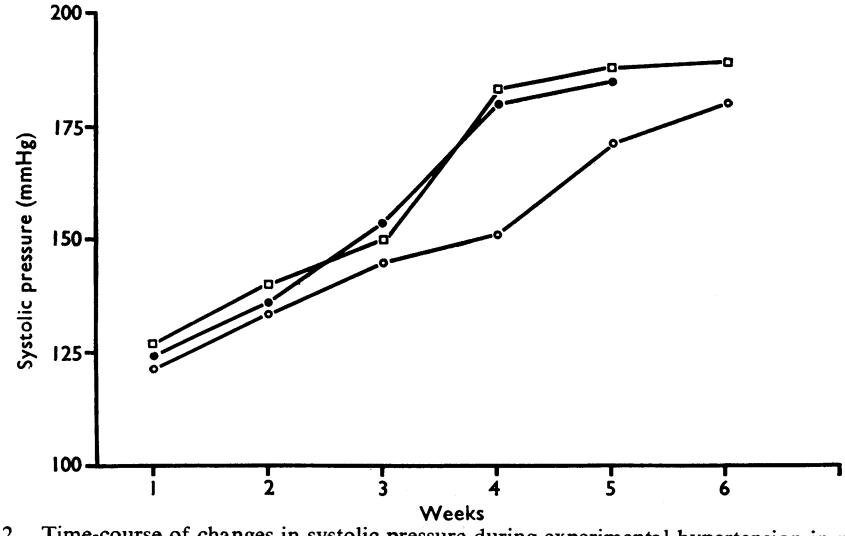

FIG. 2. Time-course of changes in systolic pressure during experimental hypertension in normal rats. ○, DOCA-NaCl treatment (unilateral nephrectomy, implantation of 40 mg DOCA pellet and maintenance on 1% sodium chloride) (twenty rats); □, DOCA-NaCl treatment after demedullation (seventeen rats); ●, renal artery constriction and removal of contralateral kidney (six rats).

There was no significant difference in the incidence of hypertension between normal rats and " partial " immunosympathectomized rats. Demedullation increased the incidence of hypertension from 56% to 93% (Fig. 3B).

Since systolic blood pressure of the " total " immunosympathectomized rats could not be determined by indirect means, the effect of DOCA-NaCl treatment and of renal artery constriction was assessed by the measurement of blood pressure directly from the femoral artery while the rats were either under chloralose-urethane anaesthesia or, alternatively, after recovery from ether anaesthesia. Additional normal rats were used as controls in these studies. The blood pressure of these control rats was $120 \pm 4{\cdot}5$ mmHg. Fourteen of the twenty-eight (50%) control rats developed DOCA-NaCl hypertension and had a mean blood pressure of $152 \pm 2{\cdot}1$ mmHg. None of the eighteen " total " immunosympathectomized rats developed DOCA-NaCl hypertension ; this group had a mean arterial pressure of $87 \pm 6{\cdot}3$ mmHg under chloralose-urethane anaesthesia (Fig. 4A). In order to find out if the difference in the blood pressure between the two groups was due to the chloralose-urethane anaesthesia, the blood pressure of five controls and five " total " immunosympathectomized rats was measured after recovery from ether anaesthesia and was found to be $173 \pm 4{\cdot}0$ mmHg and $119 \pm 3{\cdot}2$ mmHg respectively (Fig. 4B). The difference between the blood pressures of the two groups was statistically significant ($P<0{\cdot}001$). Furthermore, direct measurement of arterial pressure after recovery from ether anaesthesia showed that six demedullated " total " immunosympathectomized rats did not develop hypertension after DOCA-NaCl treatment, while the demedullated normal rats became hypertensive (Fig. 4B).

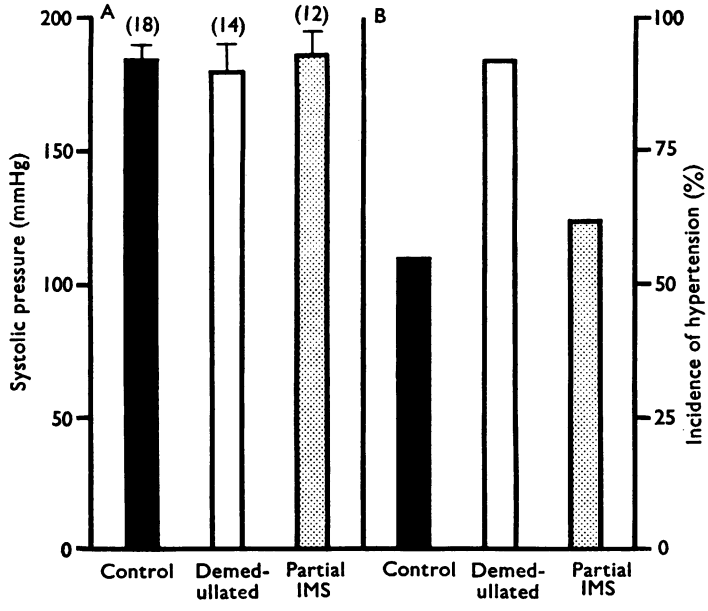

FIG. 3. Severity and incidence of DOCA-NaCl hypertension in normal (control), demedullated and " partial " immunosympathectomized (partial IMS) rats. The severity (A) is indicated by the level of systolic pressure and the incidence (B) is expressed as % of rats developing hypertension. Numbers in parentheses are the number of animals which developed hypertension. Vertical lines represent one half of the S.E.

The effect of renal artery constriction on the mean arterial pressure of normal and both " partial " and " total " immunosympathectomized rats is shown in Fig. 5. The blood pressure was again determined after recovery from ether anaesthesia. " Total " immunosympathectomized rats showed hypertensive changes at a period of 30 days after renal artery constriction but the blood pressure had returned to normotensive levels by 50 days after renal artery constriction (Fig. 5). On the other hand, both the normal and " partial " immunosympathectomized rats were still hypertensive 50 days after the renal artery constriction.

Electrolyte changes

The result of these measurements is shown in Fig. 6. There was a significant increase in the potassium content of the aortae of " partial " immunosympathectomized rats following renal artery constriction (Fig. 6B). There was no significant change in the potassium content of aortae of rats of other groups. DOCA-NaCl treatment did not lead to significantly greater excretion of sodium in the urine of normal rats, but the " total " immunosympathectomized rats after similar DOCA-NaCl treatment excreted significantly greater amounts of sodium than did the

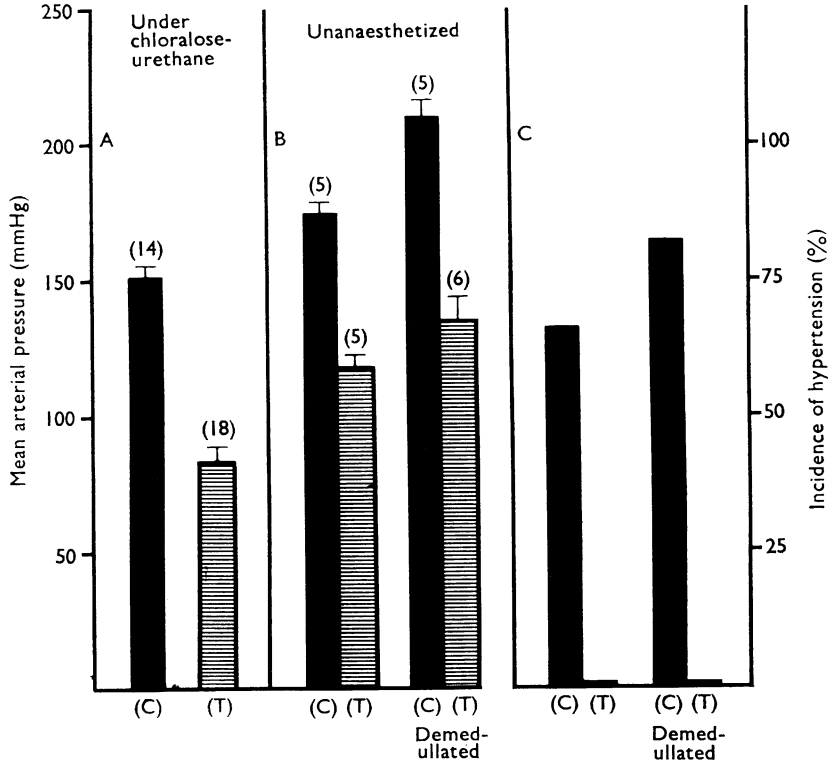

FIG. 4. Mean arterial pressure (A and B) and the incidence of hypertension (C) in normal (C) and " total " immunosympathectomized (T) rats after DOCA-NaCl treatment and DOCA-NaCl treatment plus demedullation. " Unanaesthetized " refers to measurements made after recovery from ether anaesthesia. The numbers in parentheses against control and demedullated control rats are the number of rats which developed hypertension. Vertical lines represent one half the S.E. The numbers in parentheses against " total " immunosympathectomized rats are the total number of rats used for this study.

former group (Fig. 6A). However, DOCA-sodium chloride treatment led to significantly greater urinary excretion of potassium in normal rats and in "total" immunosympathectomized rats (Fig. 6A). Fifty days after renal constriction, the aortic sodium of the normal hypertensive and "partial" immunosympathectomized hypertensive rats had increased significantly (Fig. 6B). However, there was no significant increase in the aortic sodium of the "total" immunosympathectomized rats (normotensive). Furthermore, untreated "total" immunosympathectomized rats excreted significantly more sodium per day than the normal untreated rats (Fig. 6C).

Discussion

These results show that two injections of the antiserum to nerve-growth factor caused more intensive destruction of peripheral sympathetic innervation to the cardiovascular system than was caused by one injection. Hence, the uptake of ^{3}H-noradrenaline by the aorta, heart and spleen of rats injected once was signifi-

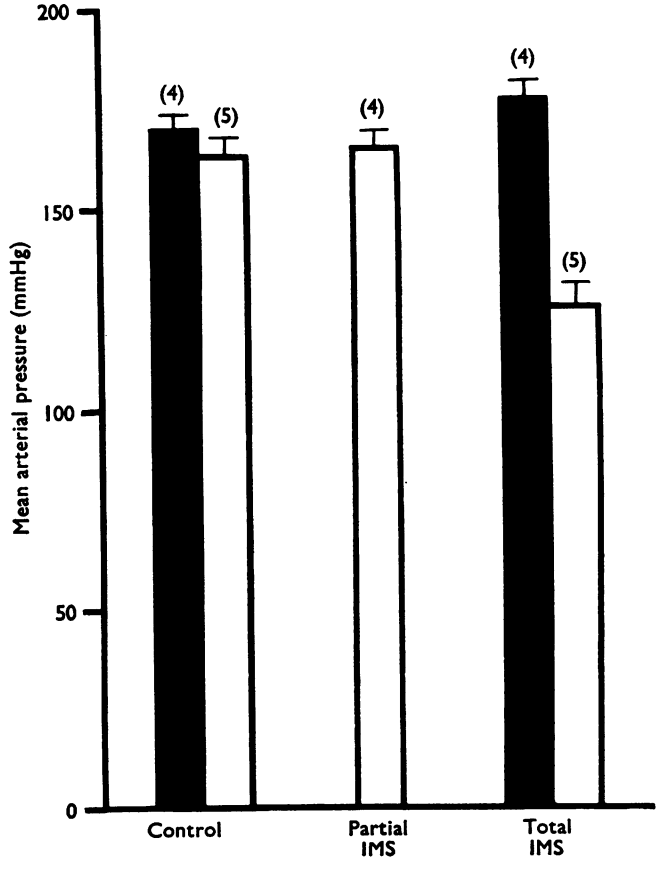

FIG. 5. Mean arterial pressure of normal (control), "partial" immunosympathectomized (partial IMS) and "total" immunosympathectomized (total IMS) rats after attempting to produce renal hypertension. Femoral arterial pressure was measured after recovery from ether anaesthesia. Arterial pressure was determined at 30 (filled columns) and 50 days (open columns) after renal artery constriction. Vertical lines represent one half the S.E. Numbers in parentheses are the numbers of rats in the group.

cantly greater than the uptake by the respective tissues of the rats injected twice. The changes in the uptake of ^{3}H-noradrenaline reflect the destruction of the sympathetic system since it has been shown that the reduction in the uptake of tritiated noradrenaline by the tissues of immunosympathectomized animals can be correlated with a decrease in the endogenous noradrenaline and that both are due to the destruction of the sympathetic nerve terminals (Iversen *et al.*, 1966 ; Zaimis *et al.*, 1965). Further evidence of the difference between the rats that received two injections and rats that received one injection is provided by the observation that systolic pressure could not be determined indirectly in the former group. These findings are similar to those of Brody (1964), who also found that blood pressure could not be determined by indirect means in immunosympathectomized rats. The reason for this is not clear. It does not seem to be entirely related to low blood pressure of immunosympathectomized rats, for it was possible to measure indirectly the blood pressure of normal rats even after inducing severe hypotension by hexamethonium.

Even though these results, as well as the results of other workers (Brody, 1964 ; Iversen *et al.*, 1966 ; Levi-Montalcini & Angeletti, 1962 ; Zaimis *et al.*, 1965), clearly show that immunosympathectomy produces an almost complete and permanent destruction of the sympathetic innervation to some tissues (such as the cardio-

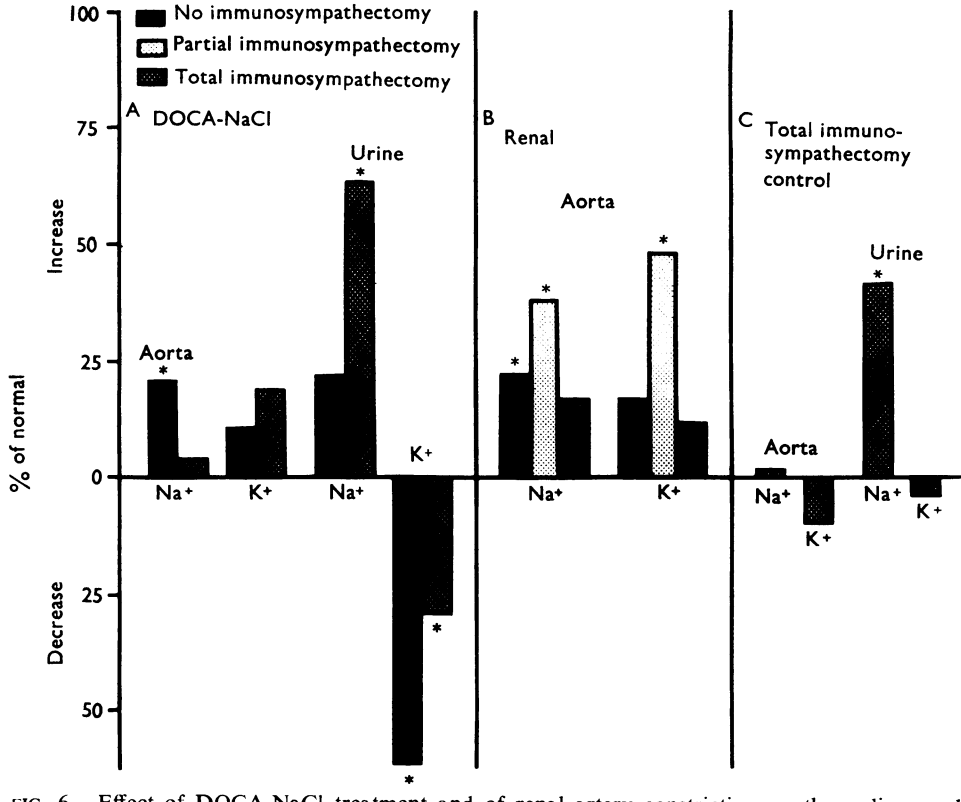

FIG. 6. Effect of DOCA-NaCl treatment and of renal artery constriction on the sodium and potassium levels in aorta and urine (24 h collection) of normal and immunosympathectomized rats. Values from untreated and unoperated normal rats were taken as 100% and values in the histogram are presented as percentage of these values. There were four to six rats in each group. The values significantly different ($P<0{\cdot}05$) from the controls are indicated by asterisks (*).

vascular system, for example), other systems, like the gastrointestinal tract, vas deferens and adrenal medulla, are scarcely affected. Therefore, the role of the sympathetic nervous system cannot be entirely excluded in any observation made on immunosympathectomized animals.

The pertinent feature of the results reported in this study is that DOCA-NaCl and sustained renal hypertension could be produced in control and " partial " immunosympathectomized rats. These results confirm our earlier findings made on rats immunosympathectomized by only one injection of the antiserum to nerve growth factor (Varma, 1967). However, neither DOCA-NaCl hypertension nor sustained renal hypertension could be produced in " total " immunosympathectomized rats. Similar findings have been made by Dorr & Brody (1964). Therefore, it seems reasonable to suggest that some degree of sympathetic control of the cardiovascular system is essential for induction of experimental hypertension with the methods used in this study, and only after destruction of the sympathetic nervous system to a certain critical degree can the determining role of this system in experimental hypertension be observed. These observations may help to explain the discrepancy in the literature (Alpert *et al.*, 1937 ; Freeman & Page, 1937 ; Goldblatt *et al.*, 1937 ; Nowak & Walker, 1939 ; Freeman & Jeffers, 1940 ; and Grimson, 1940, 1941) on the role of the sympathetic nervous system in experimental hypertension ; these discrepancies may be attributed to the level to which the sympathetic control was abolished.

It is interesting that whereas hypertension of renal origin could not be sustained in " total " immunosympathectomized rats, some degree of hypertension was observed during the first 30 days after renal artery constriction. This may mean that the initial phase of the renal hypertension is independent of the sympathetic nervous system and primarily involves the renin-angiotensin system (Laragh, 1967). The observation that renal hypertension in the " total " immunosympathectomized rats was not sustained suggests that the late phase of renal hypertension depends on sympathetic activity ; this has also been suggested by other workers (Dock, Shidler & Moy, 1942 ; Glenn, Child & Page, 1938 ; Grollman, Harrison & Williams, 1943).

The observation that both DOCA-NaCl hypertension and renal hypertension were associated with an increase in total aortic sodium and that " total " immunosympathectomized rats neither developed hypertension nor had elevated aortic sodium is of interest. On the other hand, there was no correlation between the changes in aortic potassium and the development of hypertension. Thus, there was an increase in the potassium level of aortae of " partial " immunosympathectomized rats after renal constriction but not in the aortae of normal rats after renal constriction or after DOCA-NaCl treatment although all of the three groups of rats developed hypertension. Tobian & Binion (1954) also observed that experimental hypertension in rats was associated with an increase in aortic sodium. These results strongly suggest, but do not prove, a causal relation between hypertension and the elevation of aortic sodium. Also these results do not indicate the mechanism by which lack of peripheral sympathetic system prevented the increase in aortic sodium of immunosympathectomized rats. Both the inability of the aorta to retain sodium or some mechanism leading to excess excretion of sodium may contribute to the absence of elevation of the aortic sodium in " total " immunosympathectomized rats. It was observed that " total " immunosympathectomized rats excreted more sodium in the urine than did the normal rats when both groups were maintained

on DOCA-NaCl treatment. Gill, Mason & Bartter (1964) showed that guanethidine caused increased excretion and decreased whole-body retention of sodium in man.

Furthermore, De Champlain *et al.* (1968) have suggested that the capacity of the sympathetic granules to bind and store noradrenaline is influenced by the state of sodium balance and showed that the capacity of storage could be inversely correlated with the level of blood pressure. They observed that treatment with a long-acting ganglion blocking agent could restore the blood pressure and the noradrenaline storage capacity in hypertensive animals. Although this information also failed to explain the mechanism involved, it has helped to establish that a relationship exists between the sympathetic nervous system, sodium balance and hypertension.

It has been reported that adrenal demedullation resulted in an increase in the rate of synthesis of heart noradrenaline, thus suggesting that catecholamine release from the adrenal medulla may modulate the synthesis of noradrenaline in the cardiac sympathetic neurones (Neff, Ngai, Wang & Costa, 1969). This finding together with recent observations that the turnover rate of the neurotransmitter is increased during hypertension (Krakoff *et al.*, 1967; and Dequattro *et al.*, 1969) allows the suggestion that the enhancement of the development of hypertension after demedullation may be due to a change in the condition of the neurotransmitter turnover rate. This also explains the finding that demedullation did not influence the blood pressure of " total " immunosympathectomized rats, since they did not possess functional sympathetic nerves with respect to the cardiovascular system.

In conclusion it can be said that DOCA-NaCl hypertension and sustained renal hypertension can be induced in control and " partial " immunosympathectomized but not in " total " immunosympathectomized rats. Hypertension was associated with an increase in aortic sodium. It is suggested that certain minimum control by the sympathetic nervous system is essential for experimental hypertension in rats.

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