INTERRELATIONSHIPS OF THE SYMPATHETIC NERVOUS SYSTEM, VASCULAR REACTIVITY AND ELECTROLYTES IN EXPERIMENTAL HYPERTENSION IN RABBITS

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

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May 1971

TIGHT BINDING

ACKNOWLEDGEMENTS

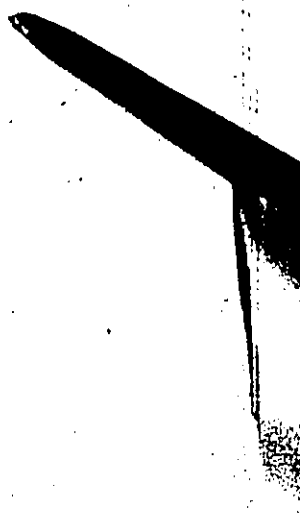
I would like to express my sincere appreciation to Dr. S. Kalsner for his guidance and encouragement throughout the course of this investigation. I am also grateful to Dr. G.M. Ling, Chairman, Department of Pharmacology for the interest he showed and the support he gave to me during this investigation.

Finally, I am grateful to Mrs. Frances Cain for her expert typing of this manuscript.

CONTENTS

LIST OF ABBREVIATIONS

DOCA	Desoxycorticosterone Acetate
DOCA-NaCl (2 weeks) ...	treated with desoxycorticosterone acetate and NaCl for two weeks.
DOCA-NaCl (5 weeks) ...	treated with desoxycorticosterone acetate and NaCl for five weeks.
NA	Noradrenaline
IPN	Iproniazid
MAO	Monoamine oxidase
IMS	Immunosympathectomized
POB	Phenoxybenzamine
S.E.M.	Standard error of the mean
Hypert.	Hypertensive
Dec.	Decentralized



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I. INTRODUCTION

Numerous factors have been implicated in the pathogenesis of hypertension. Alterations in sympathetic nerve activity, vascular reactivity, electrolyte metabolism and endocrine gland function are probably all involved in some way and it is likely that they contribute in a co-ordinated fashion to the elevation of the arterial pressure.

It is recognised that the fundamental defect in hypertension is an increased peripheral resistance due to a decreased diameter of the lumen of small arteries (Pickering, 1955; Folkow et al., 1958). This could be caused by muscular hypertrophy, an increase in the tone of the vascular smooth muscle resulting from increased sympathetic nerve activity, or to an increased responsiveness of smooth muscle cells to stimulants.

There are reports that the sympathetic nervous system plays an important role in hypertension (Smithwick and Thompson, 1953; Grimson, 1940, 1941; Willard and Fuller, 1969; Ayitey-Smith and Varma, 1970) but other reports are inconsistent with this view (Freeman and Page, 1937; Finch and Leach, 1970). However, recent observations seem to suggest that sympathetic nerve activity is increased in certain forms of hypertension (Okamoto et al., 1967; De Champlain et al., 1967, 1969; Volicer et. al., 1969; De Quattro et. al., 1969;).

In addition, increased arterial sodium (Tobian and Binion, 1952, 1954; Ross, 1956; Tobian, 1961; Ayitey-Smith and Varma, 1970) and increased vascular reactivity (Greisman, 1952, 1954; Doyle et.al., 1959; McQueen, 1961; Hinke, 1965, 1966; McGregor and Smirk, 1968) have

been linked with both experimental and essential hypertension. It has been reported that an increase in intracellular arterial sodium enhances vascular responses to pressor amines (Raab, 1952, 1953), but undoubtedly other factors could also account for enhanced responses to noradrenaline.

Although these isolated evidences have been useful in elaborating hypotheses on the pathogenesis of hypertension, the mechanisms underlying these various factors and their relationships have not been adequately examined.

The present studies were undertaken to investigate the relationship between the sympathetic nervous system, vascular reactivity and electrolyte metabolism in hypertension. DOCA-NaCl and renal hypertensive rabbits were used for this investigation. Electrolyte studies were performed on various arteries and also on certain areas in the central nervous system. Isolation of the diseased artery is the best procedure to study vascular responsiveness without complications due to nervous and humoral factors. The central ear artery of the rabbit is a small muscular artery which is responsive to both sympathetic nerve stimulation and to injected noradrenaline. It can also be deprived of its sympathetic innervation by excision of the preganglionic fibres of the superior cervical nerve (decentralization) or denervated by removal of the superior cervical ganglion. For these reasons isolated and perfused central ear artery was used as a model for the study of vascular reactivity in hypertension.

The present studies indicated that the integrated actions of the sympathetic nervous system, vascular smooth muscle and tissue electrolytes are essential for the development and maintenance of hypertension.

II. LITERATURE REVIEW

The Sympathetic Nervous System and Vascular Tone

Noradrenaline is present in the postganglionic sympathetic nerves where it acts as a neurotransmitter (Von Euler, 1946) and it is released by sympathetic nerves upon the arrival of a nerve impulse. In addition to its ability to release noradrenaline the adrenergic neuron can carry out a variety of functions related to the metabolism of noradrenaline. The adrenergic neuron stores noradrenaline within vesicles which exhibit a characteristic dense core. The noradrenaline is bound in the vesicles as a salt complex with ATP in the ratio of 4:1. Studies on dense-core vesicles have shown that they possess an active transport process requiring ATP and Mg^{++} which can accumulate noradrenaline from the cytoplasm of the neuron (Von Euler et al., 1963). Moreover, a second uptake process, which is selectively blocked by agents such as cocaine and imipramine, operates at the axonal membrane to transport noradrenaline into the nerves.

The tone of sympathetically innervated organs depends on the amount of noradrenaline available at the receptor surface. The amount of noradrenaline available to activate the receptors depends on the amount of noradrenaline released per impulse and the rate of inactivation of the amine. The released neurotransmitter may be inactivated by either local enzymatic destruction, re-uptake into nerves or diffusion into the bloodstream. Nerve uptake is abolished after denervation which also causes depletion of noradrenaline stores and the complete loss of sympathetic tone (Kirpekar et al., 1962; Trendelenburg, 1963). Decentralization, on the other hand, decreases the tonic but probably not the spontaneous

release of the neurotransmitter (Hertting et al., 1962). Some workers (Hertting et al., 1961; Whitby et al., 1961; Rosell et al., 1963; Hertting and Axelrod, 1965; Malmfors, 1965) have suggested that inactivation of noradrenaline by nerve uptake plays the major role in terminating the effect of the neurotransmitter. Recently, Kalsner and Nickerson (1969) used an oil-immersion technique to demonstrate that enzymatic inactivation of noradrenaline and adrenaline by COMT and MAO is the major process terminating the effects of these amines. Moreover, they found binding and storage mechanisms make only a minor contribution to the inactivation of low concentrations of these amines in vascular tissue.

Monoamine oxidase, an enzyme which appears to be localized in mitochondria (Schnaitman et al., 1967), can be found within the adrenergic neuron (Kopin, 1964; Snyder et al., 1965) as well as extraneuronally (Kalsner and Nickerson, 1969). This enzyme catalyses the oxidative deamination of catecholamines and is responsible for the catabolism of free intraneuronal noradrenaline. Moreover, it has been shown that deamination of noradrenaline readily occurs if uptake and storage in synaptic vesicles is blocked (Axelrod, 1964; Rutledge and Weiner, 1967). This deamination may involve also, extraneuronal MAO (Kalsner and Nickerson, 1969).

Surgical and Drug Therapy of Essential Hypertension

In the early 1920's, the treatment of hypertension with the then available drugs, the nitrites, was unsatisfactory. This led to a search for more effective therapeutic measures. It was anticipated that the increased arteriolar tone which is characteristic of essential hypertension could be reduced by section of the vasoconstrictor nerves. Consequently, bilateral lumbar sympathectomy as a means of treating

essential hypertension was introduced (Rowntree and Adson, 1925). Rowntree and Adson performed this type of surgery on a patient with malignant hypertension (225/190 mm Hg) and reported that the patient's blood pressure decreased markedly and the accompanying symptoms of the disease disappeared. However, six months later, the patient's blood pressure rose again to a hypertensive level (220/120 mm Hg), although he apparently remained in good health.

In a subsequently developed technique, the surgery was extended to the thoracic region by removing the anterior roots from the sixth thoracic to the second lumbar segments (Page and Heuer, 1935). This was found to be more effective than bilateral lumbar sympathectomy alone. For example, a young girl with persistent elevated arterial pressure underwent this type of surgery and her blood pressure fell to a normotensive level where it remained during seven months of clinical observation.

In the ensuing years, surgical therapy became popular for the treatment of essential hypertension and various modifications were attempted. Flothow (1939) instituted the following procedure: section of the splanchnic and sympathetic nerve chains above the diaphragm; removal of major, minor and least splanchnic nerves, portions of the coeliac ganglion and, in addition, of the first and second lumbar ganglia, as well as denervation of the adrenal glands. This extensive form of sympathectomy lowered blood pressure, abolished the clinical symptoms of hypertensive disease and was claimed to prolong the lives of the majority of patients. Grimson (1941) and Grimson et al. (1953)

confirmed Flothow's claim that extensive to total paravertebral sympathectomy is a more effective measure than partial or local sympathectomy in the treatment of hypertensive disease. The conclusion of Grimson and co-workers was based on their 13 years of follow-up study during which they performed total thoracic and partial to total lumbar sympathectomy, splanchnicectomy and coeliac ganglionectomy in 172 hypertensive patients. These patients were compared to hypertensive patients who were treated medically and it was found that the operated group had a higher survival rate.

The results of similar long-term studies were presented by Smithwick and Thompson (1953), and they also claimed that surgical treatment had therapeutic superiority over medical treatment in essential hypertension. They studied 1,266 hypertensive patients for a period ranging from 5 to 14 years following thoraco-lumbar sympathectomy using 467 hypertensive patients who were medically treated as the control group. Surgical treatment was found to be superior to medical treatment in alleviating the hypertensive disease and prolonging the lives of patients.

It is clear from these reports that the discrepancy in evaluating the success of surgical therapy reported by early workers was due to incomplete sympathectomy and perhaps also to partial regeneration of sympathetic nerves over the long period of observation. These clinical studies, however, served to provide evidence that the sympathetic nervous system might be involved in the pathogenesis of essential hypertension.

With the discovery of more effective antihypertensive agents,

the use of surgical sympathectomy as a treatment for hypertension declined. In 1946, Acheson and Pereira pointed out that tetraethylammonium chloride, a ganglion blocking agent, might be useful in lowering blood pressure in humans with essential hypertension by producing a "chemical sympathectomy". Ganglion blocking agents inhibit transmission of nerve impulses at sympathetic and parasympathetic ganglia. Later, Paton and Zaimis (1948) found that the methonium compounds, particularly the penta-and hexa- methonium derivatives, produced ganglionic blockade with a longer duration of action than did tetraethylammonium chloride. The application of ganglion blocking agents to the treatment of hypertension became therapeutically established but the number of adverse side effects which these drugs produced, severely limited their utility. Recently, drugs have been developed which specifically block or inhibit the activity of the sympathetic nervous system and these agents (e.g., guanethidine) are currently in wide use in the therapy of hypertension.

Although the success of surgical and "chemical" sympathectomy in the treatment of hypertension seemed to implicate the sympathetic nervous system in the pathogenesis of essential hypertension, it was impossible for investigators to use humans on an experimental basis to probe into the mechanisms involved. Hence, the need for animal studies arose.

METHODS OF PRODUCING EXPERIMENTAL HYPERTENSION

Renal Hypertension

In 1934 Goldblatt introduced a technique for the production of hypertension which was based on the premise that the disease originates in the kidneys (Goldblatt, 1934). The renal arteries of dogs were par-

tially constricted with silver clips and this resulted in persistent elevated systolic blood pressure. This form of hypertension was said to resemble the so-called benign nephrosclerosis in man. Goldblatt's technique was made more effective by removing one kidney and constricting the renal artery of the other kidney. This method was later modified by Grollman (1944) who compressed one kidney in a "figure of eight" with cotton thread and removed the contralateral kidney. These subsequent modifications of Goldblatt's technique were claimed to reduce the otherwise high mortality rate.

It was later found that renin is involved in the initial phase of the development of renal hypertension (Pickering et al., 1942, 1945) Recently, it has been shown that renin acts on angiotensionogen in blood converting it to angiotensin II and this in turn elevates the blood pressure in renal hypertension (Laragh et al., 1960; Tobian, 1960; Laragh, 1967).

Desoxycorticosterone acetate - Sodium chloride (DOCA-NaCl) Hypertension

Selye and co-workers first showed that DOCA administered to unilaterally nephrectomized rats produces severe hypertension and vascular disease (Selye et al., 1943). They found that administration of DOCA as pellets, or in the form of a daily injection, to unilaterally nephrectomized animals maintained on 1% sodium chloride solution instead of drinking water led to the rapid development of hypertension. This form of hypertension does not appear to involve a circulating pressor hormone, as is the case in renal hypertension (Koletsky and Pritchard, 1964). Moreover, it has been pointed out by Gross (1960) that this type of hypertension is fairly specific for rats because it could not be produced in the rabbit,

cat or dog (Masson et al., 1953). For instance, rabbits treated with DOCA and drinking sodium chloride solution developed polyuria, muscular weakness and paralysis but not hypertension (Masson et al., 1953).

Neurogenic Hypertension

In 1927, Hering demonstrated that the stretch receptors of the carotid sinus and aortic arch are important in the reflex regulation of blood pressure, and he speculated as to the possibility of a disturbance of this reflex in the genesis of hypertension in man. This led others to study the effects of section of the carotid sinus and depressor nerves in experimental animals. In 1929, Koch and Mies succeeded in producing neurogenic hypertension in rabbits by section of the buffer nerves. Other workers (Kremer, Wright and Scarff, 1933) have since confirmed that hypertension can be produced by section of the four buffer nerves in the rabbit. This type of hypertension is dependent on the integrity of the sympathetic nerves. Normally, impulses from the pressor receptors tonically inhibit the sympathetic centres of the medulla and thereby depress the arterial pressure. Thus, denervation of the pressor receptors in an animal releases the inhibition on the sympathetic centres which become excessively active thus increasing sympathetic nerve activity and arterial blood pressure.

Hypertension by Selective Breeding

Alexander, Hinshaw and Drury (1954) developed, by selection, a strain of rabbits showing spontaneous hypertension. Subsequently, Smirk and Hall (1958) also developed a strain of rats with spontaneous hypertension.

Recently, Okamoto and Aoki (1963) succeeded in breeding a strain of wistar rats in which the incidence of hypertension is very high. The strain was developed when a male wistar rat, discovered to be hypertensive on casual measurement, was mated with a normotensive female. Hypertensive rats of subsequent litters were then mated brother and sister. After the third generation all of the subsequent litters developed hypertension. It has since been found that the blood pressure of these rats rises progressively with age (Jones and Dowd, 1970). The cause of spontaneous hypertension is unknown but it is presumably of genetic origin and the rats have therefore been termed "genetic" or "spontaneously hypertensive" rats.

Role of the Sympathetic Nervous System in Experimental Hypertension

The results of surgical sympathectomy in patients with essential hypertension, although controversial, led investigators to examine the role of the sympathetic nervous system in various forms of experimentally induced hypertension in animals.

Goldblatt et al., (1937) undertook studies to determine whether complete sympathectomy would prevent or cure renal hypertension in dogs. In one group of dogs, the thoracic portion of the splanchnic nerves and the lower four dorsal sympathetic ganglia were removed and later the renal arteries were partially clamped. They showed that the rise in blood pressure which usually follows renal ischemia was not prevented. In another experiment, dogs with chronic renal hypertension were subjected to the same surgical sympathectomy as were those of the first group. For several weeks following the surgery, the blood pressure of the dogs was lowered but the pressure then returned to the pre-operative hypertensive level and remained elevated for over nine months.

In the same year, Freeman and Page (1937) reported similar findings on renal hypertensive dogs. They performed a more extensive sympathectomy than did Goldblatt and co-workers. In several dogs, the sympathetic chains in the thoracic and abdominal regions were removed and in a second group the hearts were denervated in addition to the sympathectomy. The purpose of denervating the heart was to prevent reflex cardiac excitation which they thought might contribute to a rise in blood pressure. Nevertheless, the extensive surgical sympathectomy did not prevent the development of renal hypertension. Freeman and Page reported that autopsies on the dogs indicated that sympathectomy was complete and no regeneration of sympathetic nerves had occurred. In their conclusion, Freeman and Page ruled out the involvement of the sympathetic nervous system in the pathogenesis of renal hypertension.

In contrast to the above observations, Introzzi et al. (1938) reported that renal hypertension was abolished by sympathectomy. Introzzi and co-workers performed unilateral lumbar ganglionectomy and splanchnicectomy and denervated one adrenal gland in a group of dogs with renal hypertension. In this group of animals, there was only a slight transitory fall in blood pressure. However, in another group of renal hypertensive dogs which were bilaterally sympathectomized, they found that hypertension was completely abolished. These observations serve to suggest that the sympathetic nervous system is involved in the maintenance of renal hypertension.

Nowak and Walker (1939) performed total sympathectomy in dogs by removing the entire thoraco-abdominal sympathetic chains and about a

month later the carotid sinus and aortic depressor nerves were sectioned. According to these workers the dogs developed neurogenic hypertension. Moreover, they reported that partial sympathectomy (bilateral splanchnicectomy, unilateral adrenalectomy and bilateral celiac ganglionectomy) did not reduce the blood pressure of dogs with neurogenic hypertension. Nowak and Walker speculated that the increase in pressure might be due to the liberation of some intrinsic factor or some other unknown mechanism.

In similar studies, Grimson (1940; 1941) showed that after complete removal of the paravertebral sympathetic chains followed by section of the buffer nerves in dogs, neurogenic hypertension did not develop immediately but after several weeks the blood pressure rose. They also reported that the blood pressure of dogs with neurogenic hypertension was initially reduced below normal by complete sympathectomy. However, after a period of time (4 to 6 weeks) the blood pressure was again elevated above normal values. The findings of Nowak and Walker (1939) and of Grimson may have an explanation in the report of Boyd and Monro (1949) that partial retention of autonomic function occurs after "complete" sympathectomy and is due to the presence of intermediate sympathetic ganglia which do not pass through paravertebral sympathetic chains and are thus inaccessible to the surgeon.

Dock et al. (1934, 1940, 1942) showed that pithing of the central nervous system lowered the arterial pressure of normotensive and renal hypertensive rats, rabbits and dogs to the same extent. In addition, a rise in arterial pressure was evoked in these animals with adrenaline and renin. They concluded that their experiments ruled out a circulating

vasoconstrictor substance and that "the renal pressor hormone" acts through the vasomotor center to maintain the hypertension. However, pithing produces such a gross lesion that it is difficult to interpret the significance of such a study.

Other workers (Reed et al., 1944; Sapirstein and Reed, 1944) have also claimed that the sympathetic nervous system is important in renal hypertension. They observed that sodium pentobarbital (anaesthetic level) and the adrenergic blocking agents, yohimbine and F883 (diethyl-amino-methyl-benzo-dioxane), lowered the blood pressure of rats with chronic hypertension, but not that of acute renal hypertensive and normal rats. They concluded that the mechanism for maintaining the later stages of renal hypertension involved the sympathetic nervous system but that this mechanism did not operate in the early stages of renal hypertension. Moreover, they suggested that at the early stages renin played a predominant role. The validity of observations on the effect of yohimbine on blood pressure of these animals is, however, questionable since its effect depends on dosage and initial pressure levels of the tested animals.

A technique has recently been introduced to eliminate the sympathetic nervous system in animals by immunological means (Levi-Montalcini and Booker, 1960). Immunosympathectomy is produced by injecting new-born animals with an antiserum to a nerve growth factor. It has been shown that this procedure results in almost total and permanent destruction of the paravertebral and prevertebral sympathetic ganglia, provided the antiserum is administered immediately after birth and injected daily for 2 to 3 days (Levi-Montalcini and Angeletti, 1966). Recently, investi-

gators have used these immunosympathectomized (IMS) animals to study the role of the sympathetic system in experimental hypertension.

Finch and Leach (1970) reported that immunosympathectomy or sympathectomy produced by the administration of 6-hydroxydopamine did not prevent the development of experimental hypertension in the rat induced either by renal artery stenosis or by treatment with DOCA. Therefore, they excluded the sympathetic nervous system as an important factor in the development of experimental hypertension. However, they did not present evidence for their claim that the rats were sympathectomized.

Dorr and Brody (1966) and Ayitey-Smith and Varma (1970) found that renal hypertension could be produced in immunosympathectomized (IMS) rats but that the hypertension was not sustained. The blood pressure of these renal hypertensive IMS rats returned to normotensive levels although their counterparts with an intact sympathetic nervous system remained hypertensive. This observation showed that the sympathetic nervous system may not be required for the development of renal hypertension but may play a role in the maintenance of renal hypertension.

Willard and Fuller (1969) and Ayitey-Smith and Varma (1970) were unable to produce DOCA-NaCl hypertension in rats which were completely immunosympathectomized. However, rats which were incompletely immunosympathectomized, because they were injected at birth only once with the antiserum, developed sustained DOCA-NaCl hypertension. It has been shown that the IMS rats which Varma (1967) reported earlier to have developed DOCA-NaCl hypertension were also incompletely immunosympathectomized. In view of this observation, Ayitey-Smith and Varma suggested that minimal control

of the sympathetic system on the cardiovascular system is sufficient for the production of experimental hypertension. Clark (1969; 1971) has implicated the sympathetic nervous system in the development of hypertension which is of genetic origin in rats. He studied the effect of immunosympathectomy in the developmental stages of this form of hypertension and reported that elevation of the blood pressure after 10 days of age depends largely, but not exclusively, on an intact nervous system.

Catecholamine Metabolism in Hypertension

The metabolism of catecholamines in essential and experimental hypertension has been studied with the aim of gaining an insight into the state of sympathetic nerve activity in hypertension.

In recent years, tritiated noradrenaline has been made available for the study of the kinetics of noradrenaline metabolism in tissues from experimental animals. Some workers (De Champlain et al., 1967, 1969; Henning, 1969; Volicer et al., 1969; De Quattro et al., 1969) have performed extensive studies on the binding, storage and synthesis of noradrenaline and on the turnover rate of this amine in various forms of experimental hypertension. These workers injected radioactive noradrenaline or a precursor intravenously into experimental hypertensive and normotensive animals which were then killed at various times after injection and tissue samples were taken for analysis of radioactivity and endogenous noradrenaline content. The rate of decline of radioactivity in the tissues was estimated and used in calculating synthesis and turnover rates of noradrenaline. It was found that the turnover of noradrenaline was higher in hearts from DOCA-NaCl, renal and neurogenic hypertensive animals than in hearts from normotensive animals (De Champlain et al., 1969; Henning, 1969; De Quattro, et al., 1969). However, the rate of synthesis of noradrenaline was normal in DOCA-NaCl and renal hypertensive animals although it was increased in neurogenic hypertensive rabbits (De Quattro et al., 1969). It was also found that the nerve uptake of noradrenaline was normal but that storage of the amine in adrenergic nerves was impaired in DOCA-NaCl hypertensive animals. It was therefore suggested, on the basis of these findings, that

there might be an increased discharge of noradrenaline by the sympathetic nerves in renal DOCA-NaCl and neurogenic hypertension. However, it is difficult to reconcile this view with the finding that the synthesis of noradrenaline is unchanged in DOCA-NaCl and renal hypertensive animals.

Louis et al., (1969), found a reduced rate of synthesis and turnover in the hearts of spontaneously hypertensive rats. They suggested that in this form of hypertension the rate of noradrenaline release was subnormal hence noradrenaline could not play a primary role in hypertension. This suggestion is in contrast with the findings by Okamoto et al., (1967) who showed marked augmentation of peripheral sympathetic activity in spontaneously hypertensive rats. In their experiments electrical activity of the sympathetic nerve, the splanchnic nerve, was recorded directly with electrodes.

In further investigations, De Champlain et al., (1966,1967) and Krakoff et al., (1967) studied the metabolism of noradrenaline in DOCA-NaCl hypertensive rats using radioactive noradrenaline. They found that the hearts and kidneys from hypertensive animals contained more deaminated and O-methylated metabolites respectively than did those from normotensive animals. They speculated that the increase in deaminated metabolites in the hearts of hypertensive animals indicated that more "cytoplasmic" noradrenaline was available for intraneuronal deamination and the increased O-methylated amines signified a greater release of physiologically active noradrenaline which was subsequently metabolized in the kidneys.

Clinical studies of noradrenaline metabolism in essential hypertension was performed by Gitlow et al. (1964) using labelled noradrenaline.

The labelled noradrenaline was infused (0.05 ug dl-B-H^3-NA per kg/min) into hypertensive and normotensive patients for 1 hour and the rate of clearance of H^3-NA from the plasma was followed for up to 3 hours. There was a significantly greater rate of clearance of labelled noradrenaline from the plasma of hypertensive than from normotensive patients. Thus, Gitlow and co-workers assumed that noradrenaline metabolism was abnormal in patients with essential hypertension.

The available evidence on noradrenaline metabolism does not clearly support the view that noradrenaline overproduction is associated with hypertension. Moreover, results from studies on urinary excretion of catecholamines in essential hypertension are also conflicting.

Von Euler et al. (1954) measured the 24 hour urinary excretion of noradrenaline in 500 unselected patients with essential hypertension. They found that in 66.2% of the patients, noradrenaline excretion figures were less than 57.4 ug per 24 hours, which is within the normal range. Excretions in the range of 57.6 to 86.4 ug were regarded as significantly increased. In 16.4% of the patients the excretion of noradrenaline was significantly greater than 86.4 ug per 24 hours. Despite the small number of patients who excreted excessive noradrenaline it was thought that this finding was of significance in the pathogenesis of essential hypertension.

Other workers (Griffiths and Collinson, 1957; Moller et al., 1957) also measured the urinary output of catecholamines (adrenaline and noradrenaline) in hypertensive patients. Whereas Griffiths and Collinson found no difference in the noradrenaline output of hypertensive and normotensive patients, Moller et al., (1957) found that hyper-

tensive patients excreted significantly less noradrenaline than did normotensive patients. However, Weil-Malherbe and Bone (1957) reported that hypertensive patients excreted significantly more noradrenaline.

The discrepancies and variations observed in studies on the urinary excretion of catecholamines might partly be attributed to the procedures used to select patients for the studies. Thus, false negative results might arise from the frequent occurence of arteriosclerosis or impaired renal function during the development of hypertensive disease and false positive results from complicating factors unrelated to the primary disease process. Ikoma (1965) carefully selected hypertensive patients for noradrenaline excretion studies, paying particular attention to renal function. Hypertensive patients with normal renal function were found to excrete more catecholamines (adrenaline and noradrenaline) per 24 hours than did normal patients or hypertensive patients with impaired renal function.

Instead of measuring urinary noradrenaline, Stott and Robinson (1967) measure normetanephrine, a metabolite of noradrenaline, in the urine of essential hypertensive patients. Hospitalized normotensive and hypertensive patients were selected for the investigation. During the investigation, patients were allowed to empty their urinary bladders completely and the urine was discarded. Later, each patient was allowed to drink a glass of water and rest in an upright position in a chair for 60 minutes, after which they emptied their bladders again. The urine samples were then analysed for normetanephrine. Hypertensive patients excreted significantly more normetanephrine than did normotensive

patients. The conclusion drawn from this study is that the elevated blood pressure in hypertensive patients is sustained by an excessive release of noradrenaline from sympathetic nerve endings. However, it is obvious that other possibilities such as an increase in the activity of the O-methylating enzyme cannot be ruled out.

Electrolytes

Salt restriction in the management of hypertension has long interested those concerned with the treatment of essential hypertension. Although low salt intake was advocated by Ambard and Beaujard as early as 1905 (Allen, 1920) it received clinical trial only after subsequent studies by Grollman et al., (1940) and Grollman and Harrison (1945). Grollman and Harrison subjected renal hypertensive rats to drastic sodium restriction and this resulted in a marked fall in blood pressure within four to six days. Addition of 0.5% sodium chloride to the diet partially prevented the hypotensive effect of the low sodium diet and 2% sodium chloride completely counteracted the effect of the sodium free diet. Two per cent potassium chloride supplement, however, did not antagonize the hypotensive effect of the sodium free diet. This finding has since been confirmed (Danford et al., 1950; Frieden et al., 1952). Frieden and co-workers also reported that they did not observe any change in the blood pressure of normotensive dogs subjected to chronic sodium depletion.

McQuarrie et al. (1936) described a fifteen year old boy with an abnormal craving for salt who would eat 80 gm of sodium chloride per day if allowed free access to it. When allowed to eat 60-80 gm of sodium chloride daily he developed a moderate hypertension (170/110) which disappeared if the intake of sodium chloride was limited to 5 gm daily.

On the other hand, the hypertensive effect of a high sodium chloride intake could be prevented by the simultaneous ingestion of 15 gm of potassium chloride.

Potassium deficiency has also been observed to have a hypotensive effect. Freed and Friedman (1950, 1951) found that normal rats on a potassium deficient diet for seven to nine weeks developed hypotension. According to these workers, simultaneous dietary withdrawal of sodium and potassium did not, however, lower the blood pressure of these animals. Moreover, Friedman et al. (1951) demonstrated the hypotensive effect of a potassium deficient diet in renal hypertensive rats and they suggested that this effect might be due to a decrease in total peripheral resistance. This suggestion is consistent with the finding of Rosenman et al. (1952) that the pressor effects of intravenously injected epinephrine, norepinephrine, angiotensin and renin are significantly decreased in rats fed on a potassium deficient diet compared to those fed on a normal diet.

Lenel et al. (1948) substituted 0.9 and 1.2% and Sapirstein et al. (1950) 1.5 to 2.5% sodium chloride solutions for the drinking water of chickens and rats respectively. They reported that the chickens and rats developed hypertension during the chronic administration of sodium chloride. Lenel and co-workers reported that the blood pressure of the chickens fell promptly after withdrawal of sodium chloride solution. Subsequent studies have shown that the elevation in blood pressure was generally proportional to the amount of sodium chloride in the diet of the animals (Meneely and Ball, 1958). Meneely and Ball

fed seven groups of normal rats diets that varied only in the amount of sodium chloride added, from 0.01 to 9.8%. After nine months on these diets, the rats showed increases in arterial pressure proportional to the dietary content of sodium. The groups of rats eating 2.8 to 5.6% sodium chloride in the diet developed mild hypertension (130-140 mm Hg) while those on the 7.0 to 9.8% sodium chloride diet often had moderate to severe hypertension (150-160 mm Hg). In addition, it was observed that a high intake of potassium in the diet prevented the hypertensive effect of a high sodium chloride intake.

Later, it was found that the hypertensive effect of a high sodium chloride intake could be intensified by the simultaneous administration of desoxycorticosterone acetate to nephrectomized animals, (Selye et al., 1943; Knowlton et al., 1947; Tobian and Redleaf, 1957; De Champlain et al., 1969). In addition, it was shown that rats on a diet practically devoid of sodium but injected with high doses of desoxycorticosterone acetate did not become hypertensive. This observation suggests that desoxycorticosterone acetate plays a secondary role in the induction of this form of hypertension.

Gaudino and Levitt (1949), Darrow (1944) and Fereebee et al. (1941) studied the effect of chronic administration of desoxycorticosterone on the distribution of sodium and potassium in tissues of animals. They estimated the extracellular and intracellular distribution of the ions using inulin and chloride to measure extracellular space. Desoxycorticosterone acetate was found to cause an increase in intracellular sodium in various tissues.

As reports on the hypertensive effect of a high sodium intake

increased, investigators became interested in studies on the metabolism and distribution of electrolytes in hypertension. Tobian and Binion (1952) studied the sodium and water content of renal arteries of hypertensive and normotensive humans obtained post mortem. They found that the arteries from hypertensive patients had abnormally large amounts of sodium and water compared to those from normotensive patients.

Subsequent studies conducted by Ross (1956) were designed to find out the distribution of sodium in hypertensive patients. He used an isotope dilution technique (^{24}Na) to measure exchangeable sodium in patients with essential and renal hypertension. The exchangeable sodium was found to be within the normal range in some patients while in others it greatly exceeded the normal values. He, then, used thiosulphate to measure extracellular space in order to estimate the distribution of sodium between the extracellular and intracellular compartments in patients with high exchangeable sodium. Ross concluded from his study that the intracellular sodium in these patients was greatly increased compared to values obtained in normotensive patients. In addition, it was found that sodium deprivation caused a slight fall in exchangeable sodium which was accompanied by a decrease in blood pressure. Ross stated that the distribution of sodium in the body is a major factor in the etiology of hypertension.

Some workers have also studied the distribution of electrolytes in experimentally induced hypertension in animals. They (Tobian and Binion, 1954; Tobian, 1956; Tobian and Redleaf, 1957, 1958) determined the sodium and potassium distribution in aortas from renal and DOCA-NaCl

hypertensive rats and found that aortic sodium and potassium were higher in the hypertensive than in normotensive animals. Moreover, Tobian and Redleaf (1957) observed that if the intake of sodium was drastically restricted, both the hypertension and the related electrolyte abnormality which usually accompanied desoxycorticosterone acetate administration were prevented. It was suggested that hypertension might be fundamentally associated with an increased arterial sodium and potassium content.

Tobian and Binion (1954) and Tobian and Redleaf (1958) determined the extracellular and intracellular distribution of sodium and potassium in aortas from hypertensive animals using the "chloride space" method. They concluded that the increase in sodium and potassium was intracellular. A suggestion was then made that if similar changes occurred in arterioles it could explain the characteristic increase in peripheral vascular resistance which is observed in hypertension.

Other workers have reported either a decrease in the potassium content of tissues from renal and DOCA-NaCl hypertensive rats (Laramore and Grollman, 1950; Tobian and Binion, 1954) or no change in the body potassium content of renal hypertensive rats (Green and Sapirstein, 1952).

Since the arterioles have a greater influence than the aorta on the total peripheral resistance, Tobian et al. (1961) decided to determine the electrolyte composition of small arteries such as the mesenteric arteries of renal hypertensive rats. Sodium and potassium contents based on dry weight of the tissue were determined. They found that the arterioles from hypertensive rats had 16% more sodium per unit dry weight than those from normotensive rats and they assumed that the increase in arterial

sodium was intracellular. The potassium content of the arterioles from hypertensive rats was, however, similar to that of the arterioles from normotensive rats.

Spontaneously hypertensive rats have also been shown to exhibit marked elevations in aortic sodium and potassium, at all ages, compared to normotensive rats (Nagaoka et al., 1969).

Recently, Tobian and Chesley (1966) have studied the state of calcium in the mesenteric arteries of the renal hypertensive rats. The arteriolar calcium was found to be significantly higher in the hypertensive than in the normotensive rats. Consequently, a suggestion was made that since calcium plays an essential role in the contraction of arterial smooth muscle, the increased arteriolar calcium content of the hypertensive animals may be partially responsible for the increased peripheral resistance in hypertension.

The demonstration that arterial sodium and perhaps potassium are elevated in hypertensive animals and that the sympathetic nervous system might be involved in the etiology of hypertension led some workers to study the relationship of these factors in hypertension.

Smythe et al. (1952) and Nickel et al. (1954) undertook to investigate the influence of catecholamines on the renal system in normal subjects. The renal clearance of sodium and potassium was determined during intravenous infusion of adrenaline and noradrenaline. The urinary excretion of sodium and potassium declined sharply, although there was hardly any change in glomerular filtration rate during the infusion of the amines. Moreover, the infusion of noradrenaline into

patients with Addison's disease resulted in a decrease in the excretion of sodium. Wagner (1957) has also reported that patients with autonomic insufficiency excreted sodium at a supranormal rate during the infusion of isotonic saline. These observations suggest that the sympathetic nervous system or catecholamines can modify electrolyte excretion through an action on the renal apparatus without influencing the glomerular filtration rate.

Furthermore, Gill et al. (1964) studied the effects of adrenergic neuron blockade with guanethidine on the excretion of sodium in normal subjects during rapid infusion of physiological saline and during the administration of sodium-retaining steroids and sodium. Sodium excretion during the infusion of saline was increased 50% to 300% following guanethidine. Moreover, the mineralocorticoids caused less retention of sodium after the administration of guanethidine. It was also observed that the increased excretion of sodium did not depend on a high glomerular filtration rate. These workers concluded that the sympathetic nervous system influences sodium metabolism probably through an effect on the renal system. This suggestion is consistent with the finding of Ayitey-Smith and Varma (1970) that immunosympathectomized rats which did not develop DOCA-NaCl hypertension excreted significantly more sodium per day than did DOCA-NaCl hypertensive rats with intact sympathetic nervous system. In addition, IMS rats did not show the characteristic elevation of aortic sodium which was observed in DOCA-NaCl hypertensive rats.

Vascular reactivity in hypertension

It is well known that adrenocortical steroids modify the pressor

effects of catecholamines. Animals with deficient adrenocorticoid function have been shown to exhibit reduced cardiovascular responses to injected or infused adrenaline and noradrenaline (Remington, 1951; Fritz and Levine, 1951; Ramey et al., 1951; Cleghorn et al., 1958). In addition, there are reports that adrenocortical steroids potentiate the effects of catecholamines in vivo (Raab, 1942; Raab et al. 1950) and in vitro (Kalsner, 1969).

Kalsner (1969) has recently shown that desoxycorticosterone potentiates the effects of catecholamines in vitro by inhibiting a major mechanism for their inactivation. He carried out his studies on aortic strips from rabbits and found that desoxycorticosterone enhanced the responses to adrenaline and noradrenaline. Experiments with the oil-immersion technique revealed that desoxycorticosterone decreased the rate at which strips inactivated catecholamines by O-methylation. Kalsner has, moreover, suggested that it is possible that the steroid does not inhibit the enzyme but decreases the access of substrate to the enzyme by impairing movement across certain biological membranes. The relevance of this finding to hypertension remains to be determined.

Raab (1942) and Raab et al. (1950) studied the effects of chronic administration of DOCA on the pressor responses to catecholamines in humans with the aim of gaining an insight into the etiology of essential hypertension. Healthy patients were injected with 10 mg of DOCA subcutaneously, daily, for an average of 17 days. Control responses to subcutaneously injected or intravenously infused noradrenaline and adrenaline were first established before DOCA treatment. It was observed that some of the patients showed a moderate rise in systolic blood pressure during treatment with DOCA. Moreover, following pretreatment

with DOCA the effects of the amines were significantly potentiated. The conclusion drawn from this finding was that the etiology of essential hypertension might involve potentiation of the effects of released noradrenaline or circulating catecholamines due to overproduction of endogenous adrenal steroids. It has also been reported that responses to amines are reduced during sodium withdrawal and cannot be potentiated by DOCA (Raab, 1952).

There is now reasonable evidence which associates vascular hyperactivity with essential hypertension. This view was the outcome of a number of studies carried out in patients with essential hypertension.

Greisman (1952, 1954) studied the effects of pressor amines on the circulation of the nailfold vascular bed in patients with essential hypertension. Noradrenaline was injected or infused intravenously into the arms of hypertensive and normotensive patients and the vasoconstriction of the vessels of the nailfold vascular bed was estimated. He reported a greater constriction in the vessels of hypertensive than in those of normotensive patients. Although this finding on the capillary circulation of the nailfold links increased vascular reactivity with essential hypertension, it is not easy to extrapolate this finding to other vascular beds because of the differences that may exist between them.

However, other vascular beds in patients with essential hypertension have been studied and most of the results are quite consistent with those of Greisman, Doyle and Black, (1955); Doyle et al., (1959); Daly and Duff, (1960); Doyle and Fraser, (1961); Mendlowitz et al., (1962).

For example, Doyle and Black (1955) determined the pressor responses to intravenously injected noradrenaline (2 ug) and angiotensin (5 units) in hypertensive and normotensive patients. They observed only slightly greater responses in the hypertensive than in normotensive patients. The patients were then pretreated with 20 mg of hexamethonium (a ganglion blocking agent) every three minutes until the maximum decrease in blood pressure was achieved, and then the effects of the pressor agents were re-examined. Hexamethonium was used to eliminate reflex control mechanisms which might have opposed changes in blood pressure. It was found that the responses of the pressor agents after hexamethonium were significantly greater in the hypertensive than in the normotensive patients. The negative results obtained initially were attributed to reflex compensation which masked the direct effects of the pressor agents.

Later Doyle et al. (1959) re-investigated the question of increased vascular reactivity in patients with essential hypertension. They determined the changes in blood flow of the forearm to intra-arterial infusions of noradrenaline (0.4 ug per minute) and angiotensin (0.5 units per minute) in hypertensive and normotensive patients. In this case, they demonstrated, in the absence of hexamethonium, a greater average fall in blood flow in the forearms of the hypertensive than in the normotensive patients. Furthermore, they observed that after hexamethonium the pattern of responses to the pressor agents was not altered. Consequently, they attributed the negative results of the earlier studies (Doyle and Black, 1955) to the route of administra-

tion of drugs and concluded that intra-arterial infusion of the drugs provides a better means of assessing vascular reactivity in patients without the interference of reflex control mechanisms.

In a subsequent investigation, Mendlowitz et al. (1961) reported that increased vascular reactivity to sympathomimetic amines developed very early in the course of essential hypertension and could be demonstrated in children of hypertensive patients prior to an elevation in blood pressure. They suggested that the phenomenon might be the outcome of a chemical alteration in vascular smooth muscle. Moreover, the vascular hyperactivity may be causally related to essential hypertension. After similar studies, Maekawa et al. (1966) reported that increased vascular responses to catecholamines have a direct relationship to the progression of hypertension.

Animal studies on vascular reactivity in hypertension have proved useful but there are some inconsistencies. Thus, exaggerated responses to injected catecholamines and angiotensin have been demonstrated by some workers in various forms of experimental hypertension (Brown and Maegraith, 1941; Olsen et al., 1950; Rothman,1955; Sturtevant, 1955, 1956; Capon, 1964; Gaskell et al., 1964), while in other studies the responses to these pressor agents were similar in hypertensive and normotensive animals (Conway, 1955; Phelan et al., 1962). In most of the above studies the experiments were not designed to probe into the cause of increased vascular reactivity.

Studies on vascular reactivity in hypertension have also been done in vitro in order to eliminate complications due to nervous and

humoral factors. These studies, however, have yielded conflicting results.

Vick et al. (1956) prepared aortic strips from normal rats and from rats which had been fed on a high sodium chloride diet for ten weeks. They determined the isotonic contraction of these aortic strips to adrenaline and found that the strips from salt-fed rats responded with greater contractions than did the control strips. However, the blood pressure of the rats after the treatment was not reported. The increased contraction of the salt-fed strips was attributed to sodium deposition in the smooth muscle of the aorta.

Subsequently, Redleaf and Tobian (1957, 1958) and Mallov (1959) investigated the contractile responses to catecholamines of aortic strips from renal and DOCA-NaCl hypertensive rats. The aortas from the hypertensive rats did not show hyperresponsiveness to noradrenaline when compared to those from normotensive rats.

In another investigation Gordon and Nogueira (1962) suggested that the negative results of other workers might have been due to the fact that the aortic strips were tested at very low initial tension. They argued that if the smooth muscle of the blood vessels shows a length-tension relationship such as skeletal muscle does, the optimal initial length (which corresponds to initial tension) might be higher for the aortic strips from hypertensive than from normotensive rats. Hence, they re-investigated the problem paying particular attention to initial tension. At high initial tension, they showed that aortic strips from the renal hypertensive rats responded to noradrenaline with greater contractions than those from the normotensive rats. However, the carotid

arterial strips from renal hypertensive dogs tested at low tension were also found to be more sensitive to noradrenaline than those from normotensive dogs (Moerman et al., 1969).

Isolated perfused arterial segments and hindquarter preparations have also been used for the investigation of vascular reactivity in hypertension. McQueen (1956) provided evidence that increased vascular reactivity is a common factor in renal and renoprival (bilaterally nephrectomized) hypertension. He studied the vascular reactivity to noradrenaline of the isolated perfused hindlimbs of renal and renoprival hypertensive rats and of bilaterally nephrectomized plus adrenalectomized rats. He found responses to noradrenaline of perfused hindlimbs of renal and renoprival hypertensives greater than controls. Renoprival hypertensive rats, whether given sodium chloride solution or water to drink, exhibited greater responses to noradrenaline than did normal control rats. Moreover, bilaterally nephrectomized rats which had also been adrenalectomized and fed on sodium chloride solution showed increased responses to noradrenaline while those on drinking water did not show any increase in responses to noradrenaline. It was then suggested that vascular reactivity might be under the control of the adrenal glands through effect on sodium metabolism.

In a subsequent experiment, McQueen (1961) found that rats fed on reserpine pellets (equivalent of 1.0 to 1.5 mg reserpine per rat daily) during the induction of renal hypertension were significantly less responsive to noradrenaline than control renal hypertensive rats. Reserpine

was used to control the blood pressure of the animals at normal level during the induction of renal hypertension. The control renal hypertensive rats showed greater responses to noradrenaline than did normotensive control rats. The conclusion was drawn that vascular hyperactivity was caused by arterial hypertrophy and that reserpine prevented the development of this phenomenon. However, the effect on the vascular system of chronic depletion of noradrenaline by reserpine was not considered during the interpretation of these results.

The results of McGregor and Smirk (1968) on the responses of isolated perfused mesenteric arteries are consistent with findings on other perfused vascular beds. McGregor and Smirk studied the vasoconstrictor responses of isolated perfused mesenteric arteries from genetic hypertensive and renal hypertensive rats. Isolated mesenteric arteries from hypertensive animals gave greater responses to noradrenaline than did those from normotensive animals.

Zimmerman et al. (1969) determined the changes in skin and muscle vascular bed resistances to sympathetic nerve stimulation and to intra-arterially injected noradrenaline in renal hypertensive dogs. They found that responses to nerve stimulation and intra-arterial noradrenaline were significantly greater in the cutaneous vascular beds of the hypertensive than of normotensive dogs. However, there were no significant differences in the responses of muscle vascular beds to nerve stimulation or exogenous noradrenaline. Thus it seems that various vascular beds of dogs undergo independent changes during hypertension.

Studies on the responses of rat atria (Baum, 1969) and perfused

hindquarter preparations from renal hypertensive rats (Baum and Shropshire, 1967) to sympathetic nerve stimulation and to tyramine did not demonstrate any significant differences between hypertensive and normotensive rats.

Recently Hinke (1965, 1966) has shown that arteries from DOCA-NaCl hypertensive rats performed more work than did those from normotensive rats. He perfused the isolated ventral tail arteries from DOCA-NaCl hypertensive and normotensive rats with noradrenaline, vasopressin, rat serum and high potassium and measured the strength of muscular contraction in terms of work performed. He also studied the effect of calcium deprivation on the noradrenaline or potassium constricted perfused arteries. The arteries from hypertensive animals were found to be more reactive to noradrenaline, vasopressin and serum than those from normotensive animals. In addition, the arteries from hypertensives performed more work and the contraction induced by noradrenaline was less affected by calcium deprivation than in those from normotensive rats. He suggested that the increased reactivity of the arteries from DOCA-NaCl hypertensive rats is due to an increased efficiency in either calcium utilization during excitation-contraction coupling or in the contractile mechanism itself.

III. METHODS AND MATERIALS

Experiments were performed on New Zealand albino rabbits of either sex weighing between 2.3 - 3.0 kg. They were all kept in the animal house and maintained on a commercial rabbit diet. Tap water was allowed ad libitum unless otherwise stated.

Determination of blood pressure

Systolic blood pressure of unanaesthetized rabbits was measured from the central ear artery using Grant's capsule connected to a condon mercury manometer (Grant and Rothschild, 1934).

To dilate the ear artery for the measurement of blood pressure the unanaesthetized rabbit was warmed to about 37^{o}C while being restrained in a rabbit box holder. The hair was previously cut short over the central artery towards the base of the ear. The membrane of the capsule was moistened with 70% glycerine and the ear was slipped between the limbs of the support of the capsule until the central artery lay beneath the centre of the capsule. The intracapsular pressure was then raised until the column of blood in the artery was just broken. The systolic blood pressure in the artery was equivalent to the intracapsular pressure at the point when the arterial blood column was broken. The systolic blood pressure of control unanaesthetized rabbits was found to be between 65 to 80 mm Hg (1 mm Hg = 1.333 mbar).

Production of DOCA-NaCl hypertension

Animals were anaesthetized with sodium pentobarbital

(40 mg/kg i.p.). One kidney of each rabbit was then removed through a flank incision. Sham-operated rabbits were subjected to the same surgical procedure except for the removal of the kidney.

To prevent infection following surgery,the animals were given three daily intramuscular injections of combiotic (an aquaeous suspension containing 100,000 i.u. penicillin G and 0.125 gm dihydrostreptomycin sulphate). The unilaterally nephrectomized rabbits were maintained on a solution of 1% sodium chloride and 0.5% potassium chloride instead of drinking water and injected subcutaneously with DOCA in peanut oil, 5 mg/kg every other day (i.e. 10-15 mg/kg per week). The sham-operated rabbits were injected with an equivalent volume of peanut oil and allowed free access to tap water. The blood pressure of all rabbits was measured at weekly intervals.

In preliminary experiments 12 rabbits were unilaterally nephrectomized and an 80 mg DOCA pellet (in beeswax) implanted subcutaneously on the thigh. They were then given a 1% sodium chloride solution to drink instead of water. Some of these animals developed muscular weakness or paralysis and most of them died within two to three weeks. This finding which was indicative of potassium depletion led to a modification of the treatment. The addition of 0.5% KCl to the regimen was found to prevent the toxic effects of DOCA and to permit the development of DOCA-NaCl hypertension. All twelve rabbits which were treated with DOCA and sodium chloride alone died. However, out of 54 rabbits treated with DOCA and sodium chloride plus a potassium supplement only 8 rabbits died.

Renal Hypertension

Grollman's method (1944) was used to produce renal hypertension in rabbits. Rabbits were anaesthetized with sodium pentobarbital (40 mg/kg intraperitoneally) and one kidney was exposed by a flank incision. Cotton thread was tightly passed around the body of the kidney. This presented a figure of eight on the kidney which was then compressed. The contralateral kidney was then removed. The blood pressure of these rabbits was measured before the operation and at weekly intervals following surgery.

Adrenalectomy

Adrenalectomy was performed in rabbits according to the procedure described by White (1966). Two hours before the operation, 2 mg cortisone acetate and 3 mg desoxycorticosterone acetate together with combiotic (combiotic; 200,000 i.u. penicillin G and 0.25 gm dihydrostreptomycin sulphate) were given intramuscularly. Each rabbit was then anaesthetized with sodium pentobarbital (40 mg/kg i.p.). An upper midline incision from the xiphisternum to the umbilicus was made, and the small and large intestine was packed to the left, thus exposing the upper abdominal vena cava, the right lobe of the liver and the portal vein.

The posterior peritoneum was incised parallel to the liver lobe margin bound to the vena cava and the right lumbo-adrenal vein was exposed and divided between two silk ligatures. The ends of the caval stump ligature were used to apply traction for exposure of the

right adrenal gland which was beneath the vena cava. The fine lower pole vessels and fascia were ligatured and the gland was carefully dissected free from the adherent fatty tissue and the vena cava. The same procedure was followed for the removal of the left adrenal gland. The incision was then sutured.

On the first post-operative day, the animals were injected intramuscularly with 2 mg cortisone acetate and 2 mg desoxycorticosterone acetate. After the first day, 1.5 mg desoxycorticosterone acetate was administered daily as maintenance therapy for about 7 days and the animals were then taken off the steroid therapy and divided into two groups. The first group was given water and the second group saline to drink for an additional 5 to 7 days. These animals were used to study the effect of adrenalectomy with or without high dietary sodium on vascular reactivity.

Decentralization and denervation

A lateral incision was made on one side of the trachea of the rabbit under sodium pentobarbital anaesthesia and the superior cervical sympathetic nerve was exposed. Decentralization of one ear artery in each rabbit was performed by cutting off about 2 cm of the preganglionic nerve about 2 cm distal to the cervical sympathetic ganglion. Denervation was performed by removing the superior cervical sympathetic ganglion and a portion of the postganglionic nerve. After suturing the neck muscle and skin with silk thread, the rabbits were injected intramuscularly with combiotic as a precaution against

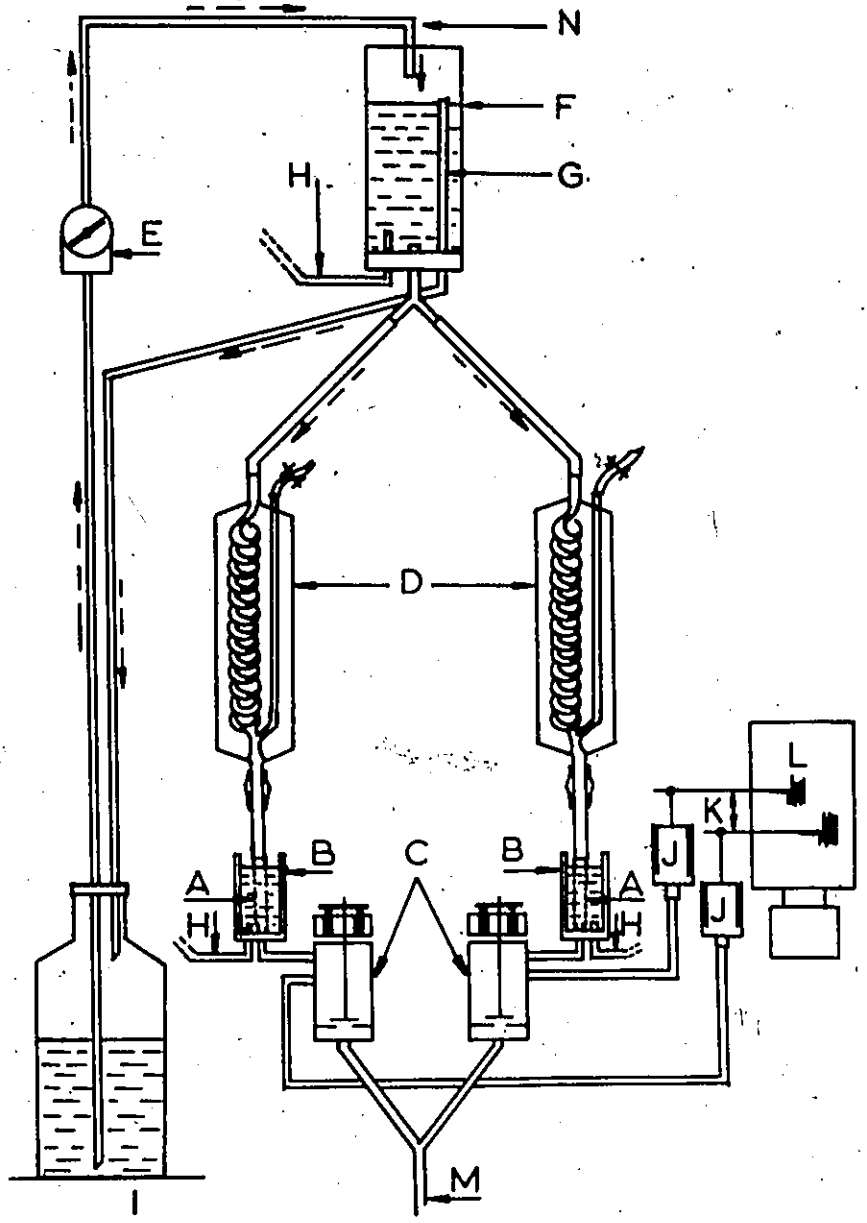


DIAGRAM OF PERFUSION APPARATUS

A, isolated central ear artery; B, organ bath at 37°C; C, Andrews outflow recorder; D, heat exchangers at 37°C; E, roller pump; F, reservoir of Krebs solution; G, overflow outlet; H, air line (for 95% O_2 and 5% CO_2); I, container of Krebs solution; J, piston recorder; K, writing lever, L, kymograph; M, outlet for perfusate and N, inlet for perfusion fluid (Krebs). The arrows with the broken lines indicate the direction of flow of perfusion fluid (Krebs).

infection.

PERFUSION OF ISOLATED RABBIT CENTRAL EAR ARTERY

Perfusion procedure

The method of perfusing the isolated central ear artery of the rabbit was a modified version (Kalsner, to be published) of that of de La Lande and Rand (1965). Krebs bicarbonate solution at 37°C flowed at a constant pressure of 85 cm H_2O through the lumens of two isolated arterial segments. The Krebs-Henseleit (Krebs) solution used contained: NaCl, 115.3 mM; KCl, 4.6 mM; $CaCl_2$, 1.8 mM; $MgSO_4$, 1.1 mM; $NaHCO_3$, 22.1 mM; KH_2PO_4, 1.1 mM; glucose 7.8 mM; to which disodium EDTA (0.01 g/L) was added. The Krebs solution was bubbled with 95% O_2 and 5% CO_2 in a reservoir which was 85 cm above two double-jacketed organ baths. After leaving the reservoir, the Krebs solution passed through two parallel heat exchangers maintained at 37°C before entering the lumens of the arterial segments. The arterial segments were suspended in Krebs solution in organ baths maintained at 37°C. The perfusate was led out from the arterial segments by outflow cannulas into Andrews outflow recorders and the flow was recorded using piston recorders on kymographs.

The rabbits were anaesthetized with 7 ml/kg of 25% solution of urethane injected intraperitoneally. The hair was clipped from the skin over the base of the ear and along the course of the central artery. The skin of the ear was incised adjacent to the central artery and vein and dissected back to expose these structures. After identifying the central artery in the base of the ear, ligatures

were placed around it preparatory to cannulation. Each ear artery was cannulated at both ends with polyethylene tubing, PE 60, and the central artery was dissected free from the cartilagenous bed of the ear. The arterial segments were about 4-5 cm in length. The two isolated arteries were suspended separately in Krebs solution, in two organ baths. The double cannulation of the artery enabled drugs to be applied either intraluminally (to the intima) or extraluminally (to the adventitia). Absence of leakage between the intraluminal and extraluminal fluid compartments was routinely tested at the beginning of each experiment by carefully observing the level of the extraluminal fluid in the organ baths.

An equilibration period of 45 to 60 minutes with frequent changes of the bathing medium was allowed before the frequency-response and dose-response curves were determined. Drugs were applied intraluminally by injections into a rubber connection close to the perfusion cannula and the volume of drugs injected was between 0.05 to 0.2 ml. The volume of drugs added to the extraluminal fluid compartment of 30 ml capacity was between 0.15 to 0.6 ml. Drugs applied extraluminally were allowed to be in contact with the tissue for two minutes after which the chamber was flushed twice with Krebs. Four minute intervals were allowed between doses in the determination of responses to extra- or intraluminally applied drugs.

Nerve stimulation

Sympathetic nerves in the artery wall were stimulated periarterially (second method of de La Lande and Rand, 1965). A pair of

platinum electrodes were set up surrounding the proximal end of the segment of artery where the perfusion cannula lay within the artery. Stimulation was done with an S5 stimulator (Grass Instruments Inc.) by biphasic square wave pulses, usually 1 msec duration and the voltage of stimulation was adjusted to be supramaximal. Frequency-response curves of the isolated perfused arteries were then determined. The nerves were stimulated at each frequency (0.5, 1.0, 2.0, 5.0, 10.0 pulses/sec) for 2 minutes and intervals of 4 minutes were allowed between frequencies.

Vasoconstrictor responses to drugs and nerve stimulation were determined and calculated as inhibition of flow through the lumen of the perfused arterial segment in terms of percent of control flow.

Inhibition of monoamine oxidase (MAO) with iproniazid (IPN)

Iproniazid was added to the organ bath to give a final concentration of 2×10^{-4} g/ml. After 30 minutes, the inhibitor was washed out and an additional 30 minutes, with frequent washes, was allowed to elapse before the responses to tyramine was tested.

Blockade of alpha-receptors with phenoxybenzamine (POB)

Phenoxybenzamine was added to the organ bath to give a concentration of 5×10^{-8} g/ml. After 15 minutes, the phenoxybenzamine was washed out and this was followed by a 30 minute period of frequent washes before drugs were tested.

The following drugs were used:

1. (-) Artérenol bitartrate hydrate: Calbiochem, Los Angeles, California, U.S.A.

2. Cortisone acetate (saline suspension): Merck, Sharp and Dohme, Division of Merck and Co. Inc., West Point, Pa.
3. Combiotic (aqueous suspension of penicillin and dihydrostreptomycin sulphate): Pfizer Company Ltd., Agricultural Division, Montreal, Quebec, Canada.
4. Desoxycorticosterone acetate: Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.
5. Ethyl Carbamate (Urethane): The British Drug House Ltd., Poole, England.
6. Iproniazid phosphate: Hoffmann-La Roche Ltd., Montreal, Quebec, Canada.
7. Kynuramine dihydrobromide: Calbiochem, Los Angeles, California, U.S.A.
8. Pentobarbital sodium (sterile solution): Abbott Laboratories Limited, Montreal, Quebec, Canada.
9. Phenoxybenzamine hydrochloride: Smith Kline and French, Philadelphia, U.S.A.
10. Tyramine Hydrochloride: Calbiochem, Los Angeles, California, U.S.A.

Concentrations of l-noradrenaline bitartrate and tyramine HCl are expressed as the base. Iproniazid phosphate and phenoxybenzamine HCl are expressed as the salts. Noradrenaline, tyramine and iproniazid were dissolved in acid saline (0.9% NaCl in 0.01N HCl). Phenoxybenzamine was first dissolved in propylene glycol and then in acid saline.

Fluorometric determination of monoamine oxidase using kynuramine as substrate

Monoamine oxidase activity was determined according to modified methods of Weissbach et al. (1960) and Squires and Lassen (1968).

The rabbits were killed by a blow on the neck. The central ear arteries, the hearts and the hypothalamic region of the brain were quickly dissected out and chilled on crushed ice. The tissues were homogenized in 5 volumes of cold distilled water. The homogenate was centrifuged at 500 rpm for 15 minutes to remove cellular debris. Aliquots of the supernatant were then diluted 1:3 with 0.5 M phosphate buffer at pH 7.4.

200 µl aliquots of supernatant were then pre-incubated in a water bath at 37°C for 10 minutes. 200 µl of kynuramine dihydrobromide (50 µg/ml in distilled water) was then added, and the reaction mixture in duplicate tubes was incubated for various times; 5, 10, 15 and 20 minutes. The reaction was stopped by adding 1.0 ml of NaOH at the end of the various times.

It is known that monoamine oxidase oxidises kynuramine to an aldehyde which condenses to form 4-hydroxyquinoline. The 4-hydroxyquinoline fluoresces intensely in strong base (Kraml, 1965).

Monoamine oxidase activity was determined using Aminco Bowman spectrophotofluorometer to measure the fluorescence produced by 4-hydroxyquinoline at activating and fluorescing wavelengths of 318 mµ and 382 mµ respectively. Blanks were also prepared in the same way but the homogenates were heated before incubation to destroy the enzyme.

Determination of Electrolytes in Tissues

The sodium and potassium content of the ear artery, femoral artery, aorta, hypothalamus and the cerebral cortex of hypertensive, adrenalectomized and control rabbits was determined by flame photometry, using modified methods of Hald (1947) and Boling (1963).

Rabbits were killed by a blow on the neck. Ear arteries, femoral arteries and aortas were dissected free from all adhering connective and fatty tissues, blotted clean between filter paper and immediately weighed on a torsion balance. The brains were removed and the hypothalamus and cerebral cortex were located and dissected out. The tissues were then weighed and kept for ashing. Each tissue was put in a vitrous crucible and ashed in a furnace at about 600^{o}C. When the tissues were completely ashed the electrolytes of each sample were extracted with 2N HCl using a volume of the acid which depended on the wet weight of each tissue sample. For every 100 mg wet weight of the artery, 10 ml of the acid was used, and for every 100 mg wet weight of brain tissue, 3 ml of acid was used. The samples were then stored for the determination of sodium and potassium.

Sodium and potassium concentrations were determined on a Jarrell-Ash flame photometer (Model 830). Before determining sodium and potassium, each extracted sample was diluted with glass distilled water. In the case of the artery, the dilution was 1:10 and the brain the dilution was 1:100. The samples were then run through the burner of the Jarrell-Ash flame photometer and the readings were recorded. Working standard solutions containing various concentrations of sodium

and potassium (0.02, 0.04, 0.06, 0.08 and 0.1 mEq/L) were used for the construction of calibration curves which were prepared daily with each set of samples. The calibration curves were used for calculating the concentration of ions in the tissue samples.

Differences between means were compared according to student's 't' test (Steel and Torrie, 1960) and were considered statistically significant when $p \leq 0.05$.

IV. RESULTS

The time course of the development of DOCA-NaCl hypertension in rabbits is shown in Fig. 1.

Rabbits which were unilaterally nephrectomized, treated with DOCA and given a solution of 1% sodium chloride and 0.5% potassium chloride to drink showed a significant increase in systolic blood pressure as early as two weeks after the initiation of the treatment. The initial systolic blood pressure of these rabbits was 76.3±0.8 mm Hg and after two weeks of DOCA-NaCl treatment it had increased to a mean of 88.0±1.5 mm Hg ($p<0.01$). The blood pressure continued to rise and reached 102.3±1.2 mm Hg by the fifth week of treatment. In contrast, sham-operated rabbits had an initial mean systolic pressure of 77.3±0.8 mm Hg, which hardly increased by the fifth week to 79.5±0.9 mm Hg.

Seven rabbits on the DOCA-NaCl regimen were injected subcutaneously with noradrenaline in oil (1 mg as the salt per rabbit for 10 days) starting on the fourth week, at a time when the blood pressure was progressively rising. Chronic administration of noradrenaline to these animals did not augment the degree of hypertension. These rabbits had a mean initial systolic blood pressure of 76.0±0.1 mm Hg and at the end of the noradrenaline treatment it was 99.9±1.8 mm Hg, which did not differ significantly from that of DOCA-NaCl hypertensive animals which were not treated with noradrenaline.

The body weight of the DOCA-NaCl treated rabbits decreased significantly over the five week period from 2.4±0.1 Kg to 2.2±0.1 Kg ($p<0.05$), whereas that of the sham-operated rabbits increased from 2.4±0.3 Kg to 3.1±0.1 Kg ($p<0.01$).

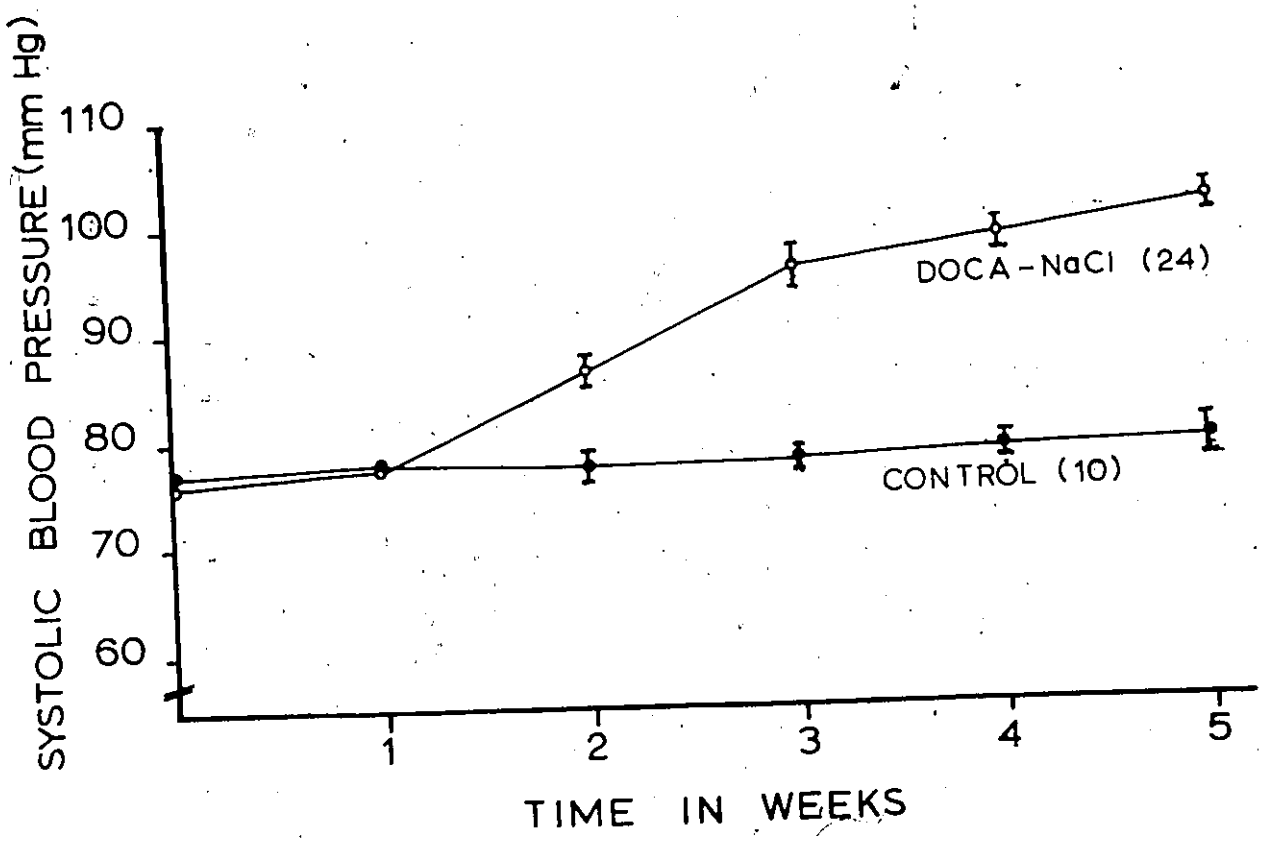


Fig. 1. Time-course of the development of DOCA-NaCl hypertension in rabbits. The systolic blood pressure was measured prior to and at weekly intervals following the initiation of treatment with DOCA-NaCl. The systolic blood pressure of control sham-operated rabbits (normotensive) is shown for comparison. Values in parentheses indicate the number of animals in each group and the vertical bars represent the S.E.M.

Effect of chronic sodium chloride intake on the blood pressure of nephrectomized rabbits

The preceding results demonstrated that unilaterally nephrectomized rabbits treated with DOCA and fed a solution of 1% NaCl and 0.5% KCl showed a significant increase in systolic blood pressure as early as two weeks after the beginning of the treatment. However, four unilaterally nephrectomized rabbits given a solution of 1% NaCl to drink but not treated with DOCA showed only a slight change in blood pressure by the second post-operative week. These animals had an initial systolic pressure of 69.0±2.4 mm Hg which increased slightly to 74.3±5.1 mm Hg at the end of two weeks on sodium chloride regimen. This treatment was undertaken in order to study the effect of sodium alone on the vascular reactivity of isolated ear arteries.

Electrolyte distribution

In order to correlate electrolyte metabolism in hypertensive animals with other parameters, the arterial sodium and potassium contents of normotensive and DOCA-NaCl hypertensive rabbits were studied. The sodium and potassium contents of the hypothalamus and the cerebral cortex were also determined for comparative purposes. Furthermore, the effect of sympathetic decentralization on sodium and potassium distribution in the ear artery of normotensive and DOCA-NaCl hypertensive rabbits was studied. The results are shown in Table 1A and B.

Ear artery

As shown in Fig. 2, the sodium and potassium contents of the

TABLE 1A

MEAN VALUES (±S.E.M.) OF TOTAL SODIUM AND POTASSIUM CONTENTS

OF ARTERIES FROM NORMOTENSIVE AND DOCA-NaCl HYPERTENSIVE RABBITS

	No. of Values	Tissue and Treatment	Sodium mEq/Kg Wet Weight	P Value	Potassium mEq/Kg Wet Weight	P Value
		1. EAR ARTERY				
(a)	6	Normotensive Control	38.3±2.5		6.2±0.6	
(b)	6	Normotensive Decentralized (5 weeks)	39.9±1.7		7.9±0.7	
(c)	7	DOCA-NaCl (5 weeks) Hypert.	81.9±3.6	<0.001	30.7±1.7	<0.001
(d)	7	DOCA-NaCl (5 weeks) Hypert.-Dec.	70.9±7.5	<0.01	14.5±2.2	<0.01
		2. AORTA				
(a)	8	Normotensive Control	27.2±1.3		11.0±2.3	
(b)	5	DOCA-NaCl (2 weeks) Hypert.	68.5±7.1	<0.001	17.2±2.2	
(c)	8	DOCA-NaCl (5 weeks) Hypert.	55.1±8.5	<0.01	13.3±2.7	
(d)	3	DOCA-NaCl (5 weeks) Hypert. (NA treated)	49.7±3.1	<0.001	9.7±1.0	
		3. FEMORAL ARTERY				
(a)	6	Normotensive Control	39.3±3.4		17.1±6.3	
(b)	5	DOCA-NaCl (2 weeks) Hypert.	91.0±6.6	<0.001	33.0±5.2	<0.05
(c)	9	DOCA-NaCl (5 weeks) Hypert.	66.2±5.8	<0.01	20.0±5.2	
(d)	3	DOCA-NaCl (5 weeks) Hypert. (NA treated)	88.7±2.1	<0.001	21.8±1.8	

S.E.M. = Standard Error of the Mean

P Values indicate significant differences between control and treated groups.

TABLE 1B

MEAN VALUES (±S.E.M.) OF TOTAL SODIUM AND POTASSIUM CONTENTS OF BRAIN TISSUES FROM NORMOTENSIVE AND DOCA-NaCl HYPERTENSIVE RABBITS

	No. of values	Tissue and Treatment	Sodium mEq/Kg Wet Weight	P Value	Potassium mEq/Kg Wet Weight	P Value
		1. HYPOTHALAMIC REGION				
(a)	7	Normotensive	45.2± 2.4		67.1±4.6	
(b)	6	DOCA-NaCl (5 weeks) Hypert.	68.5±11.8	<0.05	73.3±5.7	
		2. CEREBRAL CORTEX				
(a)	6	Normotensive	60.5±4.5		73.7±5.9	
(b)	6	DOCA-NaCl (5 weeks) Hypert.	59.8±3.5		48.0±6.7	<0.02

S.E.M. = Standard Error of the Mean

P Values indicate significant differences between control and hypertensive groups.

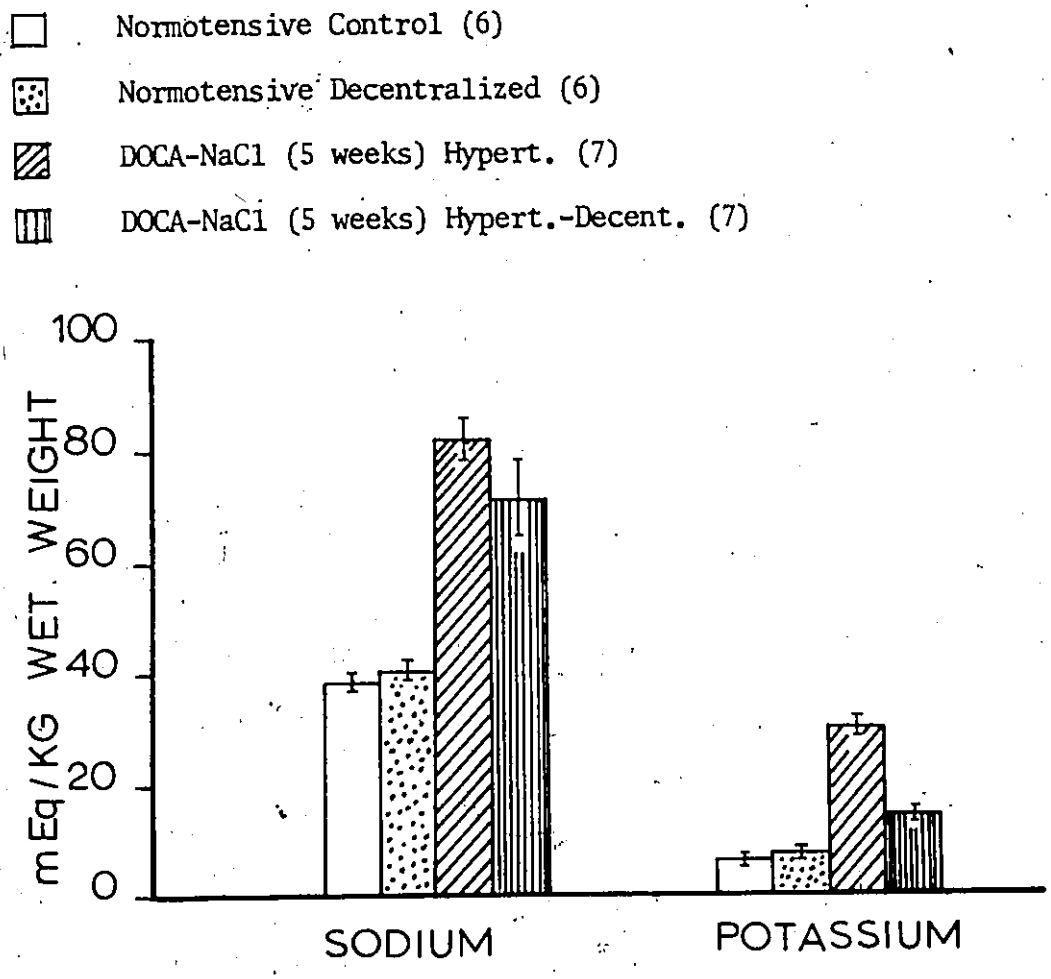


Fig. 2. Effects of DOCA-NaCl treatment and sympathetic decentralization on the electrolyte distribution in the central ear artery of rabbits. The figure shows sodium and potassium contents of control and decentralized arteries from normotensive, and from DOCA-NaCl (5 weeks) hypertensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits were significantly increased compared to those from normotensive rabbits ($p<0.001$). The sodium content of the decentralized arteries from normotensive and from DOCA-NaCl (5 weeks) hypertensive animals did not differ from that of their contralateral arteries with intact sympathetic innervation. It appears that sympathetic decentralization did not alter the sodium content of the ear arteries of DOCA-NaCl hypertensive or of normotensive animals.

The potassium content of control and decentralized arteries from normotensive rabbits did not differ significantly. In contrast, the potassium content of the decentralized arteries from DOCA-NaCl (5 weeks) hypertensive animals was significantly less than that of the contralateral arteries with an intact sympathetic innervation ($p<0.01$) but still significantly greater than that of normotensive control values ($p<0.01$).

Aorta

The sodium content of aortas from DOCA-NaCl (5 weeks) hypertensive rabbits was significantly higher than that of controls ($p<0.01$), whereas there was no change in aortic potassium content. These results are shown in Fig. 3 (left half of the graph).

Two weeks after the initiation of DOCA-NaCl treatment and when the systolic blood pressure had risen significantly above control values, the aortic sodium content was already significantly higher than that of controls ($p<0.001$). In fact, the aortic sodium content of DOCA-NaCl (2 weeks) hypertensive rabbits did not differ from that of DOCA-NaCl

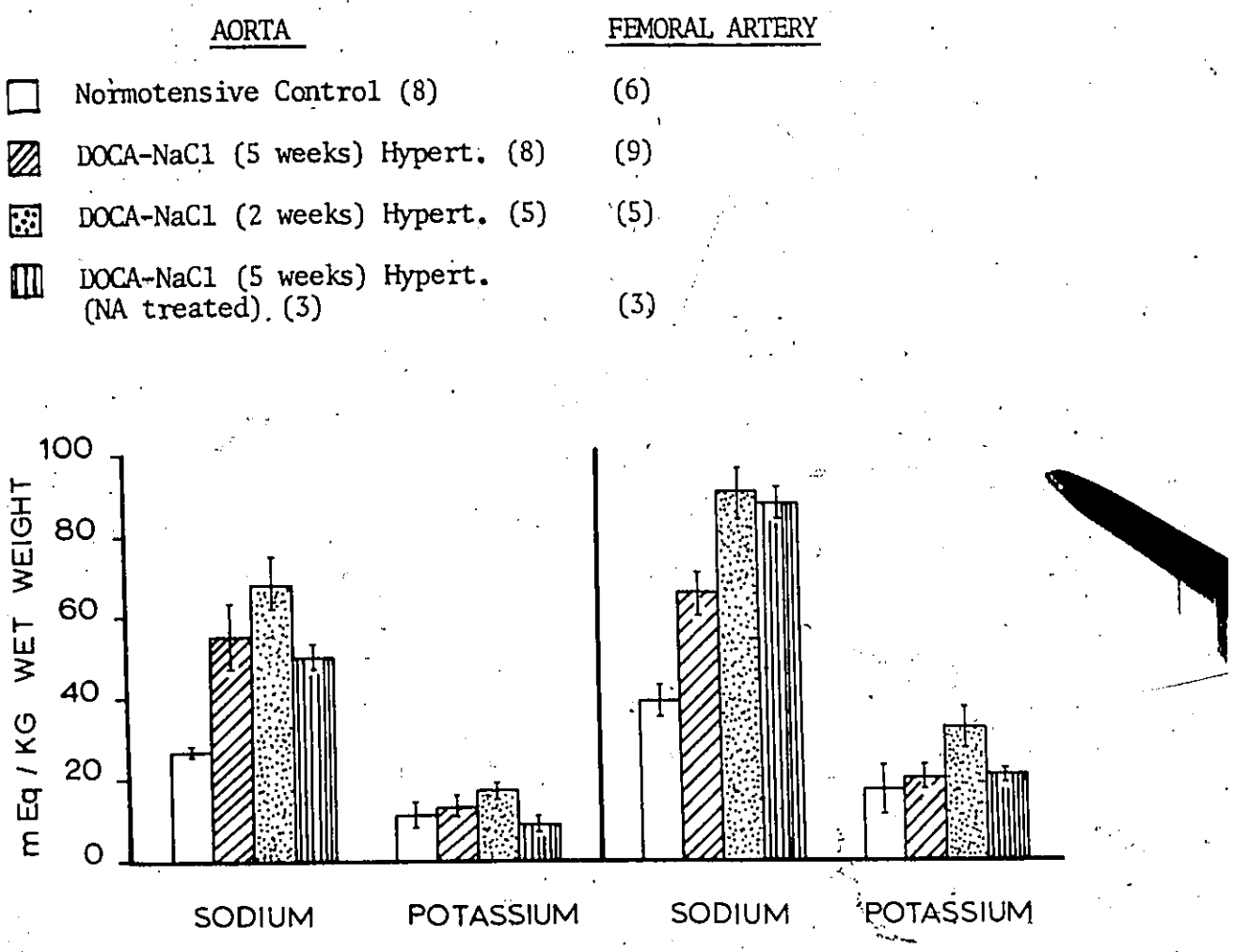


Fig. 3 Electrolyte distribution (sodium and potassium contents) in aorta (left half of graph) and femoral artery (right half of graph) in normotensive, DOCA-NaCl (2 weeks) hypertensive and DOCA-NaCl (5 weeks) hypertensive rabbits with or without noradrenaline treatment. The vertical bars represent S.E.M. and the numbers in parentheses denote the number of animals in each group.

(5 weeks) hypertensive animals. The potassium content of aortas from rabbits treated with DOCA-NaCl for 2 or 5 weeks was unchanged from control values.

Chronic administration of noradrenaline to animals treated with DOCA-NaCl (DOCA-NaCl (5 weeks) hypertensive) as described above did not alter the elevation of aortic sodium. Moreover, aortic potassium content was not influenced by treatment with noradrenaline.

Femoral Artery

Fig. 3 (right half of the graph) shows the sodium and potassium contents of femoral arteries from normotensive, DOCA-NaCl (2 weeks) and DOCA-NaCl (5 weeks) hypertensive animals and those from rabbits which received noradrenaline injections during the development of hypertension.

The sodium content of the femoral arteries from all three treated groups of rabbits was markedly increased compared to control femoral arteries ($p<0.01$). However, the sodium content of arteries from DOCA-NaCl (2 weeks) hypertensive and noradrenaline treated DOCA-NaCl (5 weeks) hypertensive animals was significantly higher than that of the arteries from DOCA-NaCl (5 weeks) hypertensive animals ($p<0.05$; $p<0.02$).

There was no change in the potassium content of the arteries from DOCA-NaCl (5 weeks) hypertensive rabbits with or without noradrenaline treatment. The femoral artery potassium content of DOCA-NaCl (2 weeks) hypertensives, however, was higher than that of controls.

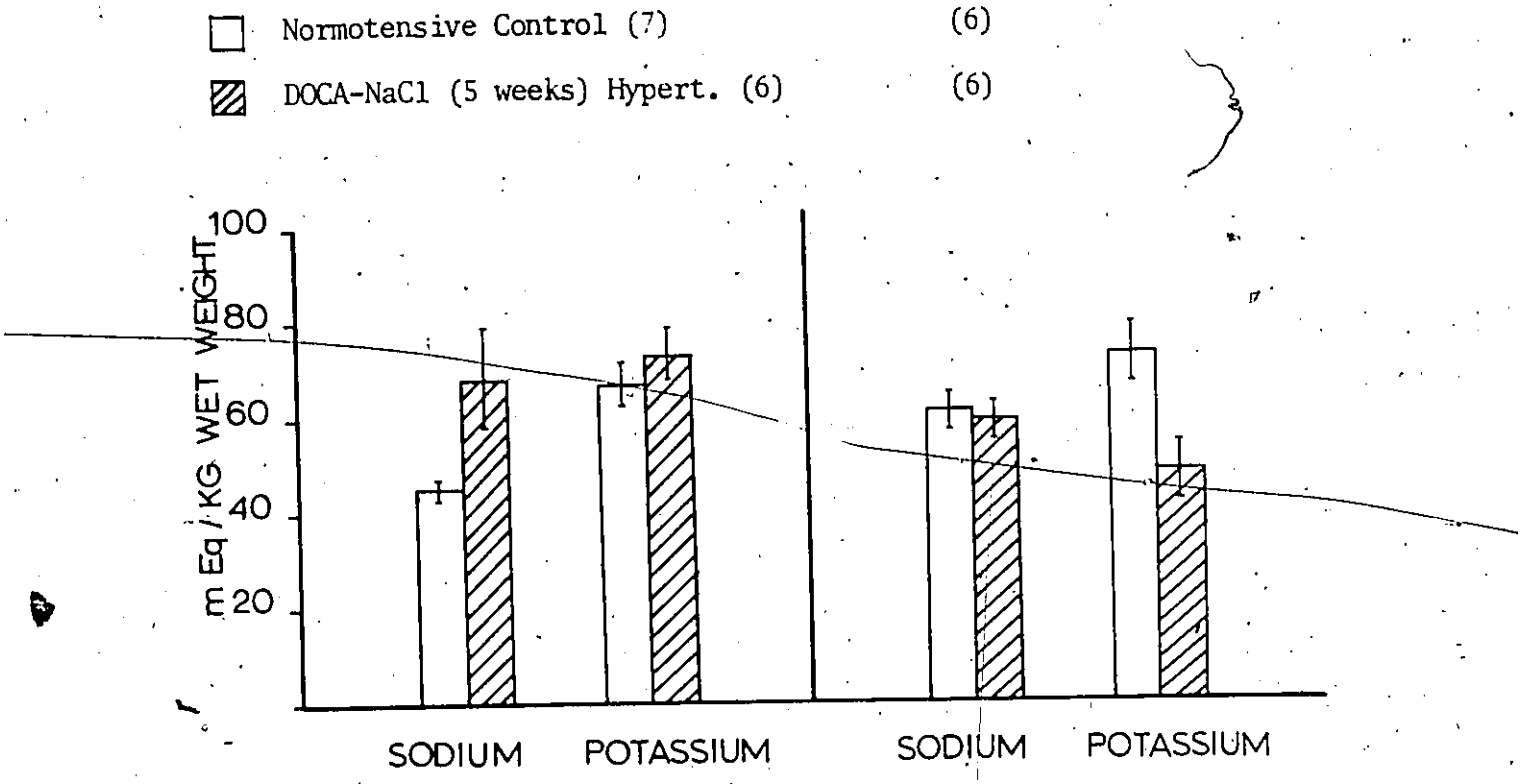


Fig. 4. Electrolyte distribution (sodium and potassium contents) in the hypothalamic region and cerebral cortex of normotensive and DOCA-NaCl (5 weeks) hypertensive rabbits. The vertical bars represent S.E.M. and the numbers in parentheses denote the number of animals in each group.

TABLE 2

MEAN VALUES (±S.E.M.) OF TOTAL SODIUM AND POTASSIUM CONTENTS OF TISSUES FROM NORMOTENSIVE AND RENAL HYPERTENSIVE RABBITS

	No. of Values	Tissue and Treatment	Sodium mEq/Kg Wet Weight	P Value	Potassium mEq/Kg Wet Weight	P Value
		1. AORTA				
(a)	8	Normotensive	27.2±1.3		11.0±2.3	
(b)	4	Renal hypertensive	35.6±3.3	<0.02	9.4±2.2	
		2. FEMORAL ARTERY				
(a)	6	Normotensive	39.3±3.9		17.1±6.3	
(b)	4	Renal hypertensive	56.5±8.1	<0.05	26.1±8.0	
		3. HYPOTHALAMIC REGION				
(a)	7	Normotensive	45.2±2.4		67.1±4.6	
(b)	4	Renal hypertensive	64.9±17.0		69.8±6.8	
		4. CEREBRAL CORTEX				
(a)	6	Normotensive	60.5±4.5		73.7±5.9	
(b)	4	Renal hypertensive	45.4±2.7	<0.05	59.6±4.6	

S.E.M. = Standard Error of the Mean

P Values indicate the significant differences between control and treated groups.

Hypothalamic region and cerebral cortex

The hypothalamic region of the brain of DOCA-NaCl (5 weeks) hypertensive animals contained a significantly greater amount of sodium than that of normotensive animals ($p<0.05$) Fig. 4 (left half of the graph). However, the potassium content was unchanged. The sodium content of the cerebral cortex from DOCA-NaCl (5 weeks) hypertensive rabbits did not differ from control values whereas the potassium content was significantly reduced ($p<0.02$) Fig. 4 (right half of the graph).

RENAL HYPERTENSION

Renal hypertension was produced in four rabbits. The initial systolic blood pressure was 77.0±1.2 mm Hg and it reached a mean of 97.0±1.2 mm Hg by the 6th week. In addition, the average body weight of the rabbits inncreased significantly from 2.4±0.1 kg to 3.1±0.1 kg ($p<0.01$) during the induction of the hypertension.

Electrolyte distribution

The sodium and potassium contents of the aorta, femoral artery, hypothalamus and cerebral cortex were studied in renal hypertensive rabbits and compared to those of normotensive animals. The data obtained are shown in Table 2.

There was a significant increase in the sodium content of arteries from renal hypertensive rabbits compared to those from normotensive animals ($p<0.05$ to <0.02). However, the potassium content of arteries from the renal hypertensives did not differ from those of the normotensive group.

The increase in the sodium content of the hypothalamus in renal hypertensive animals did not reach statistical significance and the potassium content was clearly unchanged. The sodium content of the cerebral cortex was significantly decreased whereas the potassium content was not significantly changed.

PERFUSION OF ISOLATED CENTRAL EAR ARTERY FROM THE RABBIT

Decentralization and Denervation

Nerve stimulation

Vasoconstrictor responses of the isolated perfused central ear artery to periarterial nerve stimulation were studied two and five weeks after pre-ganglionic section (decentralization) and two weeks after post-ganglionic section (denervation) of the superior cervical sympathetic innervation to the ear artery of the untreated rabbit. Contralateral arteries with an intact sympathetic innervation served as controls. Stimulation in all experiments was by square wave pulses usually of 1 msec duration and at supramaximal voltage. The magnitude of response to nerve stimulation was frequency dependent and complete cessation of flow was usually obtained at 5 pulses per second.

Decentralization

Fig. 5 shows the frequency-response curves of decentralized (2 weeks) and control arteries. The maximal response of the decentralized arteries to nerve stimulation (usually at 5.0 and 10.0 pulses/sec) was significantly less than that of the contralateral control

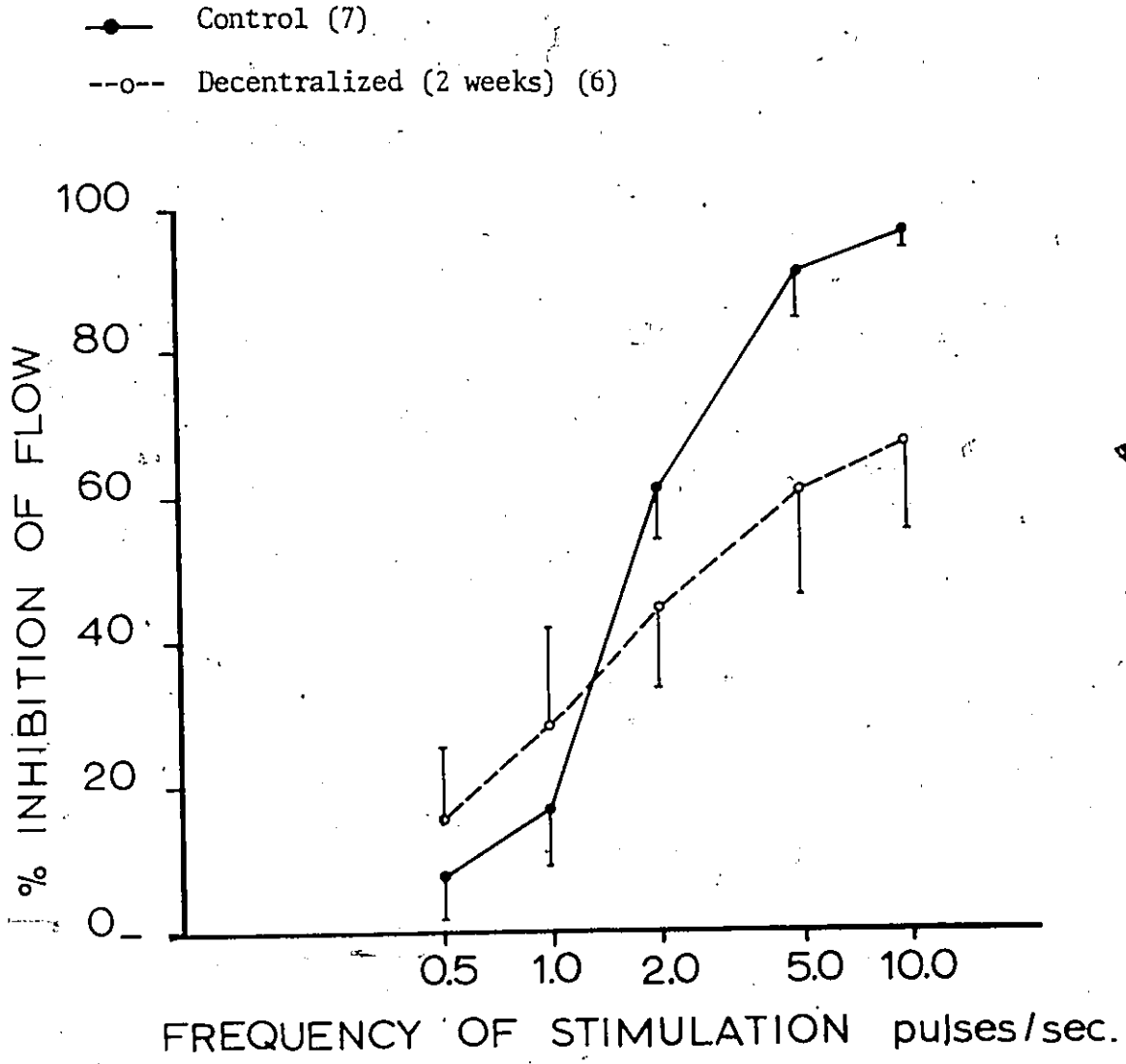


Fig. 5. Frequency-response curves to periarterial nerve stimulation of two weeks decentralized and control isolated central ear arteries from untreated rabbits. The vertical bars represent S.E.M. and the numbers in parentheses denote the number of arteries in each group.

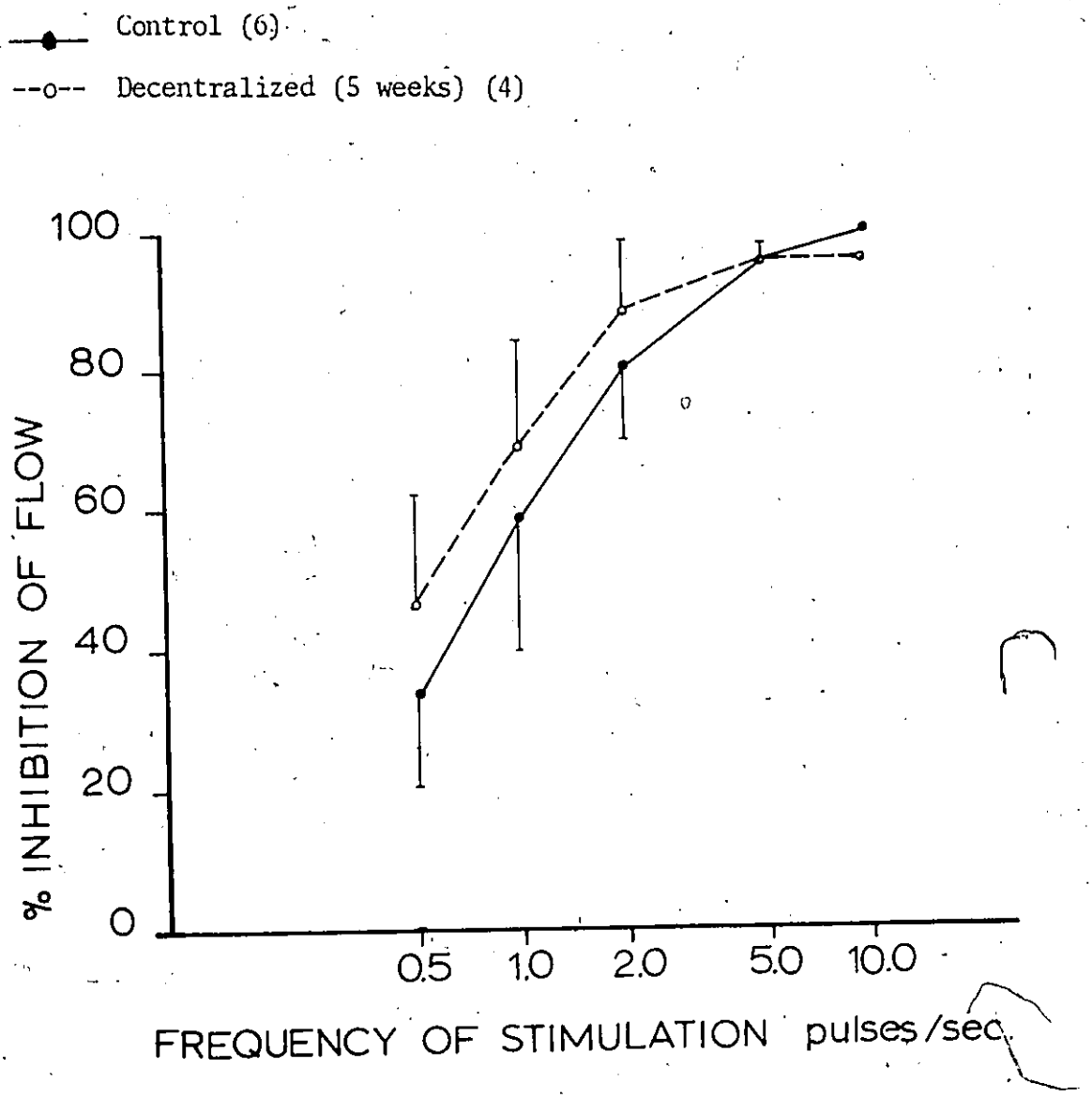


Fig. 6. Frequency-response curves to periarterial nerve stimulation of five weeks decentralized and control isolated central ear arteries from rabbits. The vertical bars represent S.E.M. and the numbers in parentheses denote the number of arteries in each group.

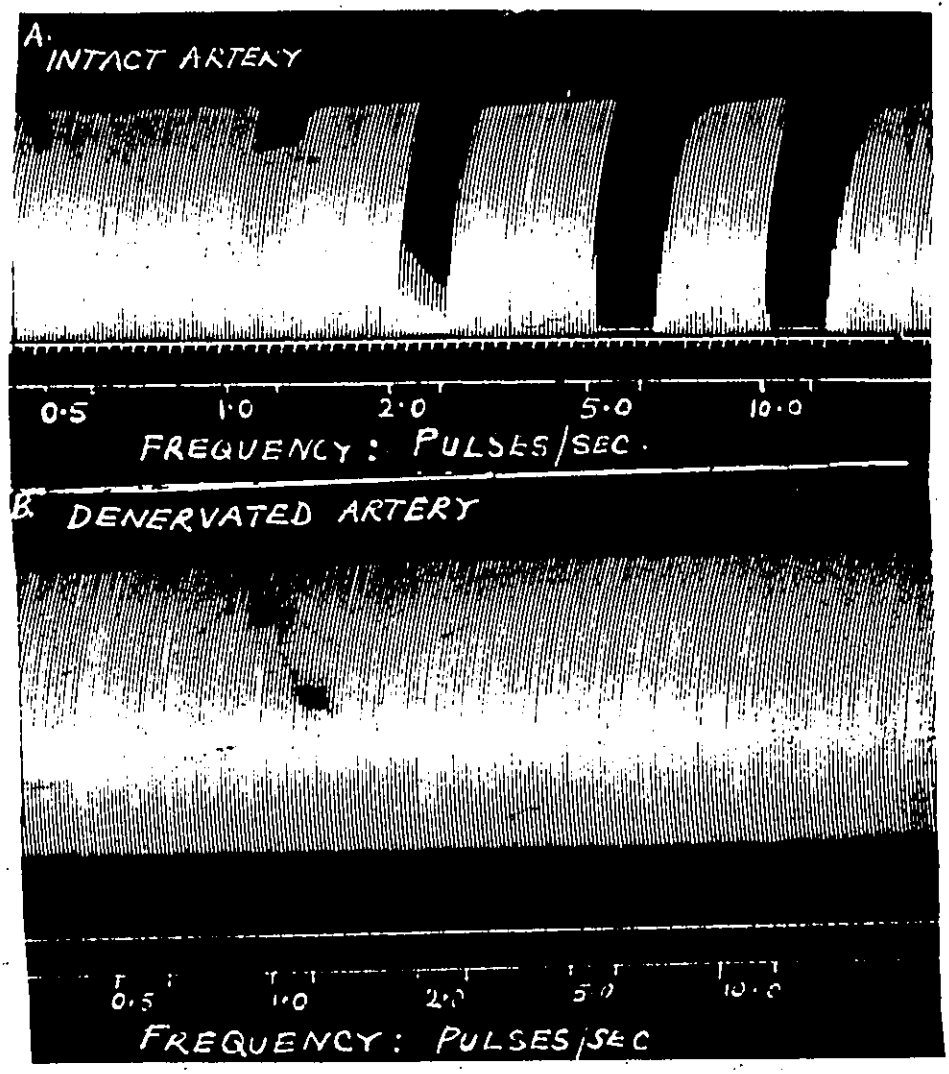


Fig. 7. Frequency-responses to periarterial nerve stimulation of control (A) and two weeks denervated (B) isolated central ear arteries from rabbits. Pulse duration was 1 msec and voltage was adjusted to be supramaximal. The frequencies of stimulation are indicated below each tracing. The kymograph tracings represent flow of fluid through isolated perfused arteries and are the deflections recorded by each writing lever attached to Andrews outflow recorder which measured flow. Each vertical stroke represents flow for a period of 8 seconds. Vasoconstrictor responses are recorded as inhibition of flow.

arteries ($p<0.01$). However, responses of the decentralized arteries at low frequencies, 0.5 to 2.0 pulses/sec did not differ from those of the control arteries. One control artery and two decentralized arteries did not respond to nerve stimulation at any test frequency.

In another set of experiments, the duration following decentralization was prolonged to five weeks and responses to nerve stimulation were again studied using the contralateral arteries as controls. The frequency-response curves are shown in Fig. 6.

Stimulation of the decentralized arteries (5 weeks) produced responses which were similar to those of the control arteries at all frequencies. Two out of six decentralized arteries did not respond to nerve stimulation at any frequency.

Denervation

Fig. 7 shows the typical effect of denervation of two weeks duration on-responses of an isolated perfused artery to nerve stimulation. Responses to periarterial nerve stimulation were abolished in a total of seven denervated arteries studied, but the contralateral control arteries gave typical responses.

Responses to noradrenaline

Effects of sympathetic decentralization and denervation on responses of isolated perfused arteries to intraluminal and extraluminal noradrenaline were also investigated.

Decentralization

Figs. 8A and B show the dose-response curves of decentralized

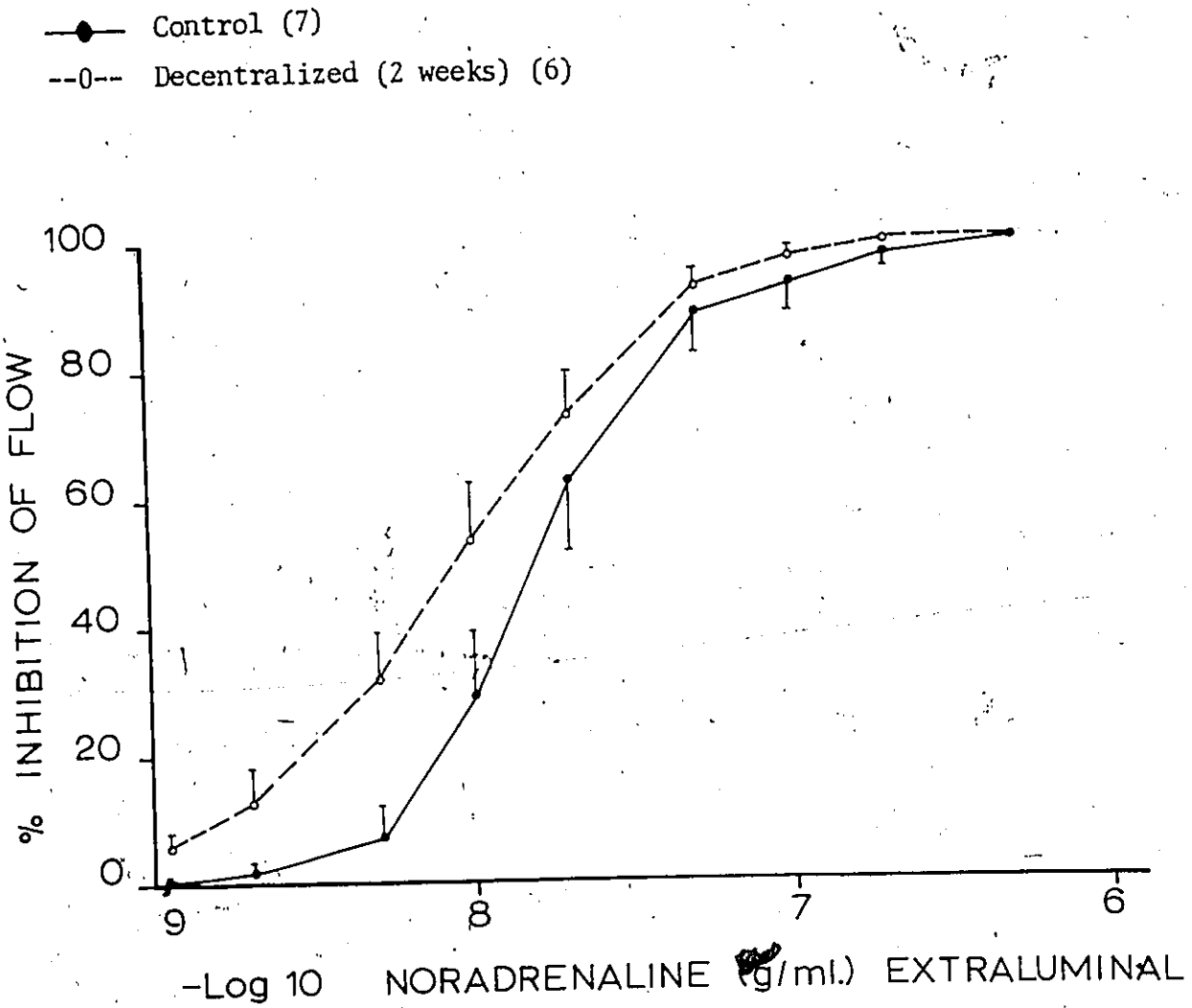


Fig. 8A. Dose-response curves of two weeks decentralized and control isolated ear arteries from untreated rabbits to extraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

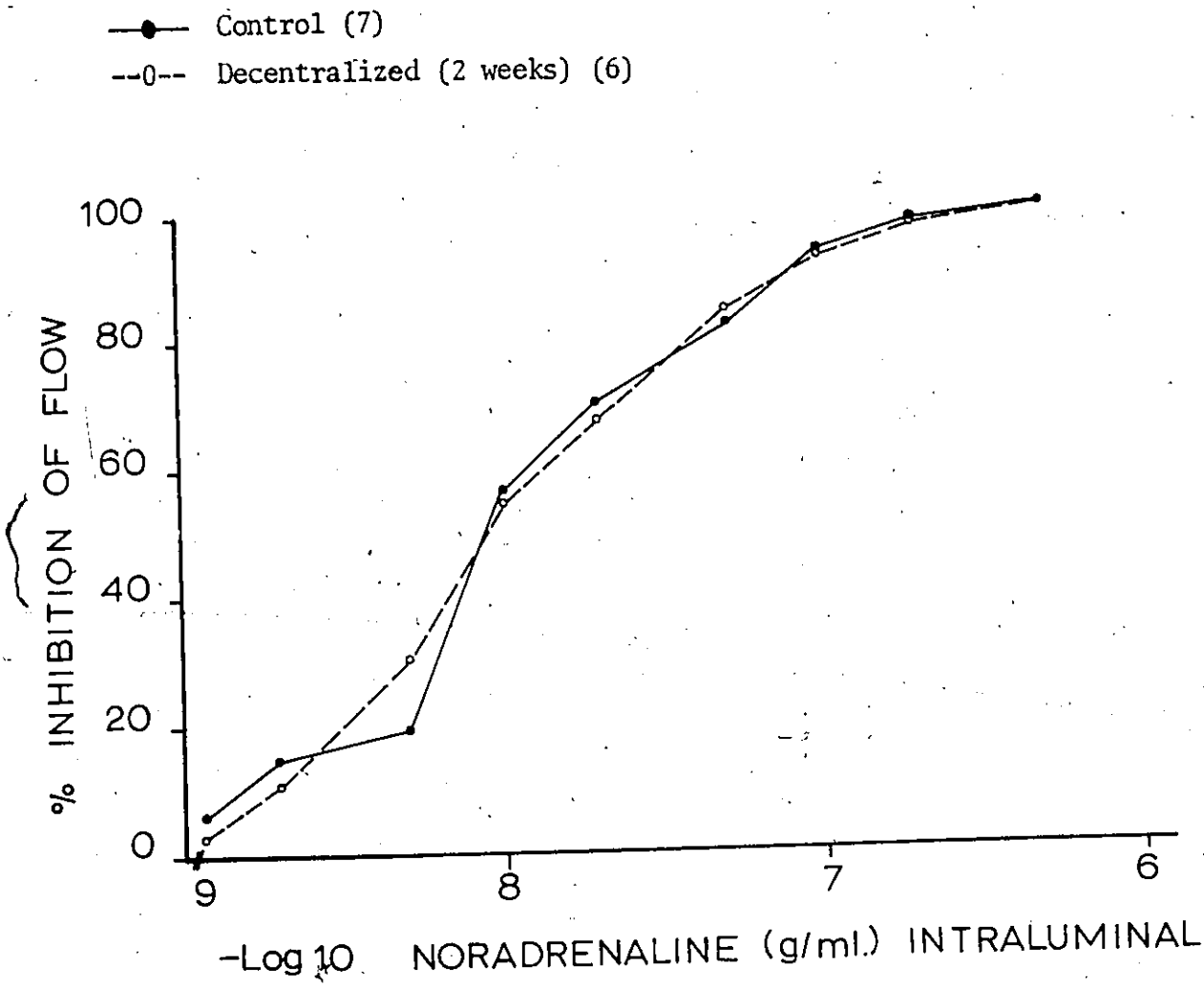


Fig. 8B. Dose-response curves of two weeks decentralized and control isolated ear arteries from untreated rabbits to intraluminal noradrenaline. The numbers in parentheses denote the number of arteries in each group.

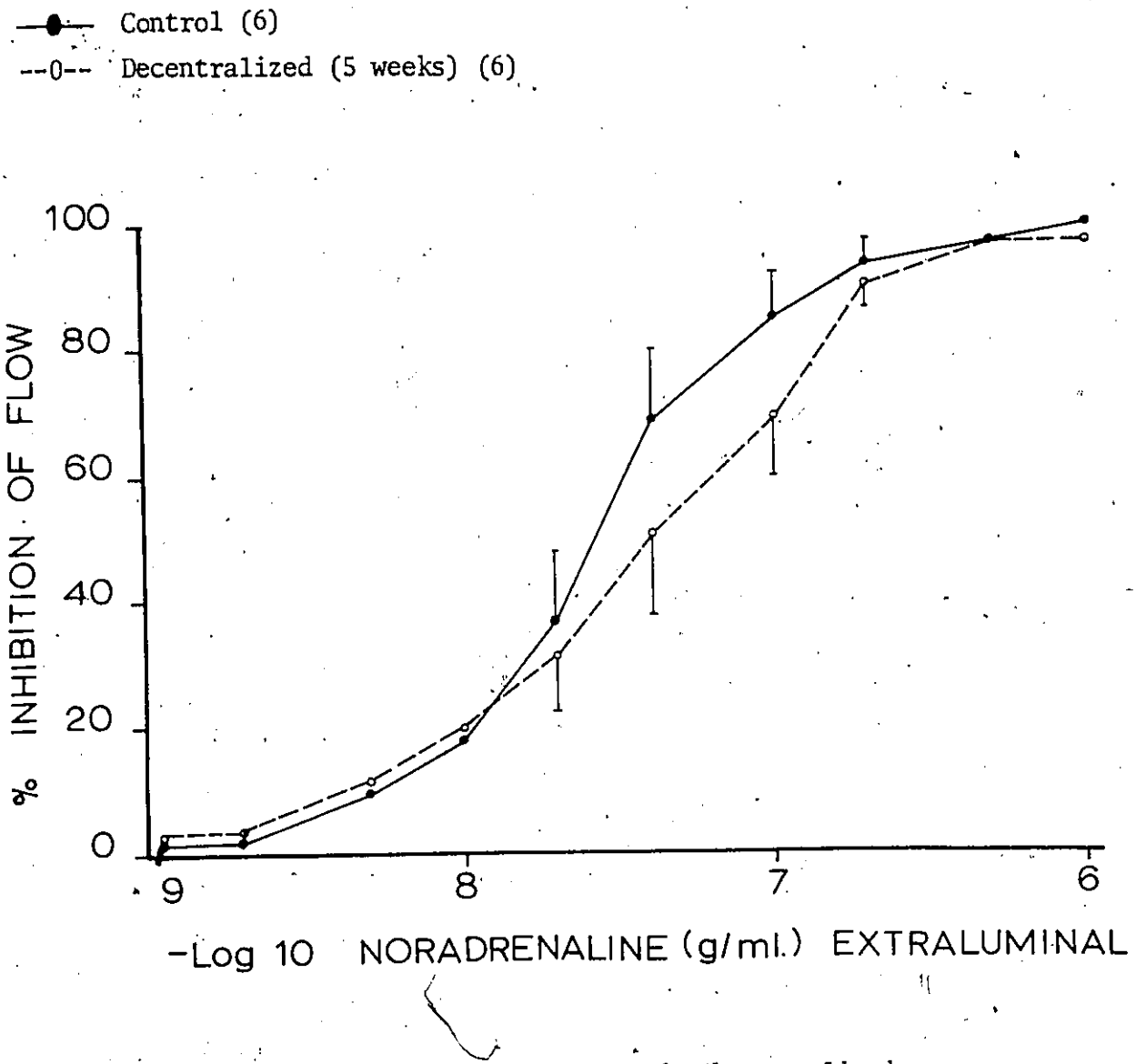


Fig. 9A. Dose-response curves of five weeks decentralized and control isolated ear arteries from untreated rabbits to extraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

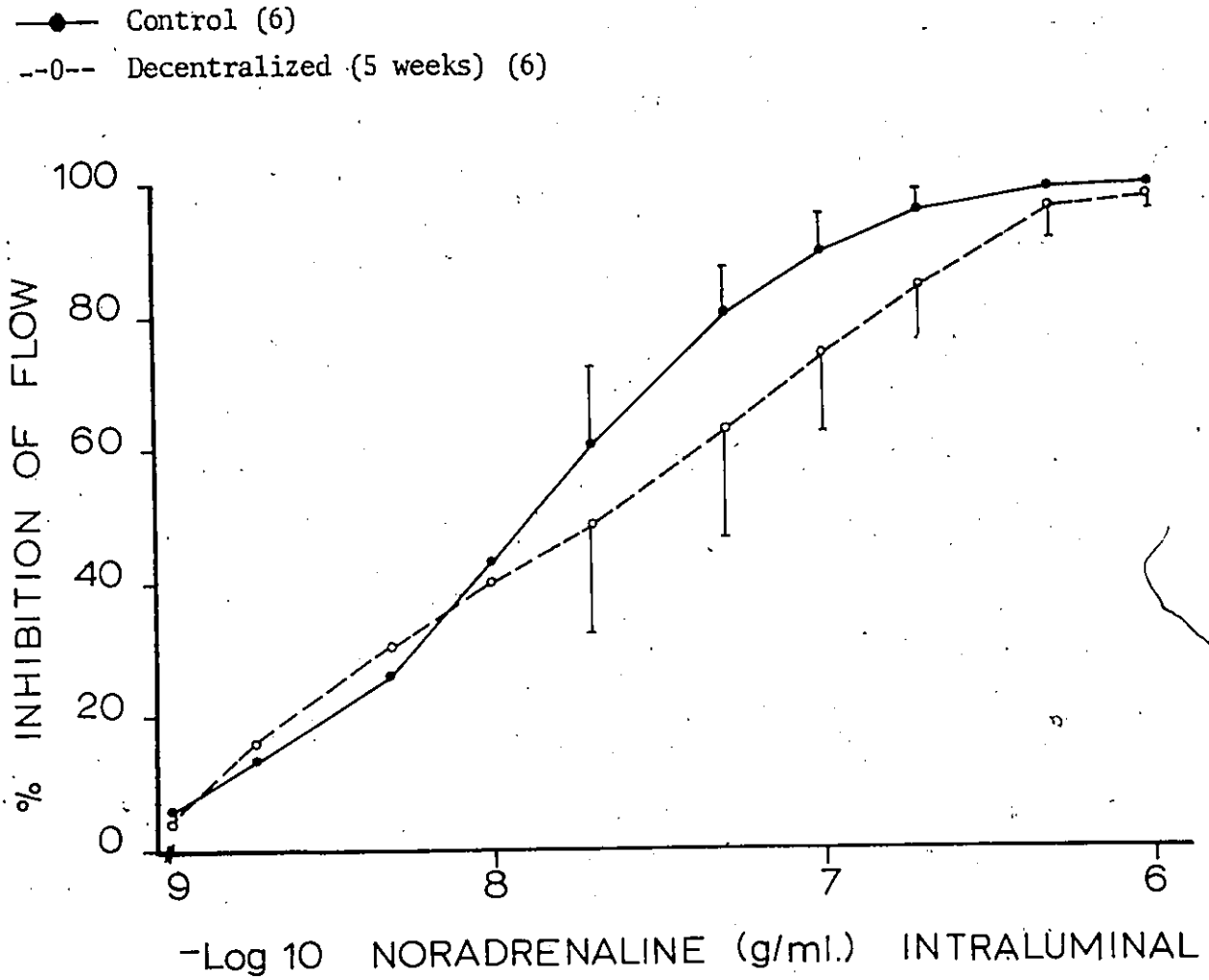


Fig. 9B. Dose-response curves of five weeks decentralized and control isolated ear arteries from untreated rabbits to intraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

(2 weeks) and control arteries from untreated rabbits to extraluminal and intraluminal noradrenaline.

Two weeks after decentralization, the arteries showed a significant increase in sensitivity of about 2-3 fold to low concentrations, 1×10^{-9} to 1×10^{-8} g/ml, of extraluminal noradrenaline ($p < 0.05$), Fig. 8A. However, the responses of the decentralized arteries to high concentrations (5×10^{-8} to 5×10^{-7} g/ml) did not differ from those of controls. The decentralized and the control arteries did not differ in their responses to intraluminal noradrenaline at any concentration tested, Fig. 8B.

Figs. 9A and B show experiments in which the interval following decentralization was prolonged to five weeks in order to study the effect of a long period of decentralization on vascular responses to noradrenaline.

Although the responses of the decentralized arteries (5 weeks) to both extraluminal and intraluminal noradrenaline did not differ significantly from those of the control arteries, a tendency for the decentralized arteries to be less sensitive to extraluminal and intraluminal noradrenaline was observed at concentrations of 2×10^{-8} to 2×10^{-7} g/ml.

Denervation

Figs. 10A and B show the vascular responses of the isolated perfused ear arteries to extraluminal and intraluminal noradrenaline two weeks after denervation compared to the contralateral control

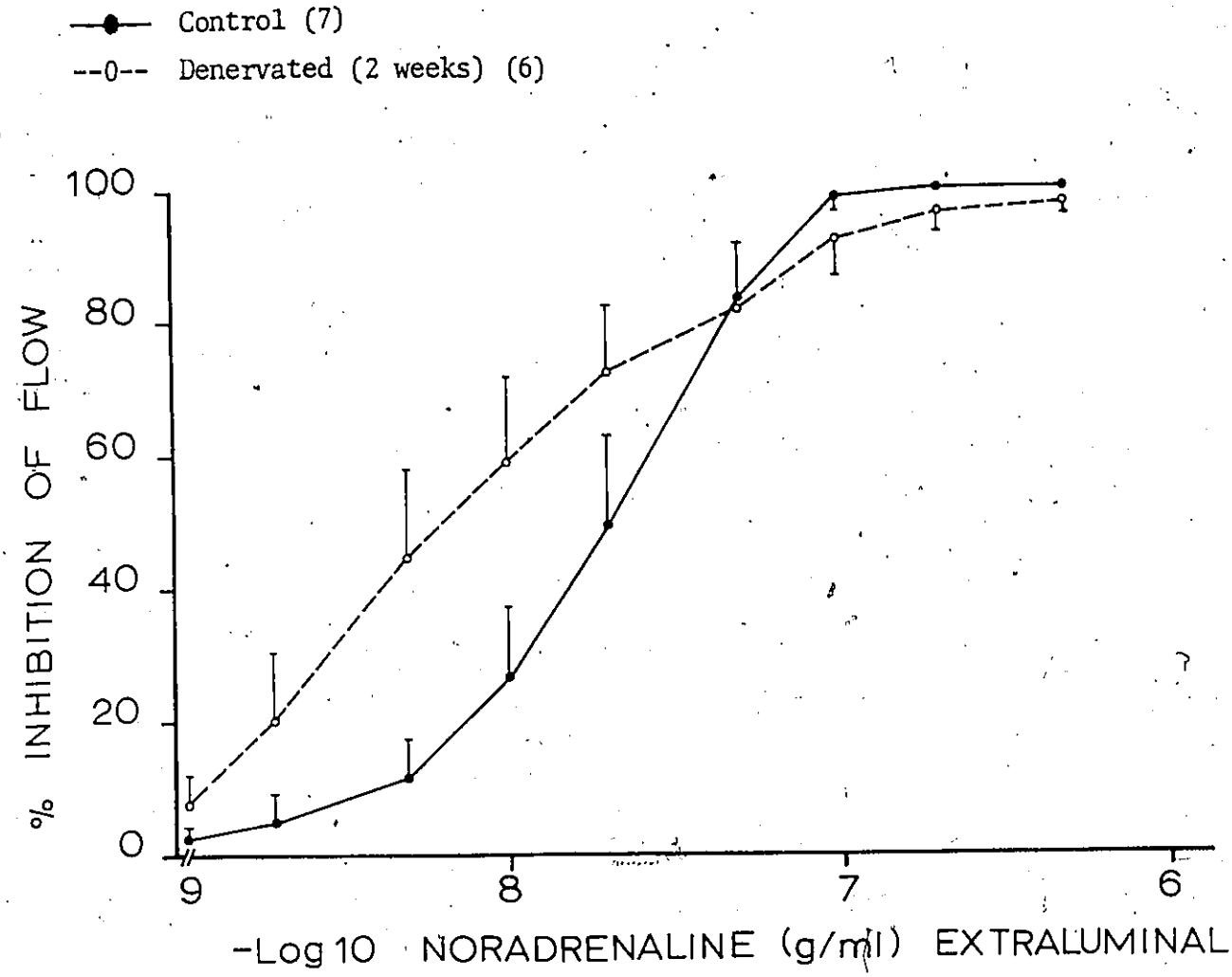


Fig. 10A. Dose-response curves of two weeks denervated and control ear arteries from untreated rabbits to extraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

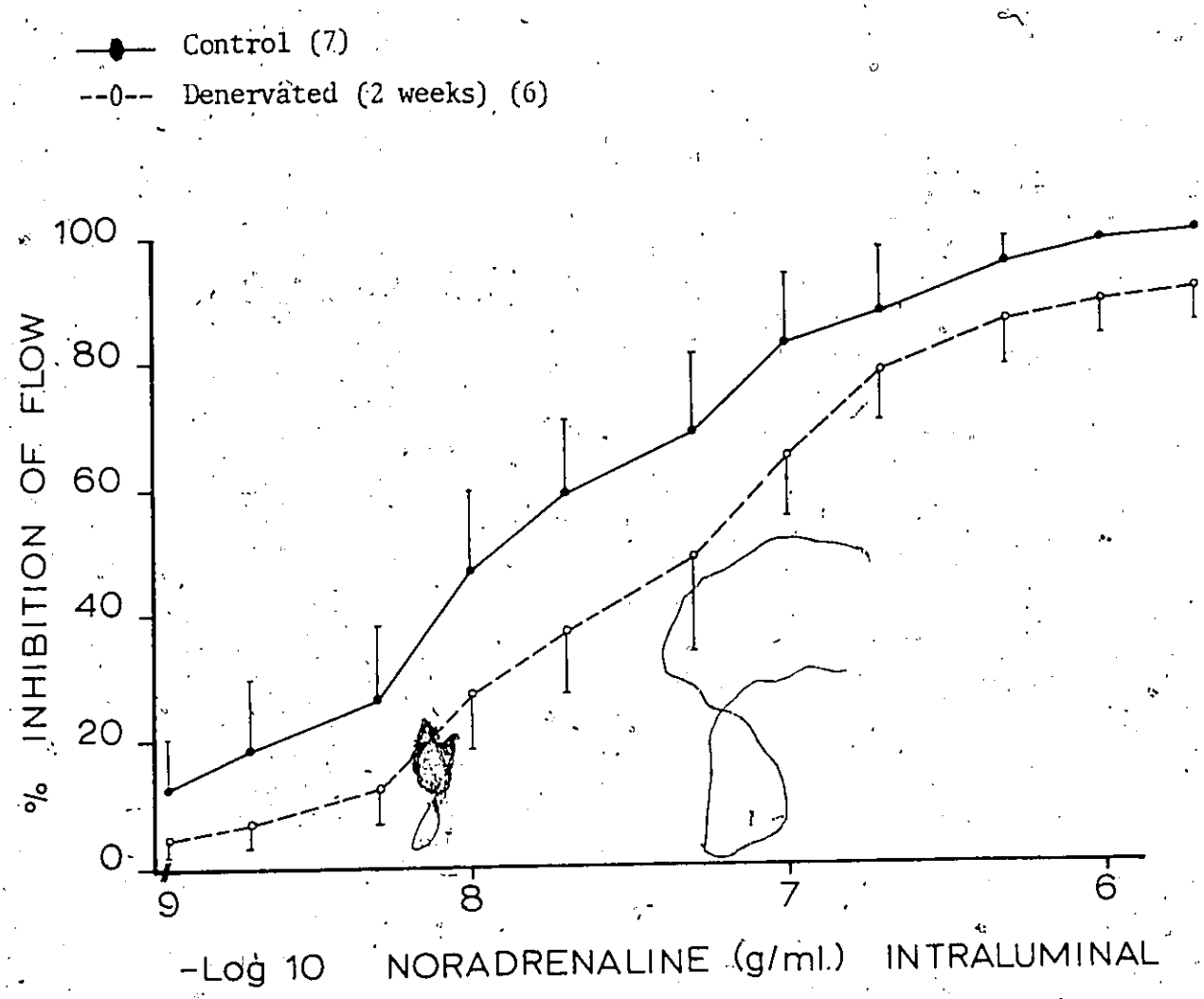


Fig. 10B. Dose-response curves of two weeks denervated and control ear arteries from untreated rabbits to intraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

arteries. The denervated arteries exhibited a significant increase in sensitivity of about 4-5 fold to low concentrations of extraluminal noradrenaline (5×10^{-9} and 1×10^{-8} g/ml; $p<0.05$) Fig. 10A. Responses to high concentrations (5×10^{-8} to 5×10^{-7} g/ml) were, however, similar in denervated and control arteries. The denervated arteries were less sensitive to intraluminal noradrenaline at all concentrations tested compared to controls Fig. 10B. However, these differences were not statistically significant.

RESPONSES OF EAR ARTERIES FROM ANIMALS TREATED CHRONICALLY WITH EXOGENOUS NORADRENALINE

Rabbits were treated with exogenous noradrenaline in order to study the effect of chronic administration of this amine on the reactivity of the ear artery deprived of the tonic release of endogenous noradrenaline by decentralization for 5 weeks. The rabbits were injected subcutaneously with 1 mg noradrenaline in oil (daily for 10 days) starting on the fourth week following decentralization. At the end of the treatment the blood pressure of the rabbits 81.3±2.0 mm Hg, did not differ from that before the treatment, 76.8±1.2 mm Hg. The contralateral intact ear arteries of these rabbits were used as controls.

Nerve stimulation

The frequency-response curves of control and decentralized (5 weeks) arteries from rabbits with and without noradrenaline pretreatment are shown in Fig. 11.

Pretreatment of rabbits with noradrenaline resulted in decreased responses of arteries with intact sympathetic innervation

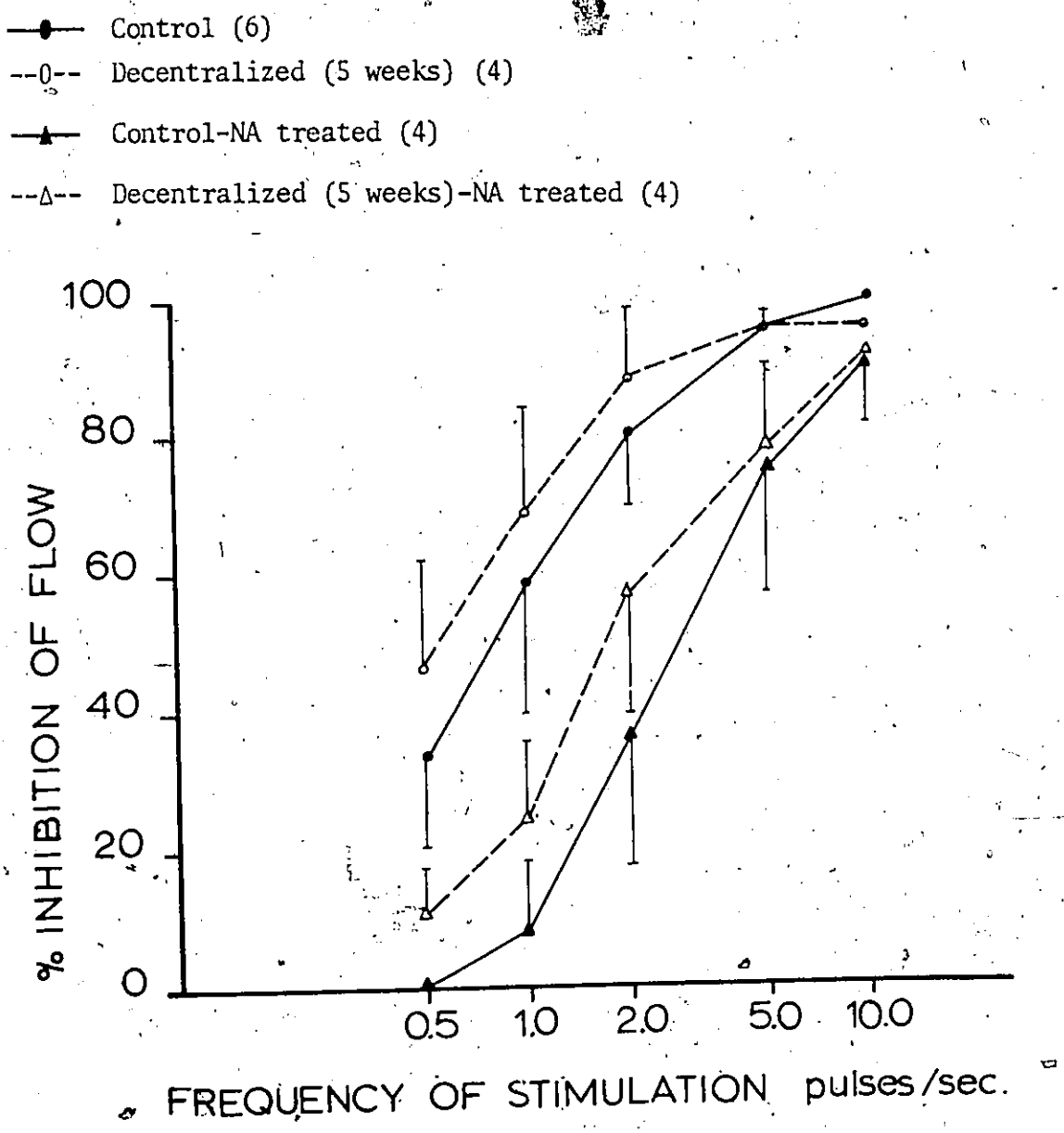


Fig. 11. Effects of the chronic administration of noradrenaline on the responses to periarterial nerve stimulation of control and decentralized isolated perfused ear arteries from rabbits. Rabbits were treated with 1 mg noradrenaline subcutaneously daily for 10 days. The figure shows the frequency-response curves of control and decentralized (5 weeks) arteries from rabbits with and without noradrenaline treatment. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

(control) to low frequency stimulation 0.5 and 1.0 pulse/sec) compared to those from untreated rabbits ($p<0.05$). Decentralized (5 weeks) arteries from treated animals also gave smaller responses to low frequency stimulation (0.5 and 1.0 pulse/sec) than did decentralized vessels from untreated animals ($p<0.05$). The responses of decentralized arteries did not differ significantly from those of their contralateral control arteries in either group of rabbits.

Responses to noradrenaline

The effects of chronic noradrenaline pretreatment on the responses of decentralized (5 weeks) and control arteries to noradrenaline are shown in Figs. 12A and B.

Following pretreatment of rabbits with noradrenaline, control arteries exhibited decreased responses to extraluminal noradrenaline, especially at concentrations of 1×10^{-8} to 5×10^{-8} g/ml ($p<0.05$) Fig. 12A. Decentralized arteries from noradrenaline treated animals were less responsive than those from untreated animals to extraluminal noradrenaline at concentrations of 1×10^{-8} to 5×10^{-8} g/ml but these differences were not statistically significant. There were no significant differences between the responses to extraluminal noradrenaline of control arteries and their contralateral decentralized arteries in either group.

Responses of control and decentralized arteries from treated animals to intraluminal noradrenaline did not differ from those of untreated rabbits, Fig. 12B. As mentioned previously, decentralized arteries from untreated rabbits showed a tendency to be less respon-

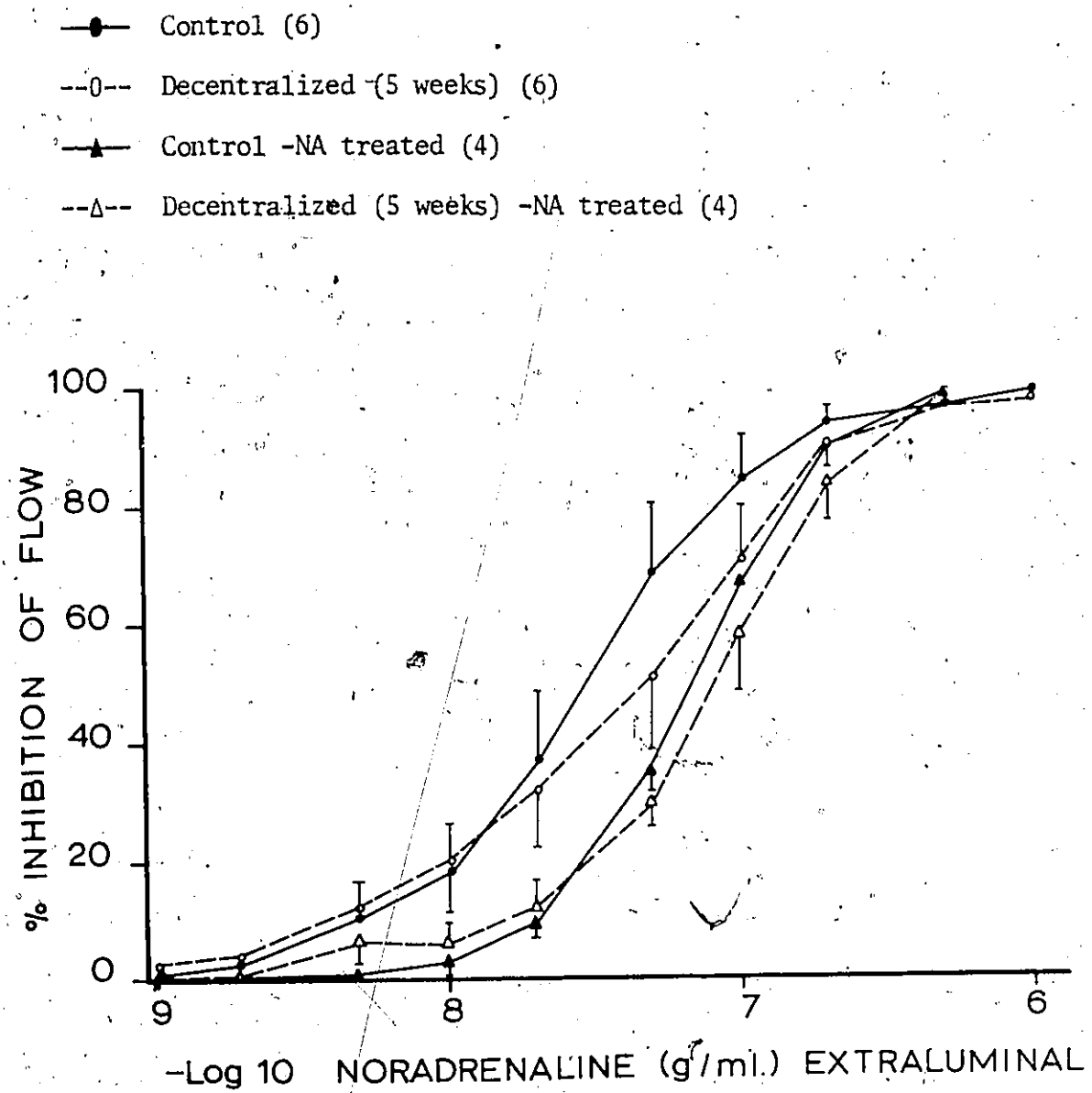


Fig. 12A. Effects of the chronic administration of noradrenaline on responses to noradrenaline of control and decentralized isolated perfused ear arteries. The figure shows the dose-response curves of control and decentralized arteries from rabbits with and without noradrenaline treatment to extraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

—●— Control (6)

--○-- Decentralized (5 weeks) (6)

—▲— Control -NA treated (4)

--△-- Decentralized (5 weeks) -NA treated (4)

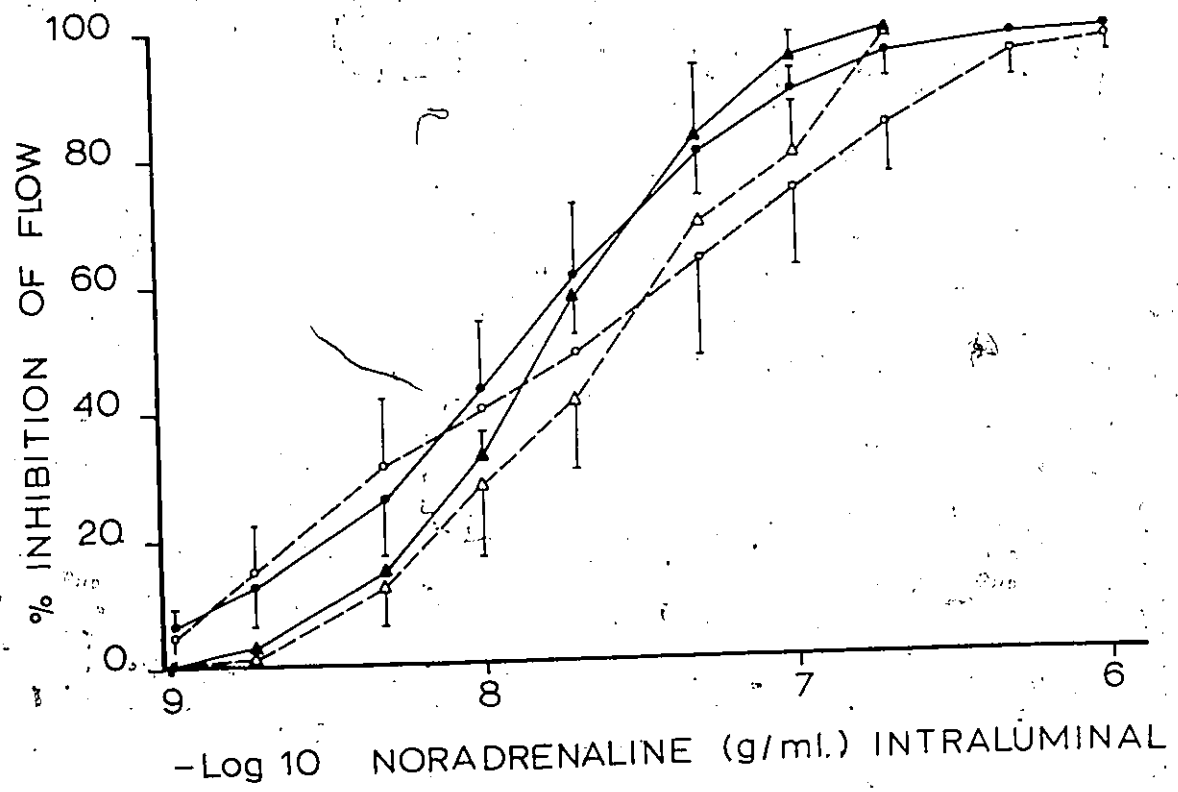


Fig. 12B. Effects of the chronic administration of noradrenaline on responses to noradrenaline of control and decentralized isolated perfused ear arteries. The figure shows the dose-response curves of control and decentralized arteries from rabbits with and without noradrenaline treatment to intraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

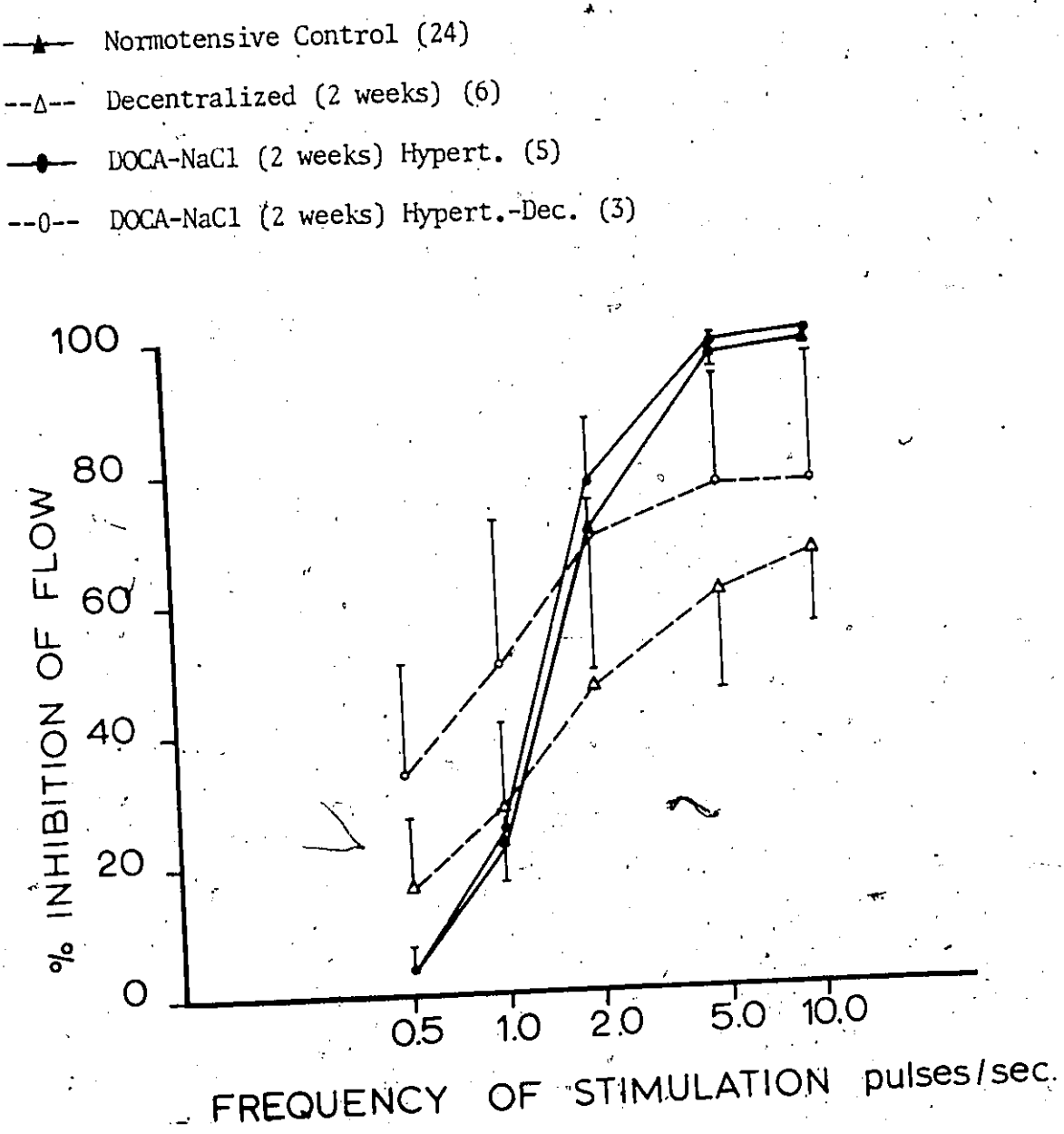


Fig. 13. Frequency-response curves to perivarterial nerve stimulation of control and decentralized ear arteries from DOCA-NaCl (2 weeks) hypertensive rabbits compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

sive to intraluminal noradrenaline as compared to contralateral arteries with an intact sympathetic innervation and a similar tendency was observed after pretreatment of rabbits with noradrenaline.

DOCA-NaCl Hypertension

Vasoconstrictor responses of isolated perfused arteries from DOCA-NaCl hypertensive rabbits to periarterial sympathetic nerve stimulation and to noradrenaline were studied. The objective of these experiments was to ascertain the condition of sympathetic transmission and the reactivity of vascular tissue from DOCA-NaCl hypertensive animals in the presence and absence of an intact sympathetic innervation.

Nerve Stimulation

Nerve stimulation was performed at 1 msec duration and at supramaximal voltage. Frequency-response curves were determined in control and decentralized arteries from hypertensive rabbits treated for two weeks and five weeks with DOCA-NaCl. Fig. 13 shows the frequency-response curves of control and decentralized arteries from DOCA-NaCl (2 weeks) hypertensive animals compared to those from normotensive rabbits. Responses of control arteries from DOCA-NaCl (2 weeks) hypertensives did not differ from those from normotensive rabbits. Decentralized arteries from the hypertensive group showed a tendency to be more responsive to low frequency stimulation (0.5 and 1.0 pulse / sec) and less responsive to high frequency stimulation than their contralateral controls but these differences were not statistically significant, probably because of the small number of arteries tested.

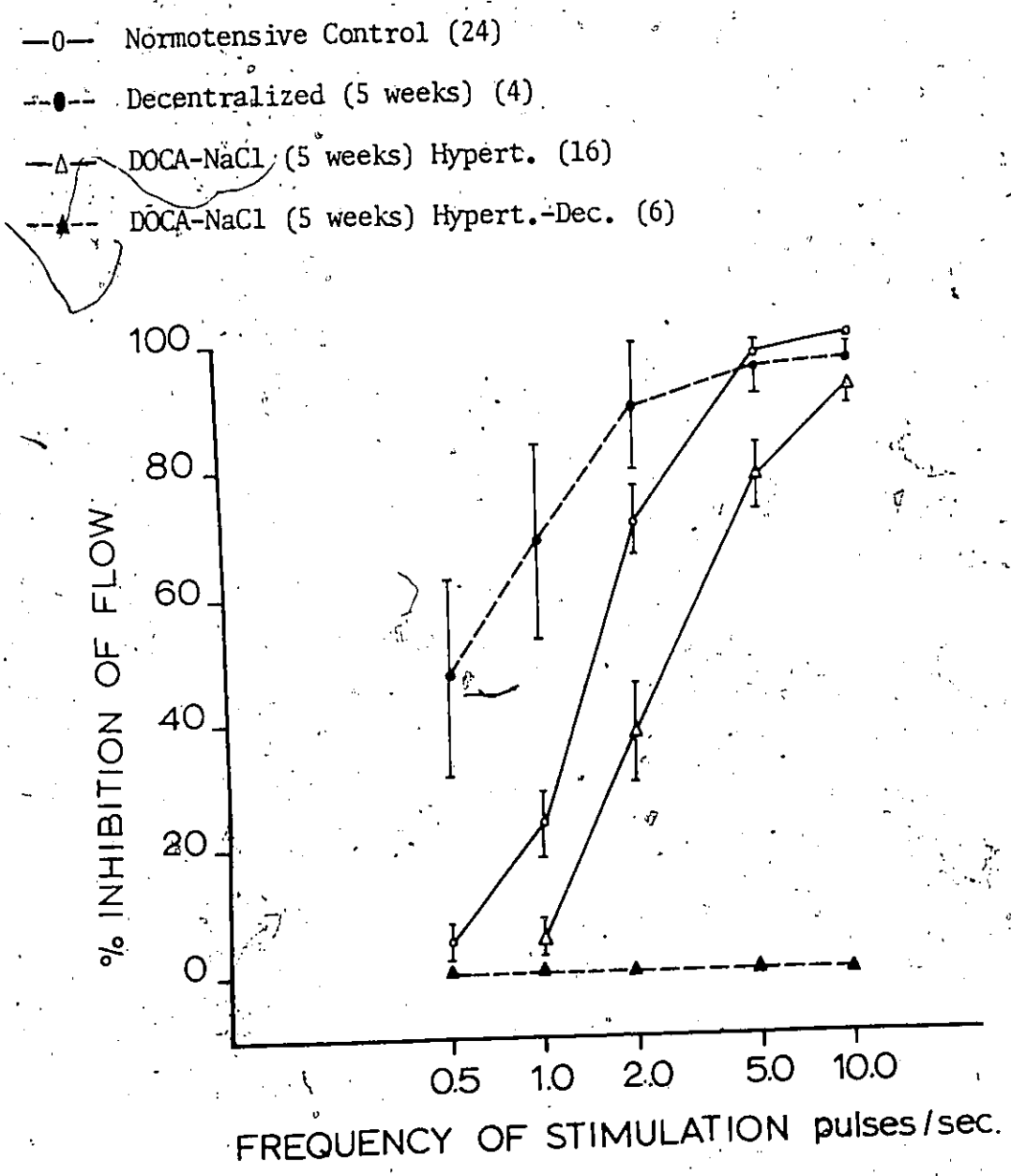


Fig. 14A. Frequency-response curves to periarterial nerve stimulation of control and decentralized ear arteries from normotensive and from DOCA-NaCl (5 weeks) hypertensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

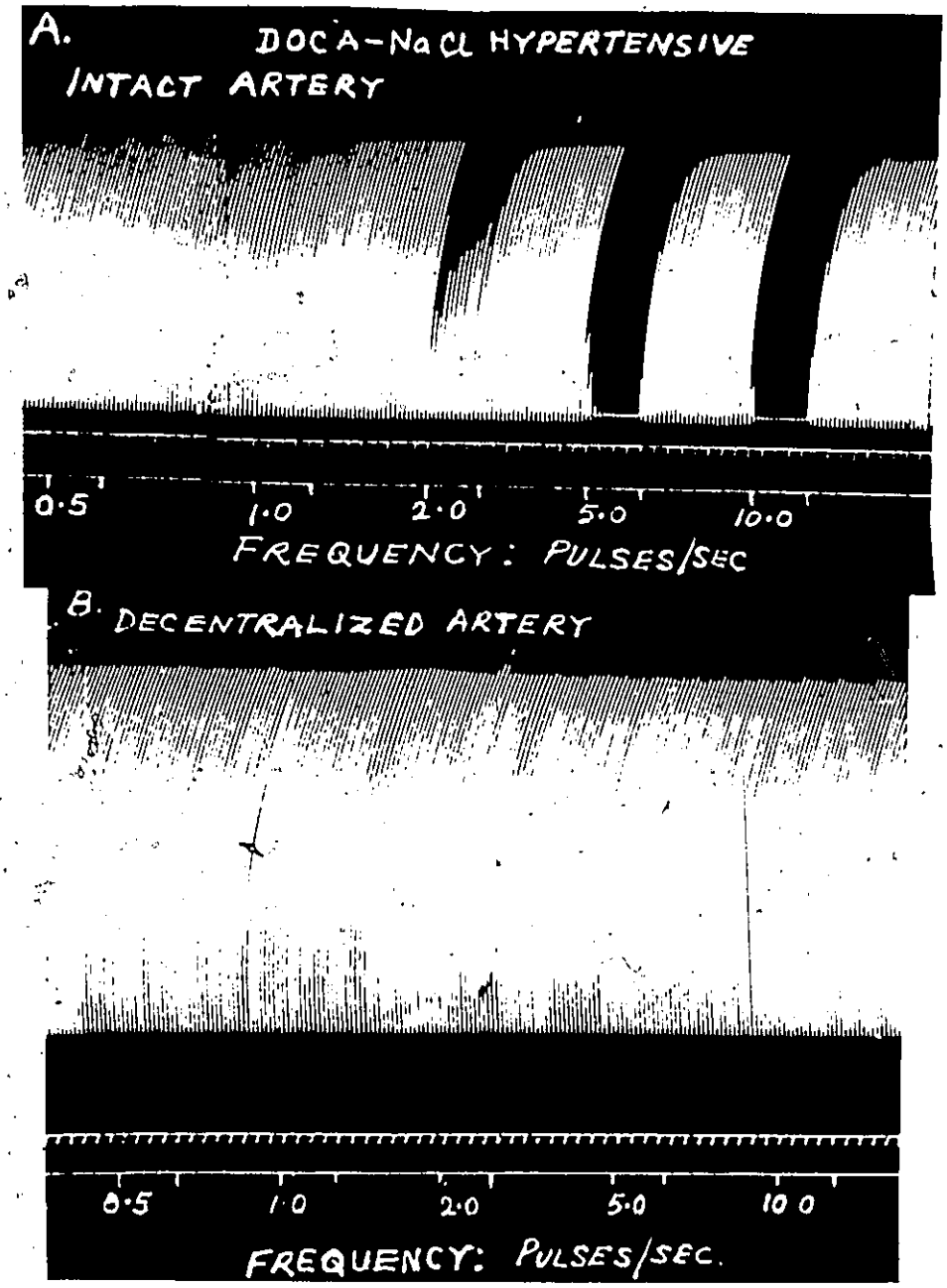


Fig. 14B., Responses of control (A) and decentralized (B) isolated perfused arteries from DOCA-NaCl (5 weeks) hypertensive rabbits to periarterial nerve stimulation. Pulse duration was 1 msec and voltage was adjusted to be supramaximal. The frequencies of stimulation are indicated below each tracing. The kymograph tracings represent flow of fluid through isolated perfused arteries and are the deflections recorded by a writing lever attached to Andrews outflow recorder which measured flow. Each vertical stroke represents flow for a period of 8 seconds. Vasoconstrictor responses are recorded as inhibition of flow.

The responses of decentralized arteries from DOCA-NaCl (2 weeks) hypertensive and from normotensive rabbits did not differ significantly.

Fig. 14A shows the frequency-response curves of arteries from DOCA-NaCl (5 weeks) hypertensive and normotensive rabbits. Fig. 14B is a kymograph tracings of a typical experiment.

Control arteries from DOCA-NaCl (5 weeks) hypertensive rabbits exhibited significantly decreased responses to nerve stimulation compared to those of normotensives ($p<0.05$). Moreover, the decentralized arteries from DOCA-NaCl (5 weeks) hypertensive animals did not respond to nerve stimulation. However, decentralized (5 weeks) arteries from normotensive animals responded to low frequency stimulations (0.5 and 1.0 pulse /sec) with significantly greater constriction than controls ($p<0.01$).

Seven of the DOCA-NaCl hypertensive rabbits were injected subcutaneously with noradrenaline (1 mg per rabbit daily) for 10 days starting on the fourth week following the initiation of DOCA-NaCl regimen. Pretreatment of hypertensive rabbits with noradrenaline resulted in the restoration of the responses to nerve stimulation of the decentralized arteries.

Fig. 15A shows the frequency-response curves of control and decentralized arteries from DOCA-NaCl hypertensive rabbits with and without noradrenaline pretreatment. The kymograph tracings from a typical experiment are shown in Fig. 15B.

After pretreating DOCA-NaCl hypertensive rabbits with noradrenaline, the decentralized arteries responded to nerve stimulation.

—●— DOCA-NaCl (5 weeks) Hypert. (16)

--○-- DOCA-NaCl (5 weeks) Hypert.-Dec. (6)

—▲— DOCA-NaCl (5 weeks) Hypert. (NA treated) (7)

--△-- DOCA-NaCl (5 weeks) Hypert.-Dec. (NA treated) (6)

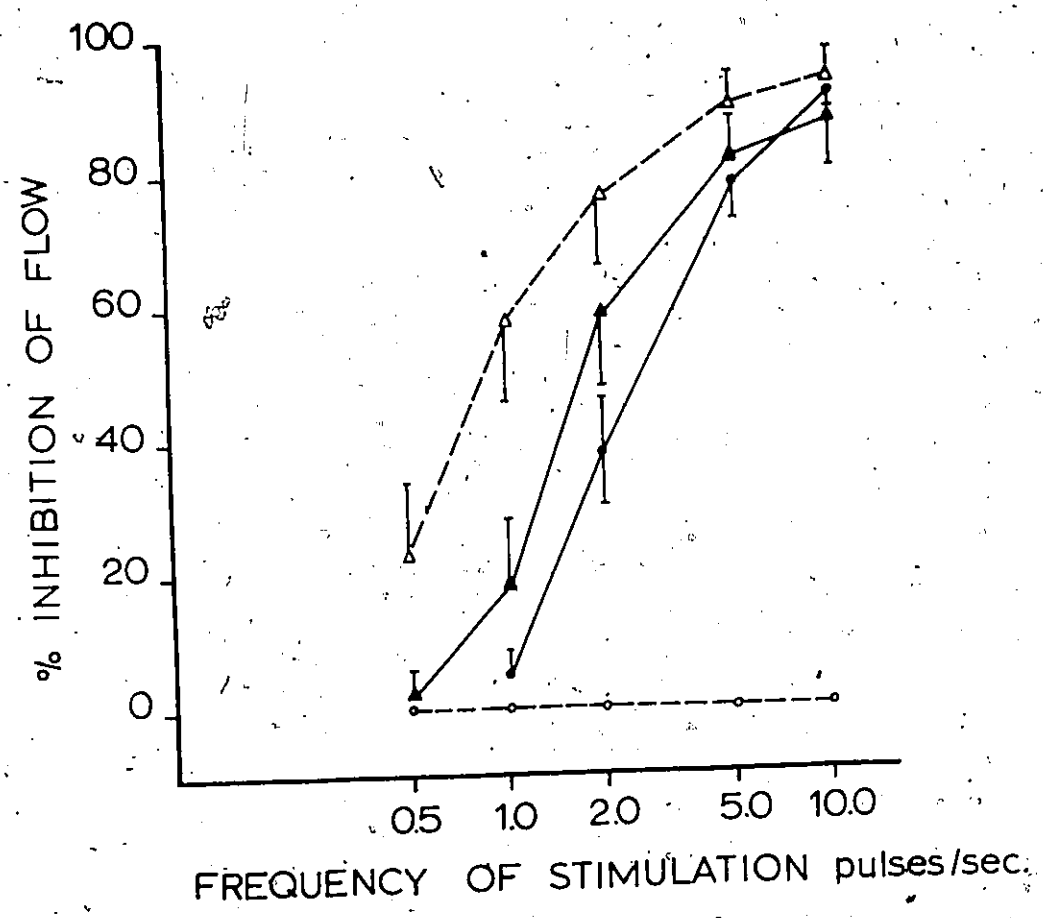


Fig. 15A. Frequency-response curves to periarterial nerve stimulation of control and decentralized arteries from DOCA-NaCl (5 weeks) hypertensive rabbits with and without noradrenaline pretreatment. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

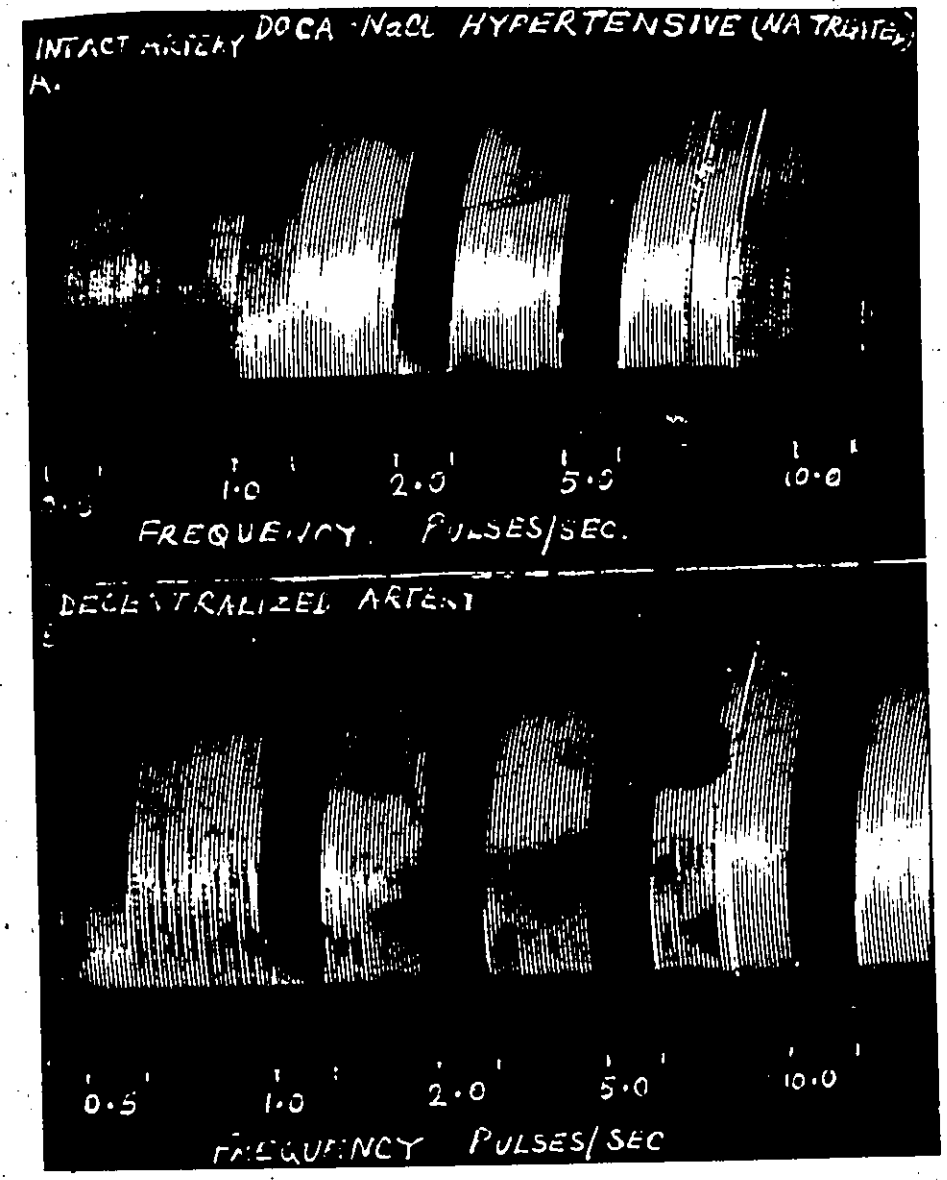


Fig. 15B. Responses to periarterial nerve stimulation of control (A) and decentralized (B) isolated perfused arteries from DOCA-NaCl (5 weeks) hypertensive rabbits pretreated with noradrenaline. The frequencies of stimulation are indicated below each tracing. Pulse duration was 1 msec and voltage was adjusted to be supramaximal. The kymograph tracings represent flow of fluid through isolated perfused arteries and are the deflections recorded by a writing lever attached to Andrews outflow recorder which measured flow. Each vertical stroke represents flow for a period of 8 seconds. Vasoconstrictor responses are recorded as inhibition of flow.

—●— Normotensive (24)

--○-- DOCA-NaCl (5 weeks) Hypert. (NA treated) (7)

-·-▲-·- DOCA-NaCl (5 weeks) Hypert. -Dec (NA treated) (6)

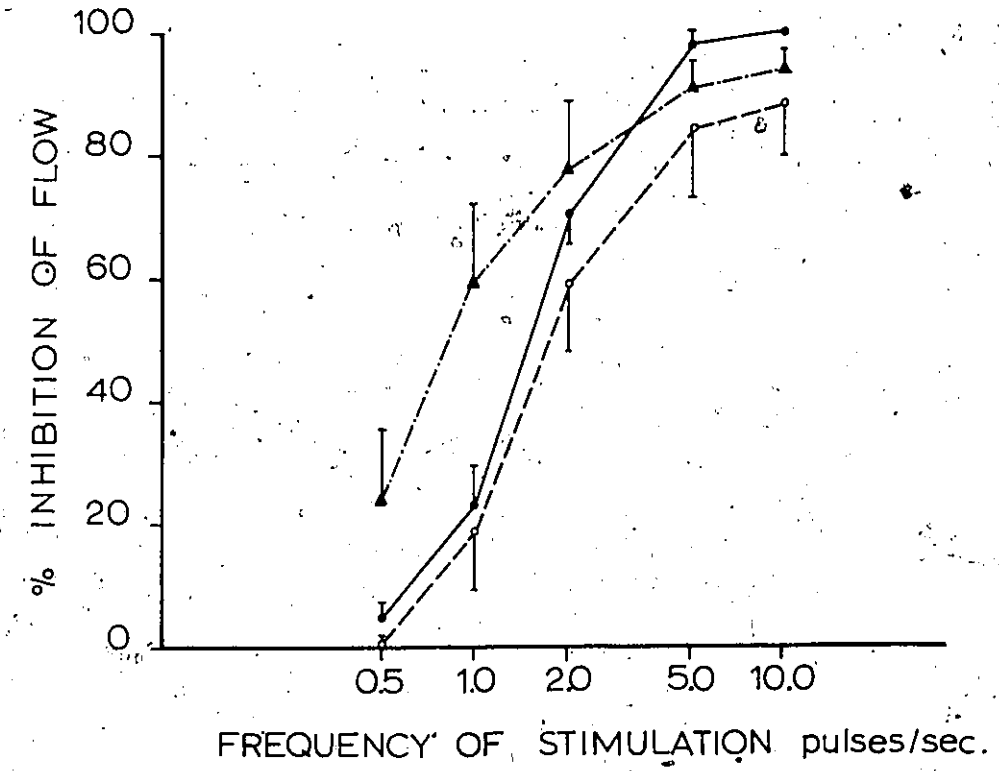


Fig. 15C. Frequency-response curves to periarterial nerve stimulation of isolated ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits pretreated with noradrenaline compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

Moreover, they gave significantly greater responses to low frequency stimulations (0.5 and 1.0 pulse /sec) than did their contralateral control arteries ($p<0.05$). Control arteries from noradrenaline pretreated hypertensive rabbits exhibited responses which did not differ from those of control arteries from untreated hypertensive animals.

Arteries with an intact sympathetic innervation from hypertensive rabbits treated with noradrenaline gave responses to nerve stimulation which did not differ from those of arteries from normotensives, except that responses to maximal stimulation (frequency of 10.0 pulses/sec) were significantly decreased ($p<0.05$) (Fig. 15C). Decentralized arteries from hypertensive rabbits pretreated with noradrenaline were significantly more responsive to low frequency stimulations (0.5 and 1.0 pulse /sec) than the contralateral control arteries or arteries from normotensives ($p<0.05$; $p<0.02$). However, responses to maximal stimulation did not differ in the three groups.

A comparison between frequency-response curves to nerve stimulation of arteries from normotensive and from DOCA-NaCl (5 weeks) hypertensive rabbits when both groups were treated with noradrenaline is presented in Fig. 15D. The frequency-response curve of control arteries from normotensive rabbits pretreated with noradrenaline did not differ from that of similarly treated arteries from DOCA-NaCl hypertensive rabbits. Decentralized arteries from noradrenaline pretreated hypertensive animals responded to nerve stimulation with greater constriction than did decentralized arteries from pretreated normotensive rabbits, but these differences were not significant

—●— Control (NA treated) (4)

--0-- Decentralized (5 weeks) NA treated) (4)

—▲— DOCA-NaCl (5 weeks) Hypert. (NA treated) (7)

--Δ-- DOCA-NaCl (5 weeks) Hypert.-Dec. (NA treated) (6)

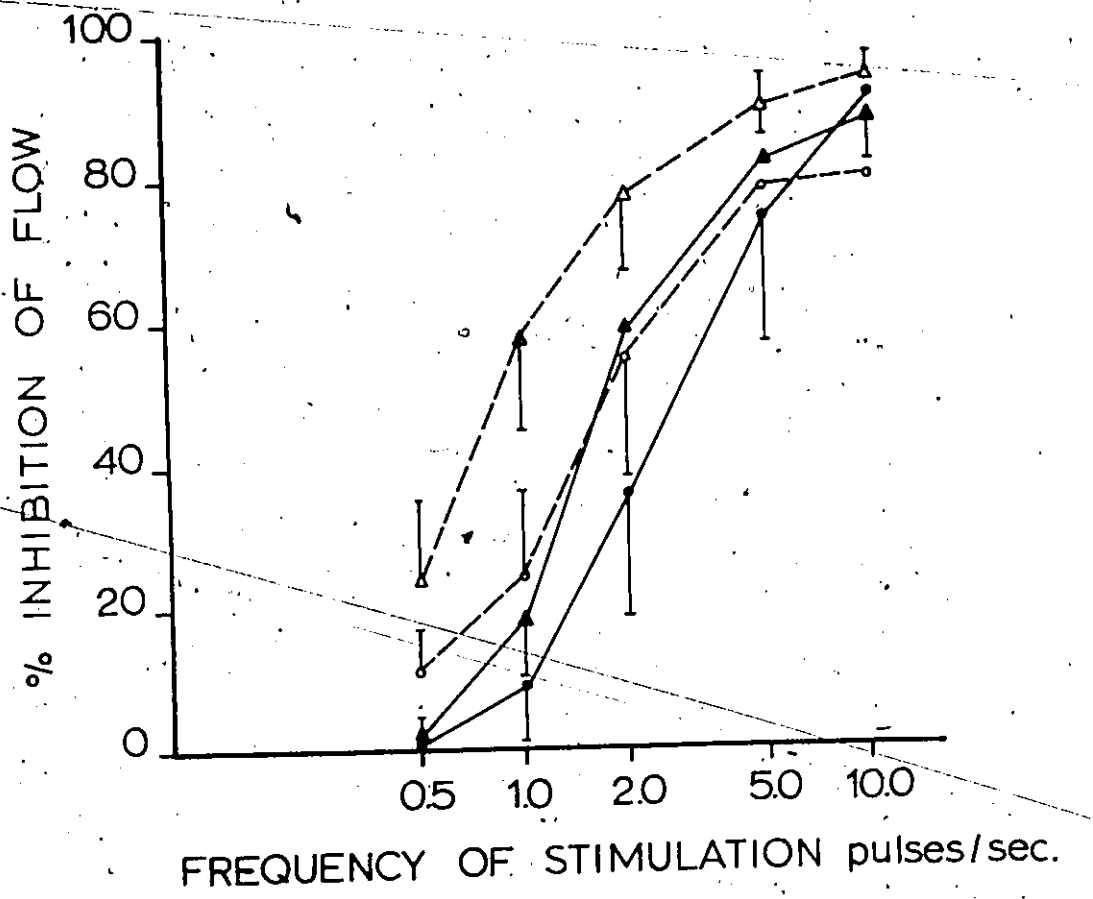


Fig. 15D. Frequency-response curves to periarterial nerve stimulation of control and decentralized ear arteries from normotensive and DOCA-NaCl (5 weeks) hypertensive rabbits when both groups were pretreated with noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

except at 1.0 pulse/sec ($p<0.05$). Although responses of decentralized arteries from normotensive rabbits pretreated with noradrenaline did not differ significantly from those of their contralateral control arteries, those from hypertensive animals were significantly more responsive to low frequency stimulations (0.5 and 1.0 pulse /sec) than the contralateral control arteries ($p<0.05$; $p<0.02$).

Responses to noradrenaline

The responses of control and decentralized arteries from DOCA-NaCl (2 weeks) hypertensive rabbits to extraluminal noradrenaline were similar, but the responses of the two groups of arteries were enhanced compared to those from normotensive animals (Fig. 16A). Control arteries from DOCA-NaCl (2 weeks) hypertensives also showed a significant increase in sensitivity to intraluminal noradrenaline compared to arteries from normotensive rabbits ($p<0.05$) Fig. 16B. Responses of the decentralized arteries from DOCA-NaCl (2 weeks) hypertensives to intraluminal noradrenaline were enhanced but not significantly so, compared to arteries from normotensive animals. The preceding results (Figs. 8A and B) showed that after decentralization (2 weeks) there was a significant increase in the responses of the operated arteries from normotensive rabbits to low concentrations of extraluminal noradrenaline but not to intraluminal noradrenaline.

Responses of control arteries from DOCA-NaCl (5 weeks) hypertensive rabbits to extraluminal noradrenaline were enhanced but those of the contralateral decentralized arteries were less sensitive to extraluminal noradrenaline than either the control arteries from

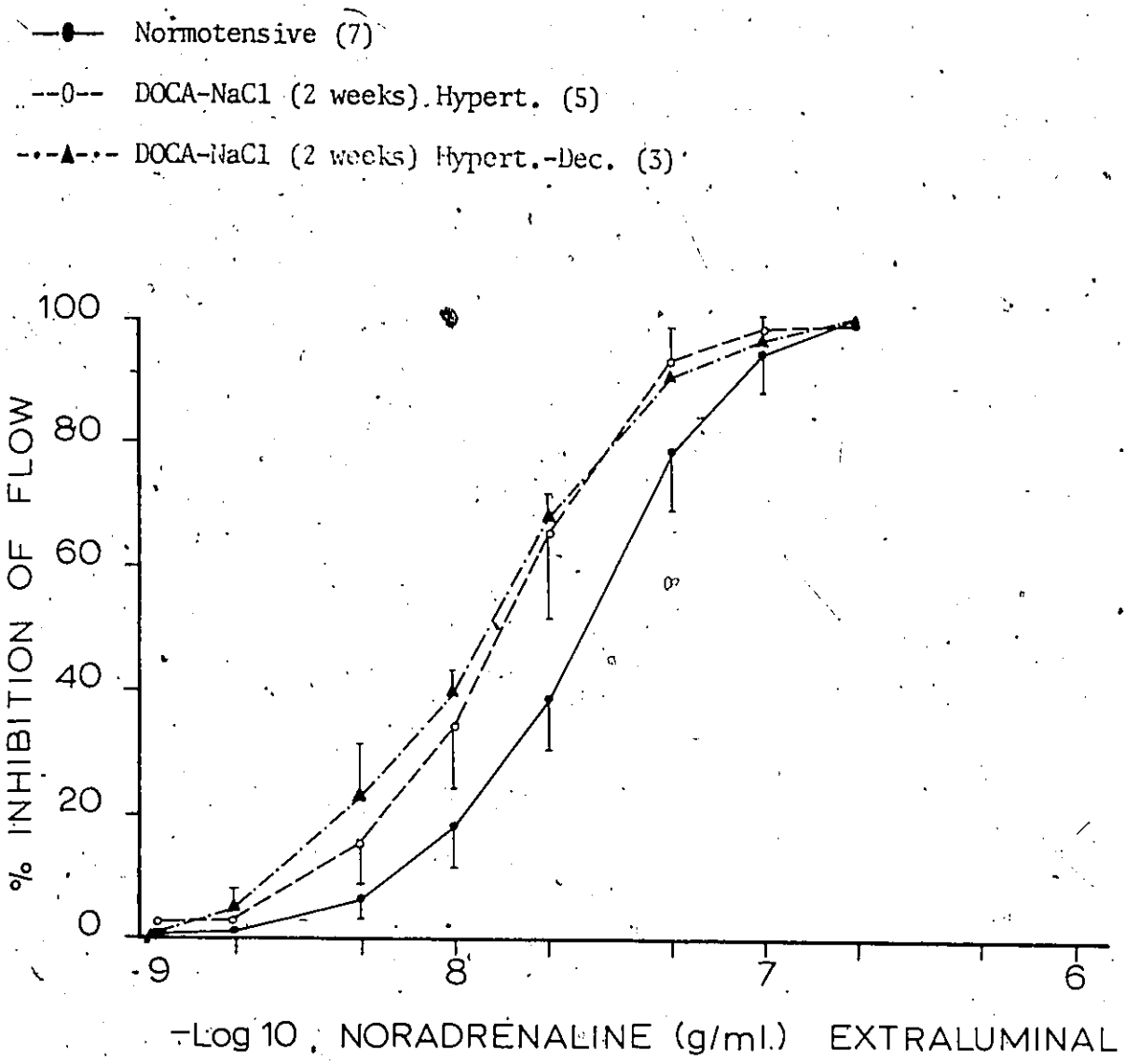


Fig. 16A. Vasoconstrictor responses to extraluminal noradrenaline of control and decentralized isolated arteries from DOCA-NaCl (2 weeks) hypertensive rabbits compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

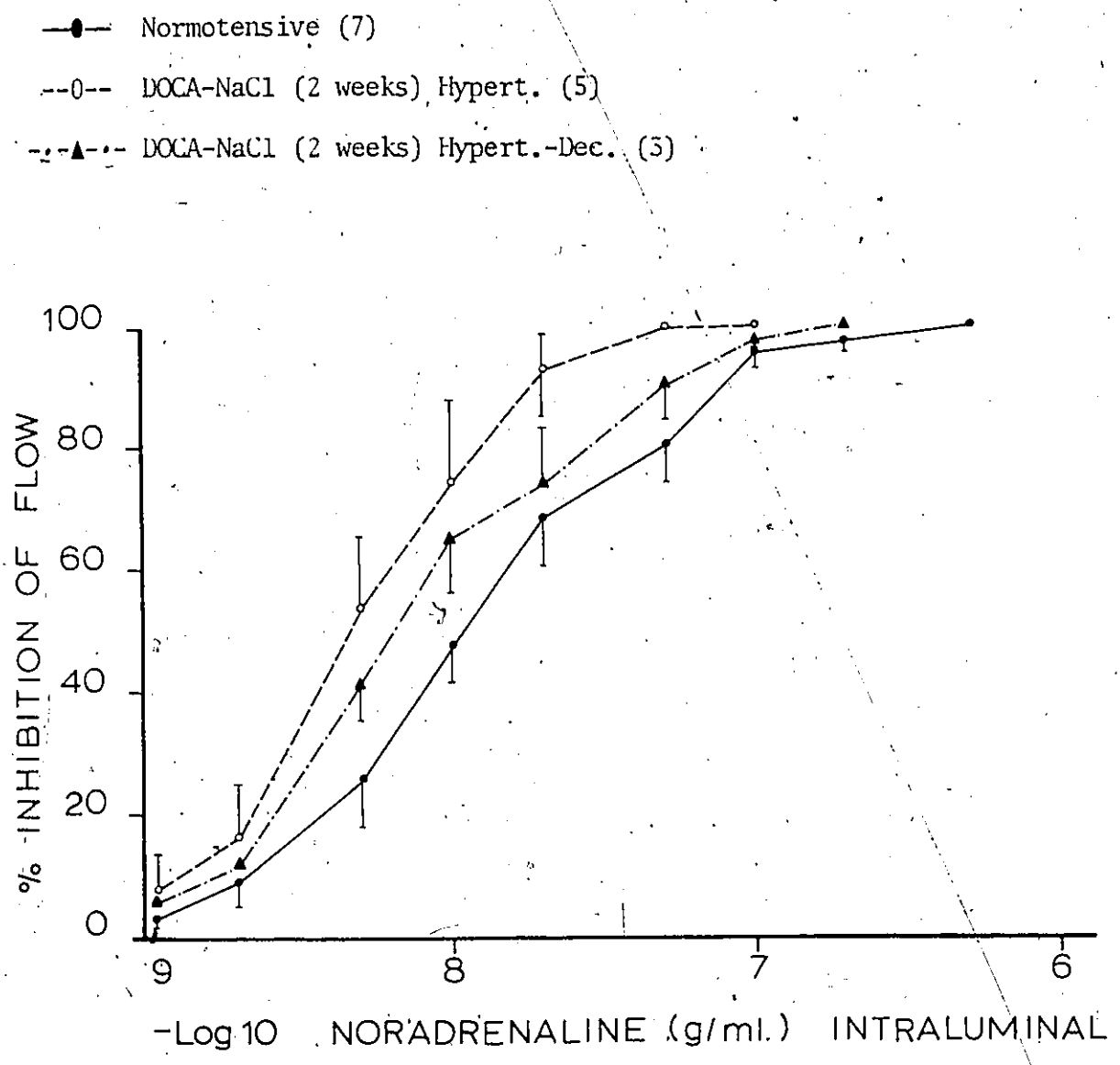


Fig. 16B. Vasoconstrictor responses to intraluminal noradrenaline of control and decentralized isolated arteries from DOCA-NaCl (2 weeks) hypertensive rabbits compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

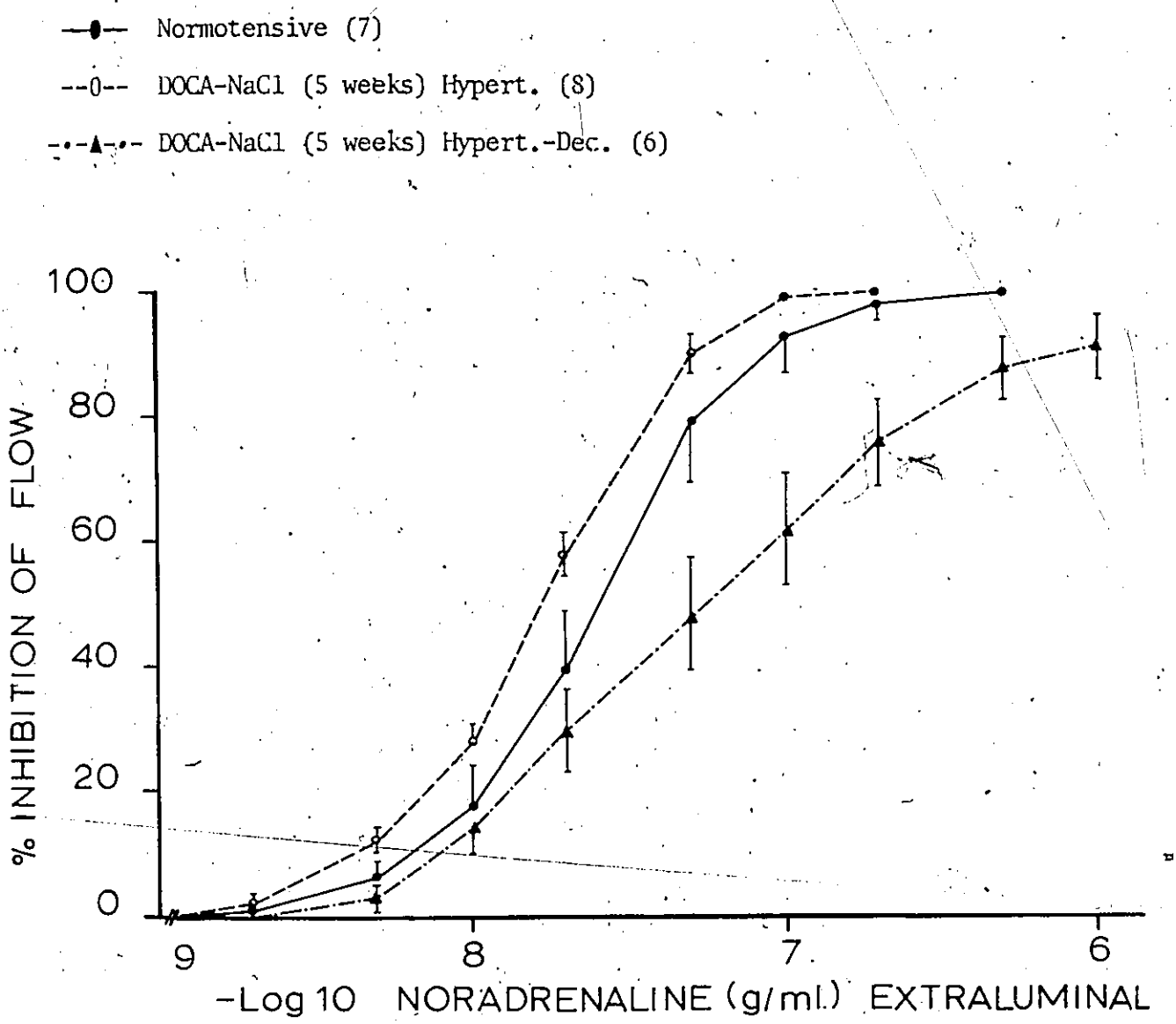


Fig. 17A. Vasoconstrictor responses to extraluminal noradrenaline of control and decentralized ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

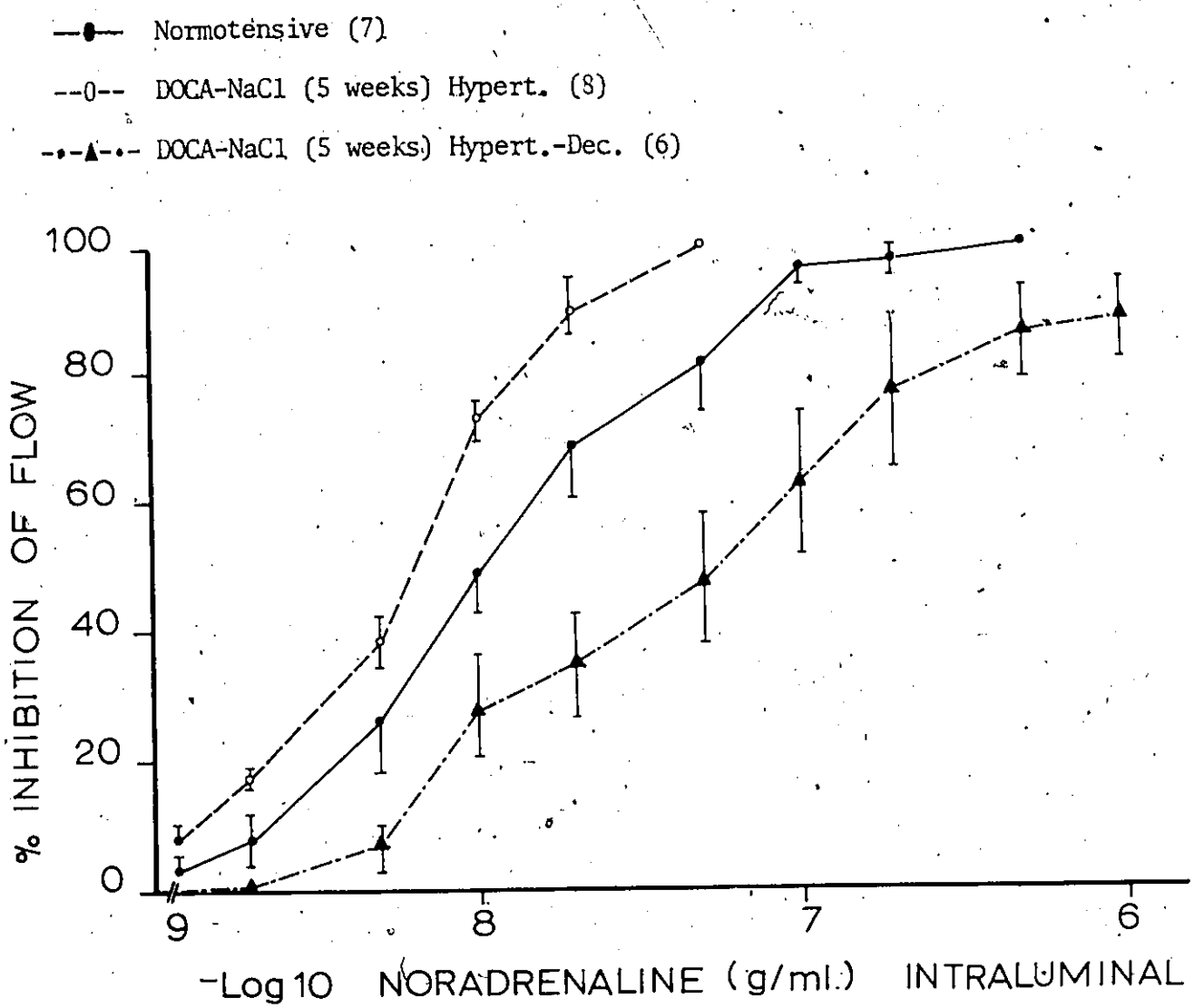


Fig. 17B. Vasoconstrictor responses to intraluminal noradrenaline of control and decentralized ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

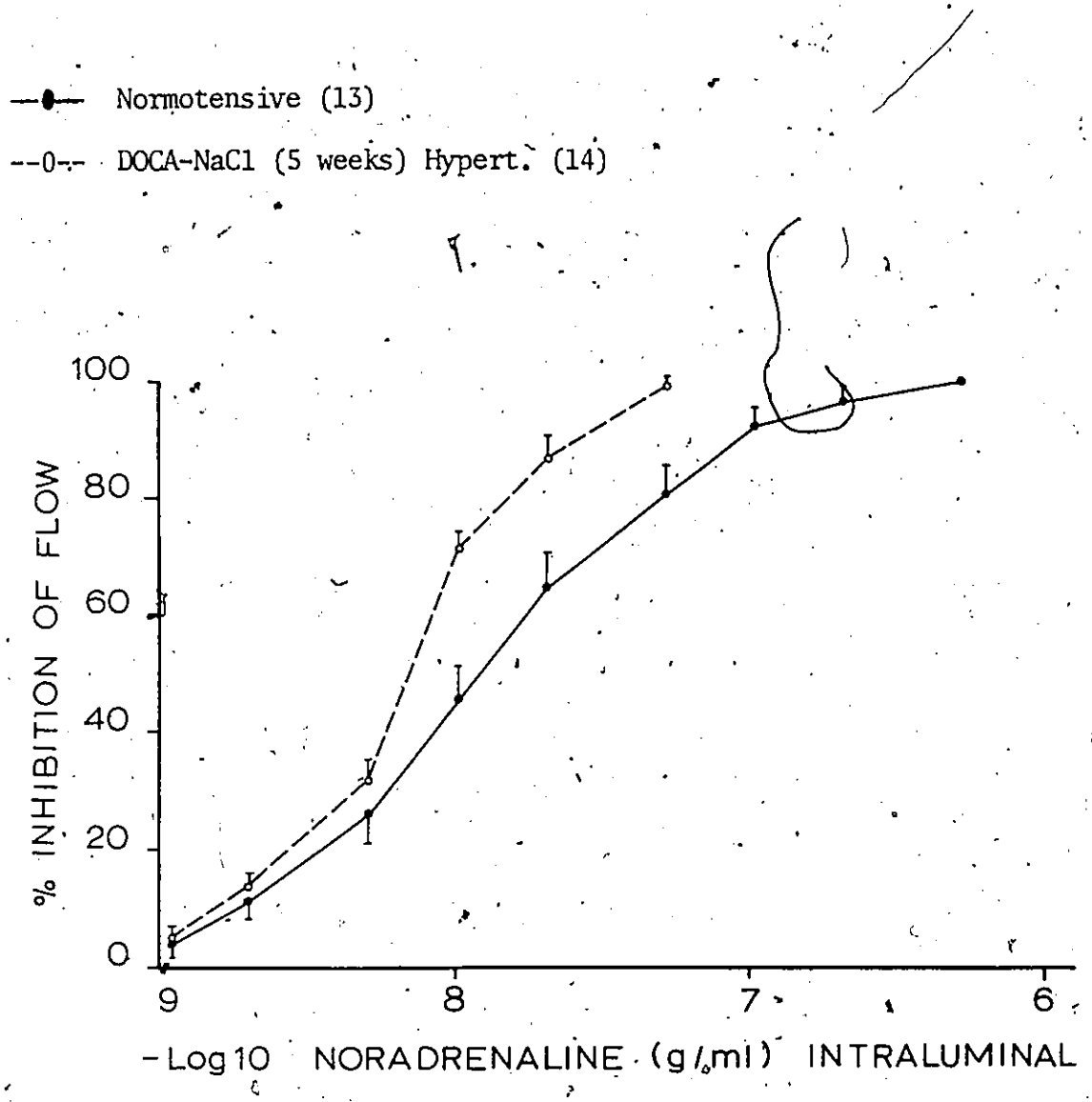


Fig. 18. Responses to intraluminal noradrenaline of isolated perfused arteries from DOCA-NaCl (5 weeks) hypertensive rabbits compared to those from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

DOCA-NaCl hypertensive ($p<0.01$ to $p<0.001$) or those from normotensive animals ($p<0.05$ to $p<0.01$) (Fig. 17A). The same situation held with respect to intraluminal noradrenaline but the responses of the control arteries were even more significantly increased ($p<0.05$ to $p<0.02$) Fig. 17B and Fig. 18). Fig. 18 shows the pooled results of several experiments.

Responses of isolated arteries from DOCA-NaCl (5 weeks) hypertensive rabbits pretreated with exogenous noradrenaline

Experiments were performed to find out if the development of subsensitivity of the decentralized arteries to noradrenaline during induction of DOCA-NaCl hypertension was related to lack of tonic release of noradrenaline onto the arterial vessels by the sympathetic nerves (decentralized arteries from DOCA-NaCl (5 weeks) hypertensives did not respond to nerve stimulation). Rabbits on DOCA-NaCl treatment were injected with noradrenaline in oil (daily for 10 days).

In Figs. 19A and B responses of control and decentralized arteries from DOCA-NaCl (5 weeks) hypertensive rabbits with and without noradrenaline treatment are compared.

Following pretreatment of DOCA-NaCl hypertensive rabbits with noradrenaline, control arteries exhibited decreased responses to extraluminal noradrenaline ($p<0.01$) (Fig. 19A). However, the decentralized arteries from hypertensive rabbits pretreated with noradrenaline gave responses which were similar to those of decentralized arteries from untreated hypertensive animals. The responses of decentralized arteries of pretreated hypertensive rabbits did not differ from those of their contralateral control arteries. As already mentioned decentralized

—●— DOCA-NaCl (5 weeks) Hypert. (8)

--○-- DOCA-NaCl (5 weeks) Hypert.-Dec. (6)

—▲— DOCA-NaCl (5 weeks) Hypert. (NA treated) (7)

--△-- DOCA-NaCl (5 weeks) Hypert.-Dec. (NA treated) (7)

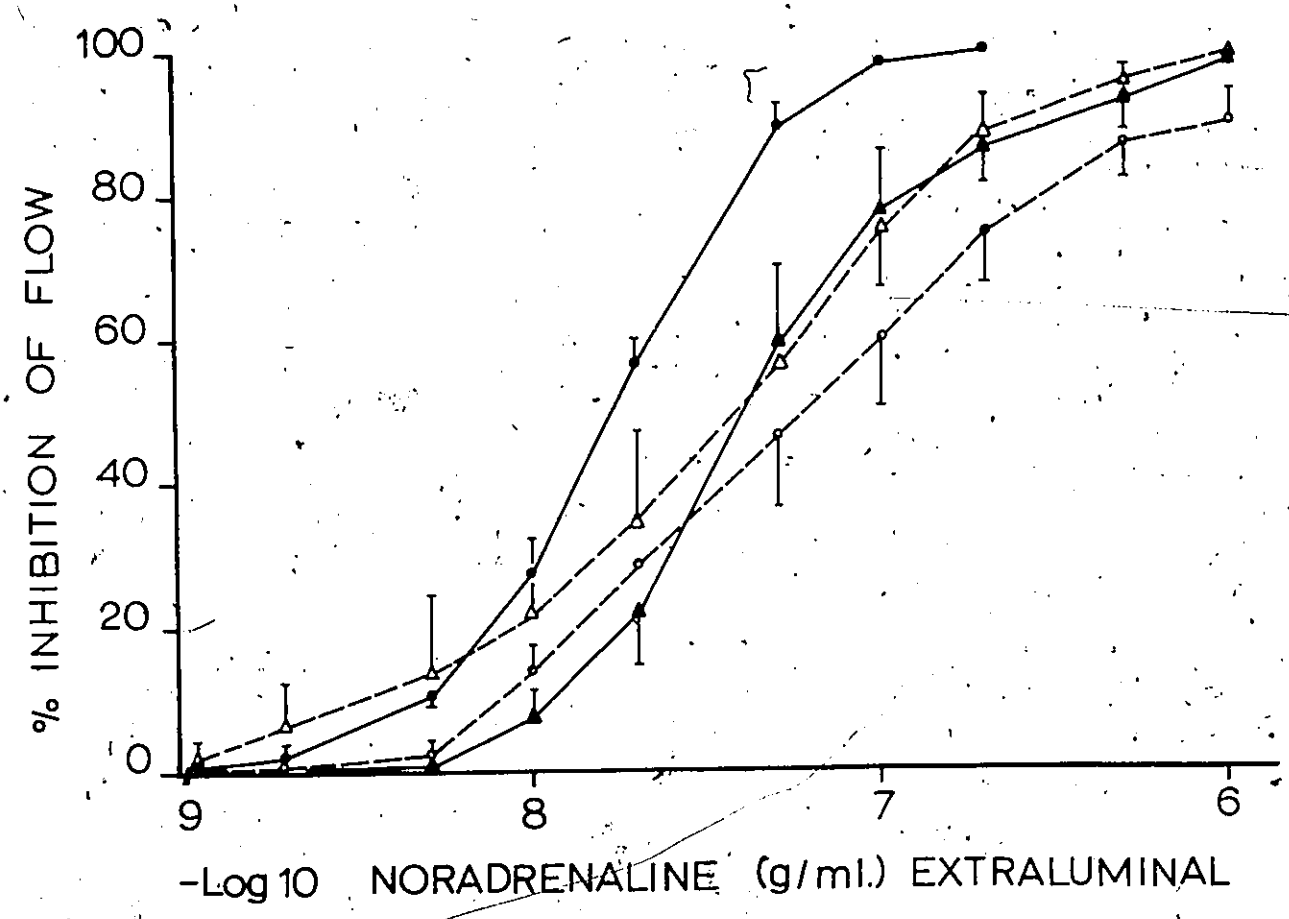


Fig. 19A. Vasoconstrictor responses to extraluminal noradrenaline of control and decentralized ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits with and without noradrenaline pretreatment. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

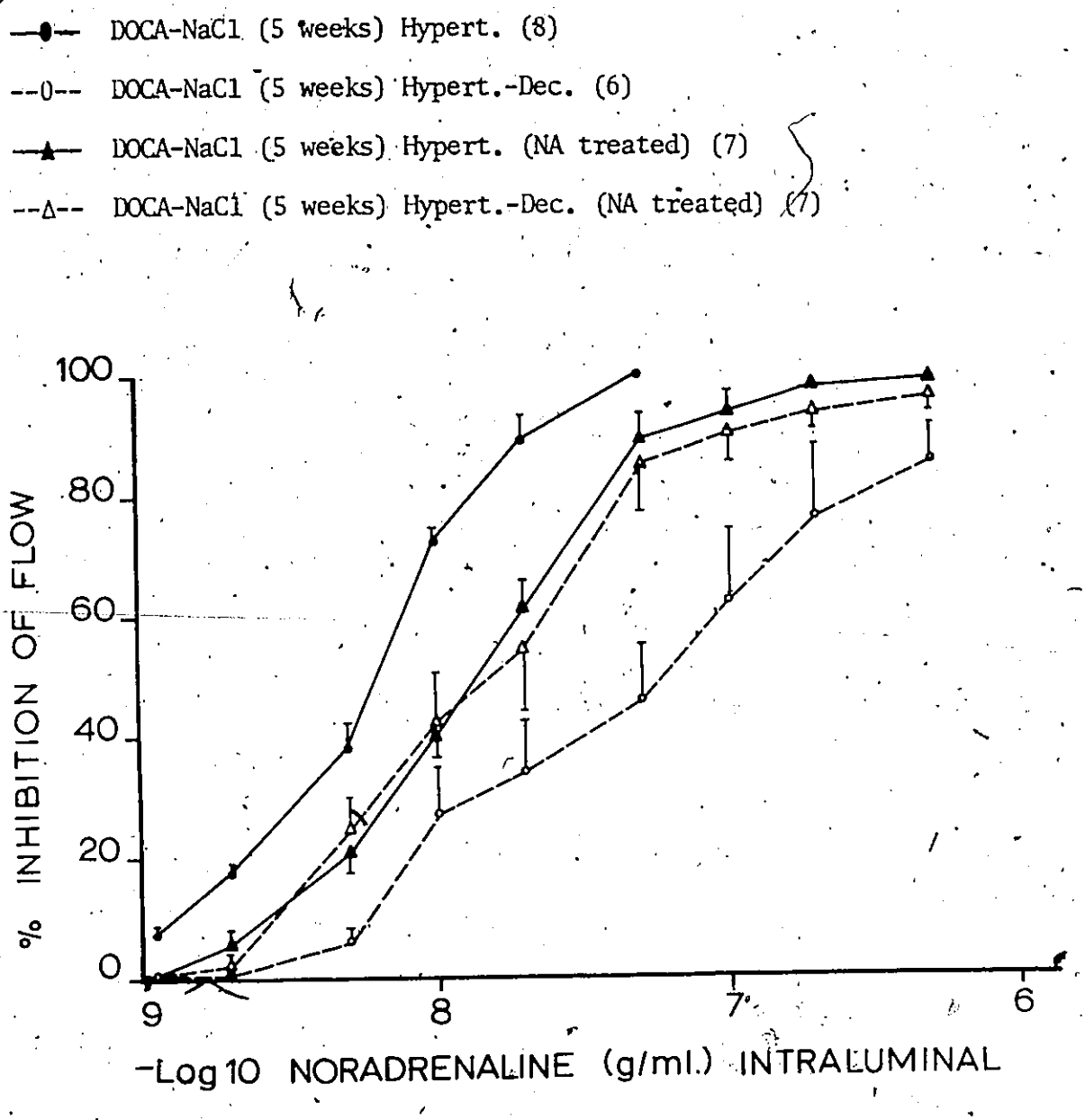


Fig. 19B. Vasoconstrictor responses to intraluminal noradrenaline of control and decentralized ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits with and without noradrenaline pretreatment. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

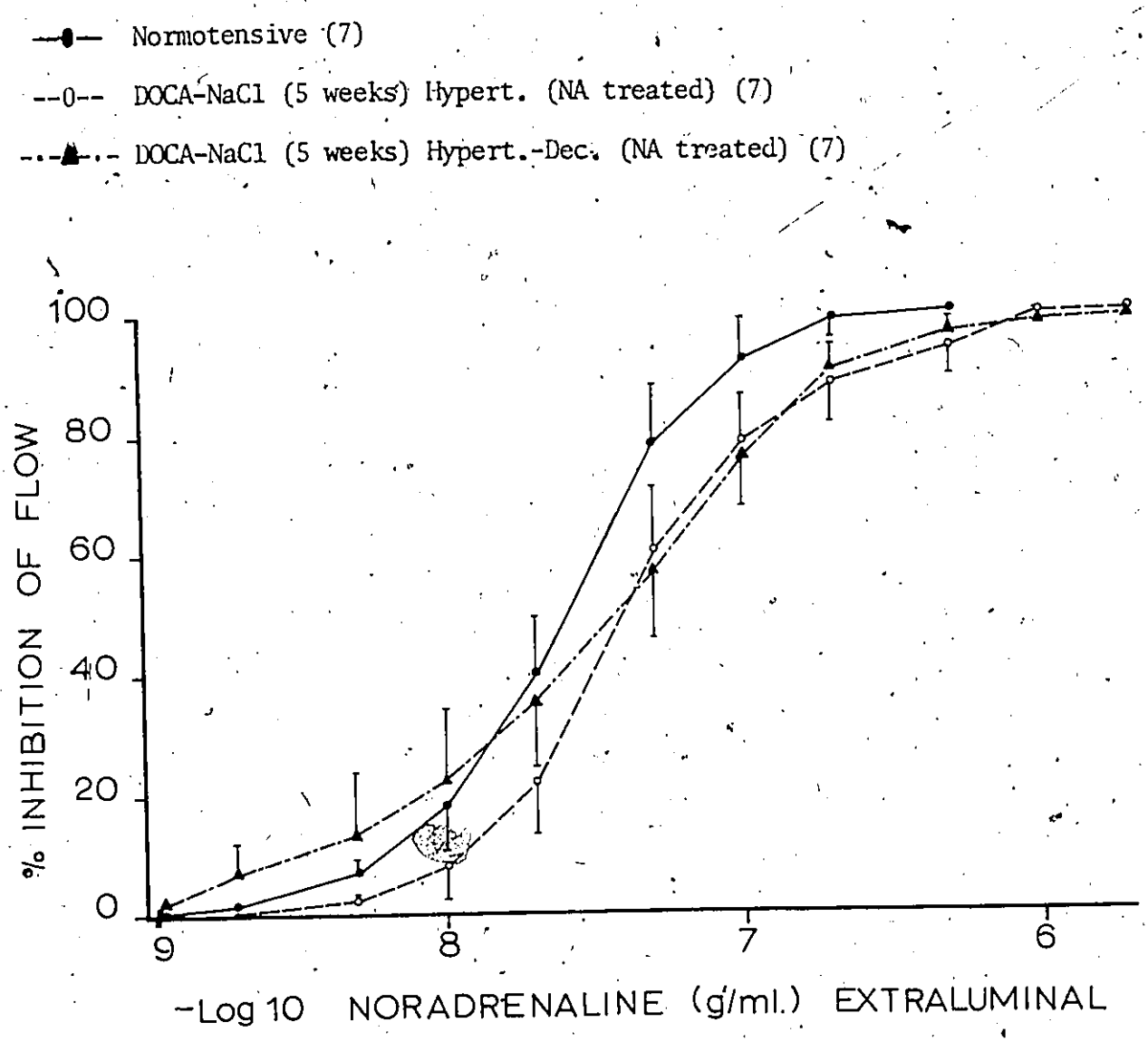


Fig. 19C. Responses to extraluminal noradrenaline of control and decentralized isolated arteries from DOCA-NaCl (5 weeks) hypertensive rabbits pretreated with noradrenaline compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

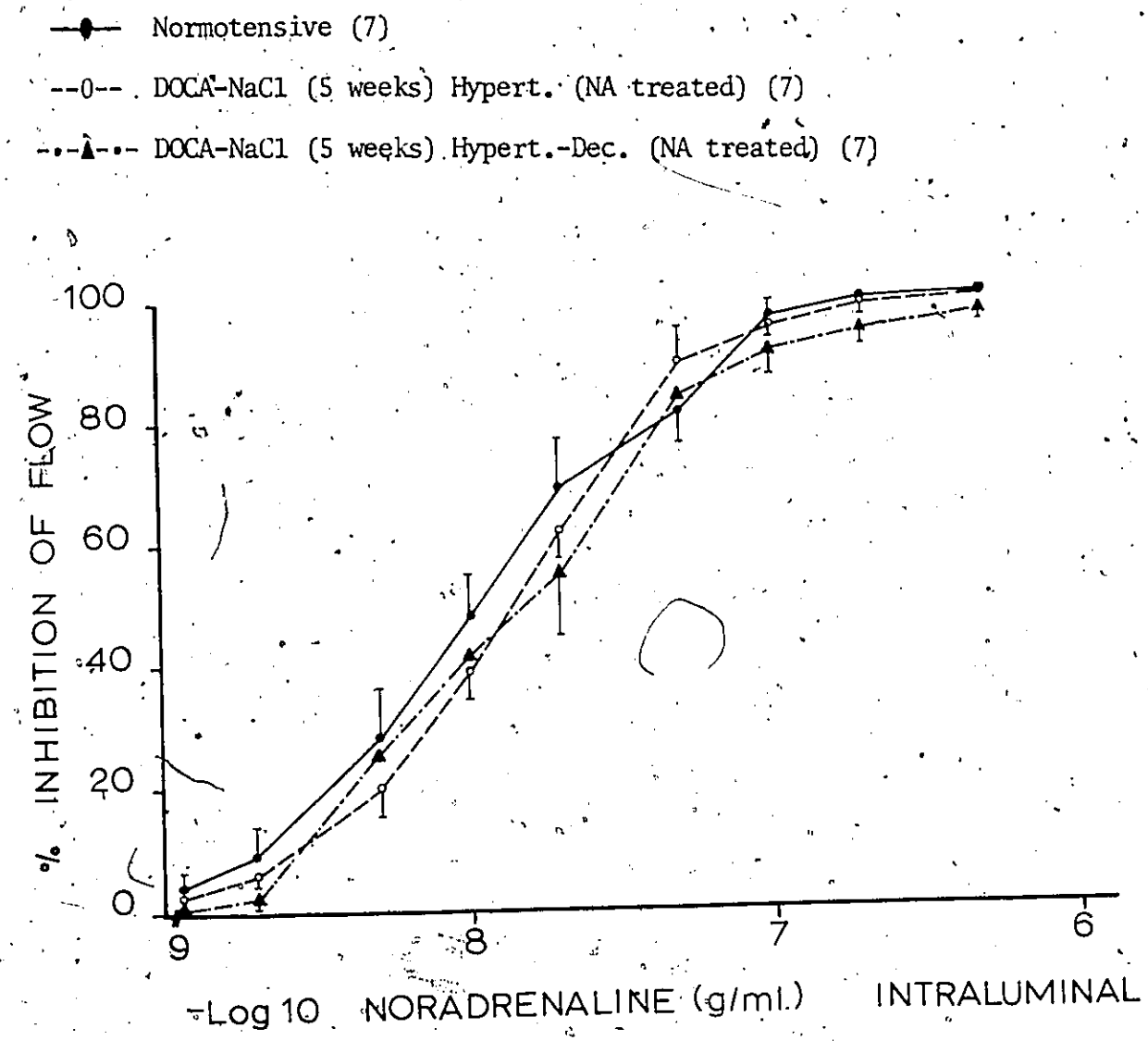


Fig. 19D. Responses to intraluminal noradrenaline of control and decentralized isolated arteries from DOCA-NaCl (5 weeks) hypertensive rabbits pretreated with noradrenaline compared to those of arteries from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

arteries from hypertensive animals without noradrenaline pretreatment were markedly less sensitive than the contralateral control arteries to extraluminal noradrenaline ($p<0.01$).

Pretreatment of DOCA-NaCl hypertensive rabbits with noradrenaline appeared to eliminate the differences between the responses of the arteries with and without a sympathetic innervation. Results are shown in Figs. 19B and D. Noradrenaline pretreatment appeared to depress responses of arteries with an intact sympathetic innervation to intraluminal noradrenaline Fig. 19B. However, responses of decentralized arteries from DOCA-NaCl (5 weeks) hypertensive rabbits treated with noradrenaline were enhanced as compared to those of decentralized arteries from untreated hypertensive animals. As already mentioned, decentralized arteries from DOCA-NaCl (5 weeks) hypertensive rabbits exhibited a marked decrease in responses to intraluminal noradrenaline compared to their contralateral control arteries ($p<0.001$). However, after pretreatment of DOCA-NaCl hypertensive rabbits with noradrenaline the responses to intraluminal noradrenaline of decentralized arteries no longer differed from those of their contralateral control arteries. The control and decentralized arteries from these treated hypertensive rabbits exhibited responses to extra- and intraluminal noradrenaline which were comparable to those of arteries from normotensive animals (Figs. 19C and D).

Figs. 19E and F show the dose-response curves of control and decentralized arteries from normotensive and from DOCA-NaCl hypertensive rabbits when both groups were pretreated with noradrenaline. (Fig. 19E) Control arteries from normotensive rabbits treated with

—●— Control (NA treated) (4)

--○-- Decentralized (5 weeks) (NA treated) (4)

—▲— DOCA-NaCl (5 weeks) Hypert. (NA treated) (7)

--△-- DOCA-NaCl (5 weeks) Hypert.-Dec. (NA treated) (7)

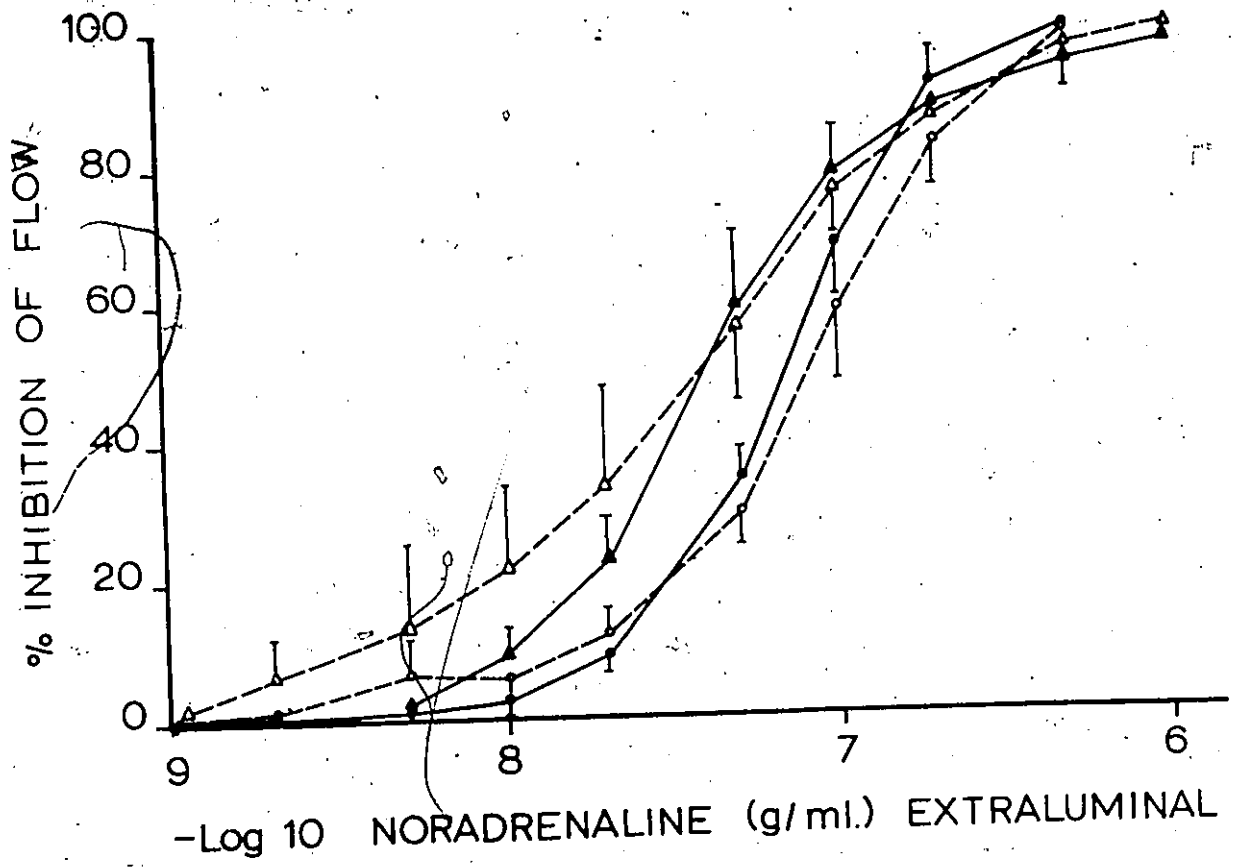


Fig. 19E. Vasoconstrictor responses to extraluminal noradrenaline of control and decentralized ear arteries from normotensive and and DOCA-NaCl (5 weeks) hypertensive rabbits pretreated with noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

—●— Control (NA treated) (4)

--○-- Decentralized (5 weeks) (NA treated) (4)

—▲— DOCA-NaCl (5 weeks) Hypert. (NA treated) (7)

--△-- DOCA-NaCl (5 weeks) Hypert.-Dec. (NA treated) (7)

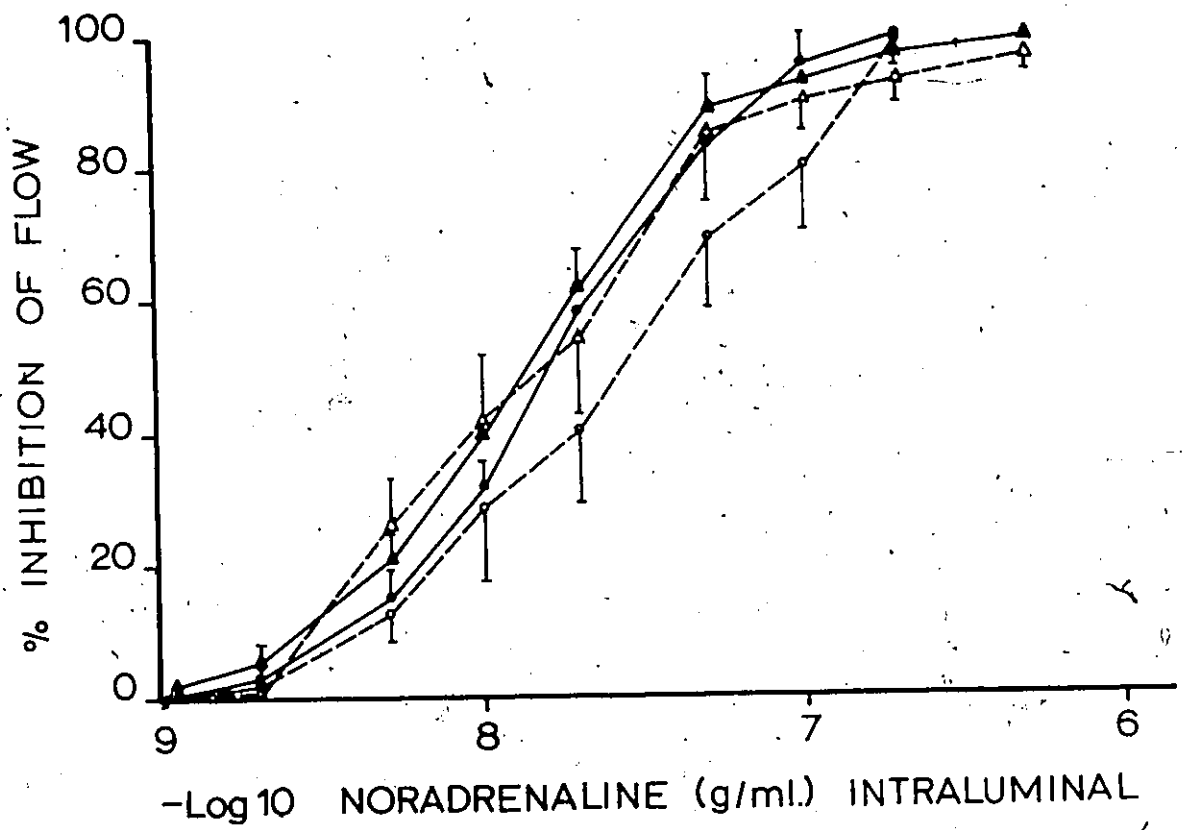


Fig. 19F. Vasoconstrictor responses to intraluminal noradrenaline of control and decentralized ear arteries from normotensive and DOCA-NaCl (5 weeks) Hypertensive rabbits pretreated with noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

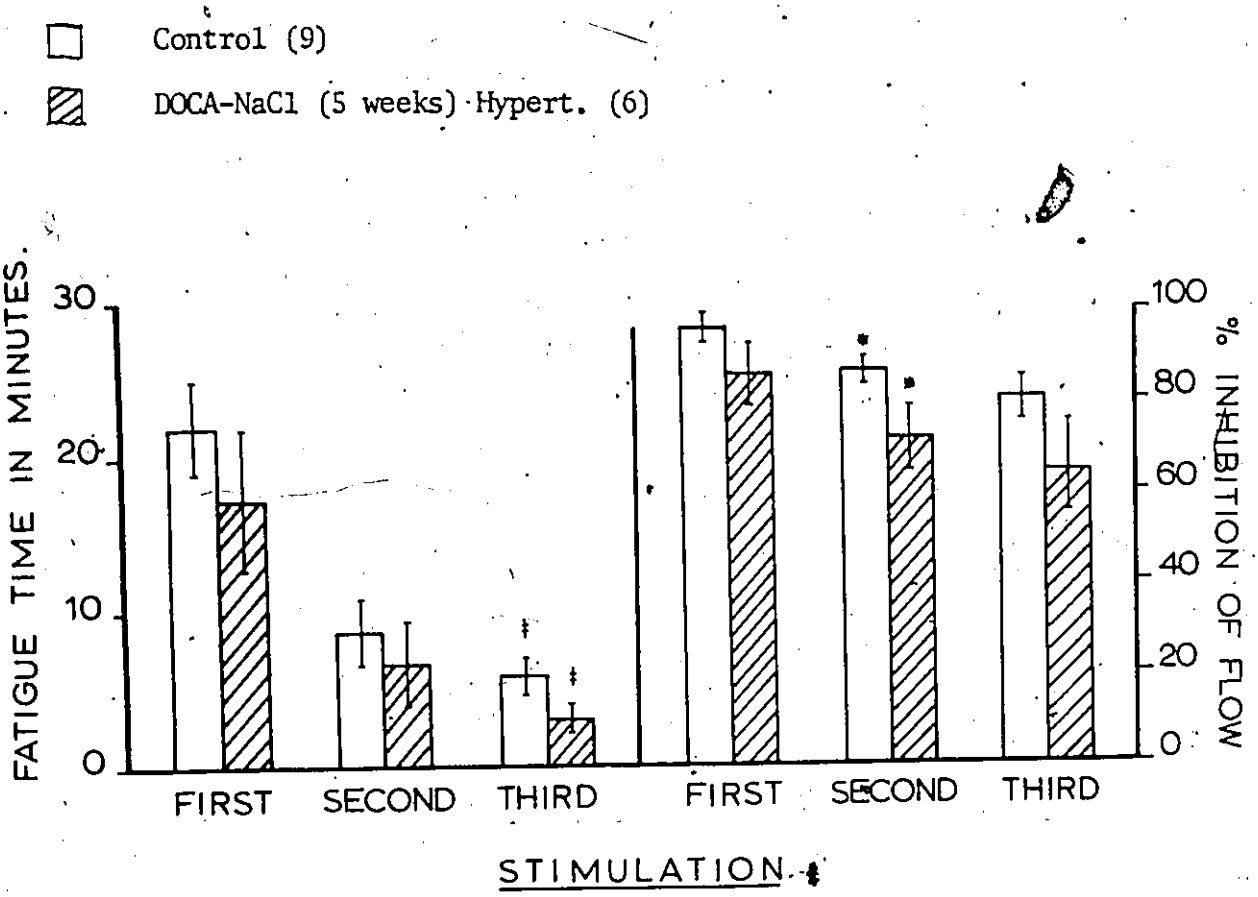


Fig. 20. Responses to prolonged stimulation of ear arteries from control and DOCA-NaCl (5 weeks) Hypertensive animals. Left half of the graph shows "fatigue time" during prolonged stimulation and the right half the vasoconstrictor responses of the arteries. Stimulation was carried out at 1 msec duration, frequency of 5 pulses/sec and at supramaximal voltage. The vertical bars represent S.E.M. and the numbers in parentheses denote the number of experiments in each group.

‡ $p<0.05$

*

noradrenaline were less responsive to extraluminal noradrenaline than those from DOCA-NaCl hypertensive rabbits at concentrations of 2×10^{-8} to 1×10^{-7} g/ml. There were no significant differences between the responses to extraluminal noradrenaline of control arteries and their contralateral decentralized arteries from normotensive or from DOCA-NaCl hypertensive rabbits pretreated with noradrenaline.

In Fig. 19F, it is shown that responses of control and decentralized arteries from noradrenaline pretreated normotensive rabbits to intraluminal noradrenaline did not differ from those of noradrenaline pretreated DOCA-NaCl hypertensive rabbits.

Effect of prolonged stimulation on responses of ear arteries from DOCA-NaCl (5 weeks) hypertensive rabbits

Isolated perfused arteries from DOCA-NaCl (5 weeks) hypertensive rabbits were stimulated to fatigue to assess sympathetic neuro-effector transmission. Stimulation of the periarterial sympathetic nerves was carried out at 1 msec duration, frequency of 5.0 pulses per sec and at supramaximal voltage. Stimulation was discontinued when the vasoconstrictor response was reduced by 50% of its initial peak response. Time taken for the peak vasoconstrictor response to decline 50% was termed 'fatigue time'. Three periods of sustained stimulation at 1 hour intervals were performed on each artery. The results of this study are presented in Fig. 20.

During the first stimulation period the 'fatigue time' of arteries from control and from DOCA-NaCl (5 weeks) hypertensive rabbits was 22.3±4.0 min. and 17.5±4.6 min. respectively. The initial peak

—●— Normotensive (5)

—○— Normotensive -IPN (5)

--▲-- DOCA-NaCl (5 weeks) Hypert. (6)

--△-- DOCA-NaCl (5 weeks) Hypert. -IPN (6)

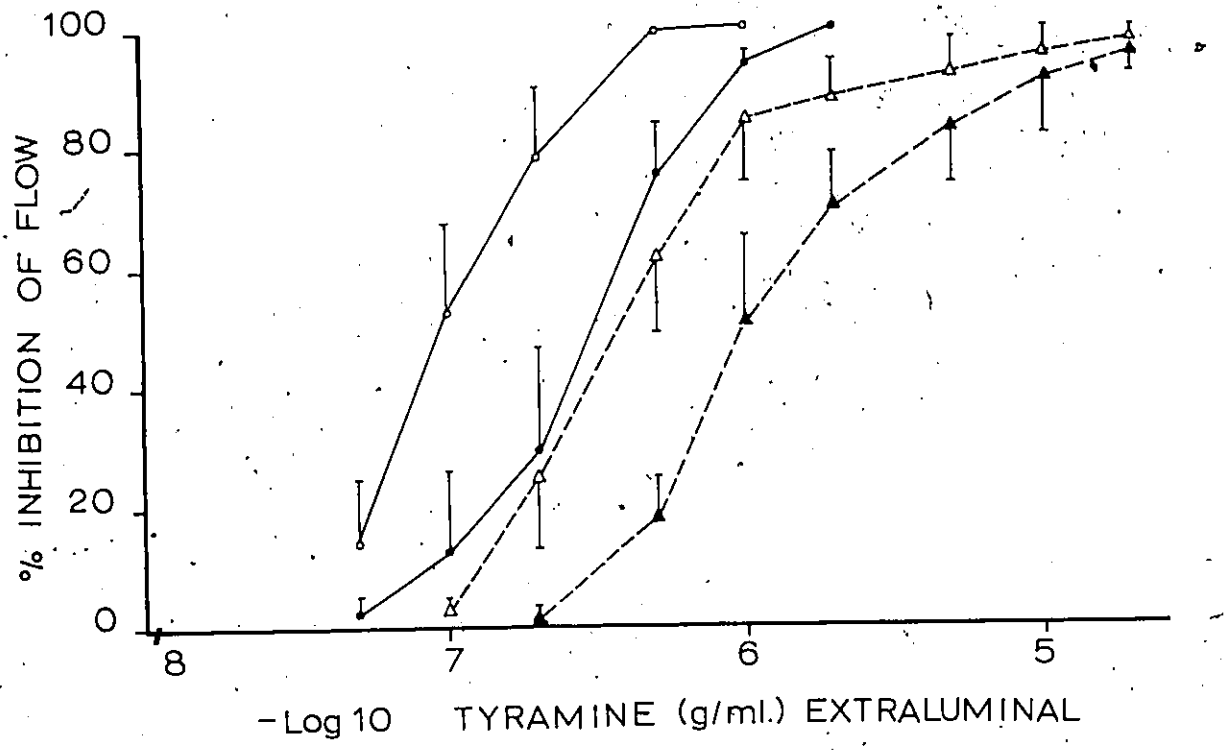


Fig. 21. Contractile effects of extraluminal tyramine on isolated perfused ear arteries from normotensive and DOCA-NaCl (5 weeks) hypertensive rabbits before and after treatment with iproniazid (IPN), 2×10^{-4} g/ml. The vertical bars represent S.E.M. and the numbers in parentheses denote the number of values in each group.

responses were a mean of 94.8±2.4% (% inhibition of flow) and 84.6±6.9%. There was no significant difference in the 'fatigue time' of the initial peak response between the two groups of arteries.

During the second stimulation period the time to fatigue for both groups of arteries decreased by more than 50% compared to the first period of stimulation. It was 8.6±2.9 min. and 6.6±2.7 min. for arteries from control and from DOCA-NaCl hypertensive animal and the corresponding peak responses were 86.3±3.4% and 70.6±7.4% ($p<0.05$).

Fatigue of the response to nerve stimulation was quite rapid during the third period of stimulation. The time to fatigue was 5.9± 1.0 min. for controls and 3.0±0.6 min. for the treated arteries ($p<0.05$) and the corresponding peak responses were 79.9±5.4% and 64.2±9.6%.

Effects of tyramine on isolated perfused ear arteries from DOCA-NaCl (5 weeks) hypertensive and normotensive rabbits

Dose-response curves to tyramine administered extraluminally were determined in arteries from DOCA-NaCl (5 weeks) hypertensive and normotensive rabbits. The results are shown in Fig. 21.

The dose-response curve to tyramine obtained in arteries from DOCA-NaCl hypertensive animals was shifted markedly to the right of controls. The threshold concentration of tyramine was about 4-fold higher and the concentration producing maximal inhibition of flow was about 20-fold higher in the arteries from hypertensive rabbits.

The arteries were then exposed for 30 minutes to iproniazid (IPN) (2×10^{-4} g/ml), a monoamine oxidase inhibitor, as described in Methods, and then retested to tyramine. The tyramine curves for both groups of arteries appeared to be shifted to the left to a similar extent. However, while the concentration of tyramine required to produce maximal

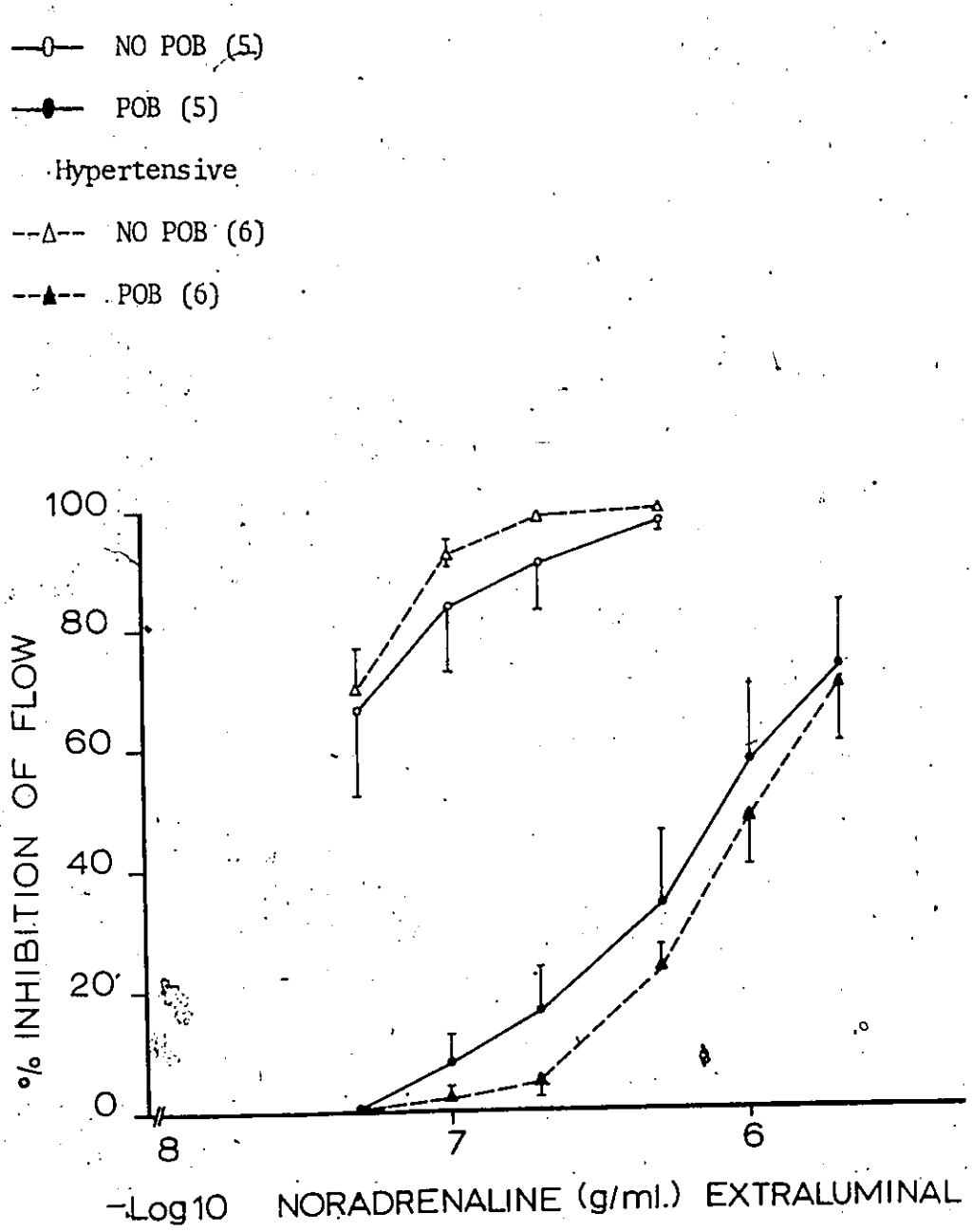


Fig. 22A. Effect of phenoxybenzamine (5×10^{-8} g/ml) on responses of isolated ear arteries from normotensive and DOCA-NaCl (5 weeks) hypertensive rabbits to extraluminal noradrenaline. Vertical bars represent S.E.M. and the numbers in parentheses denote the number of arteries in each group. POB = phenoxybenzamine.

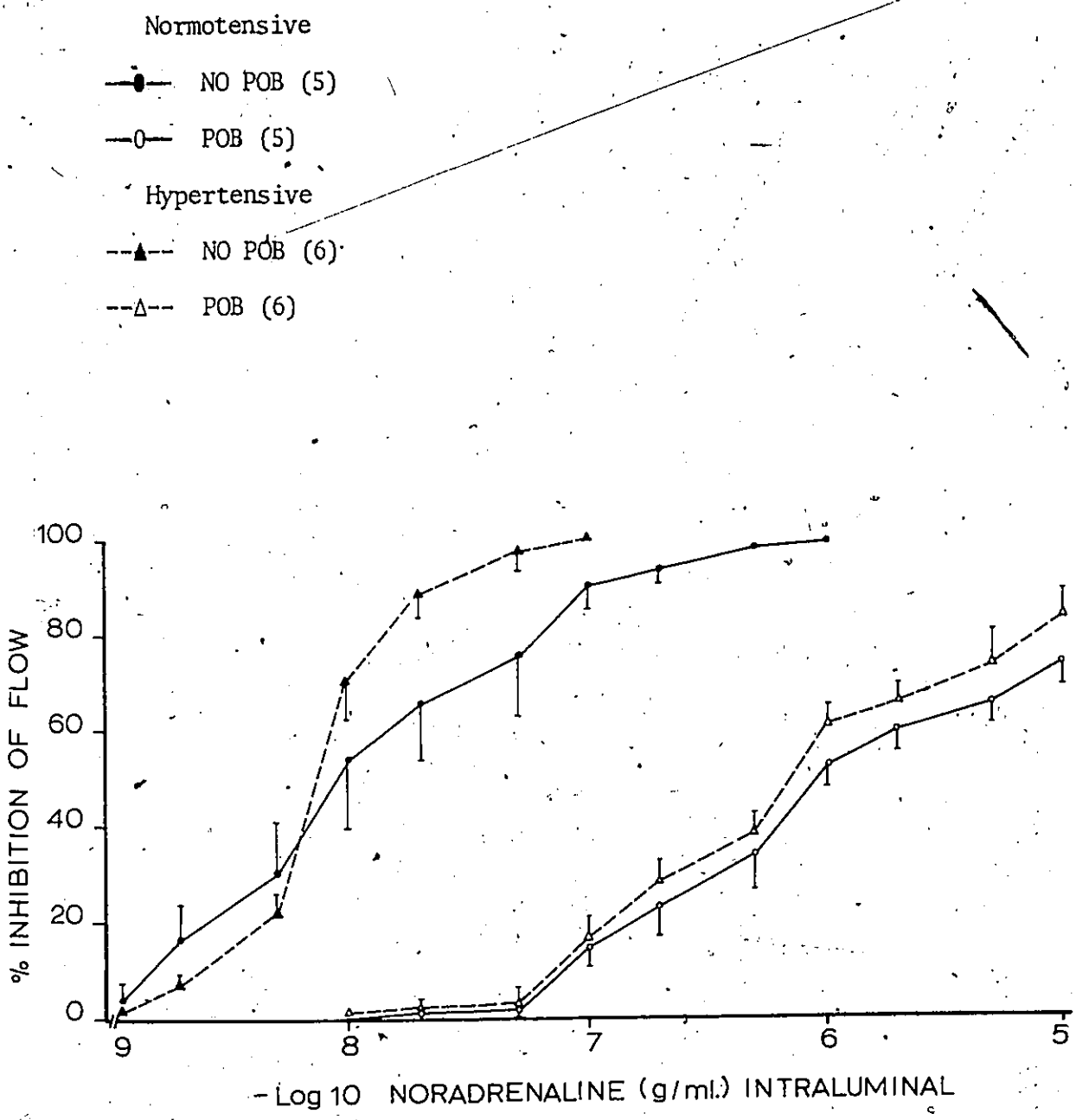
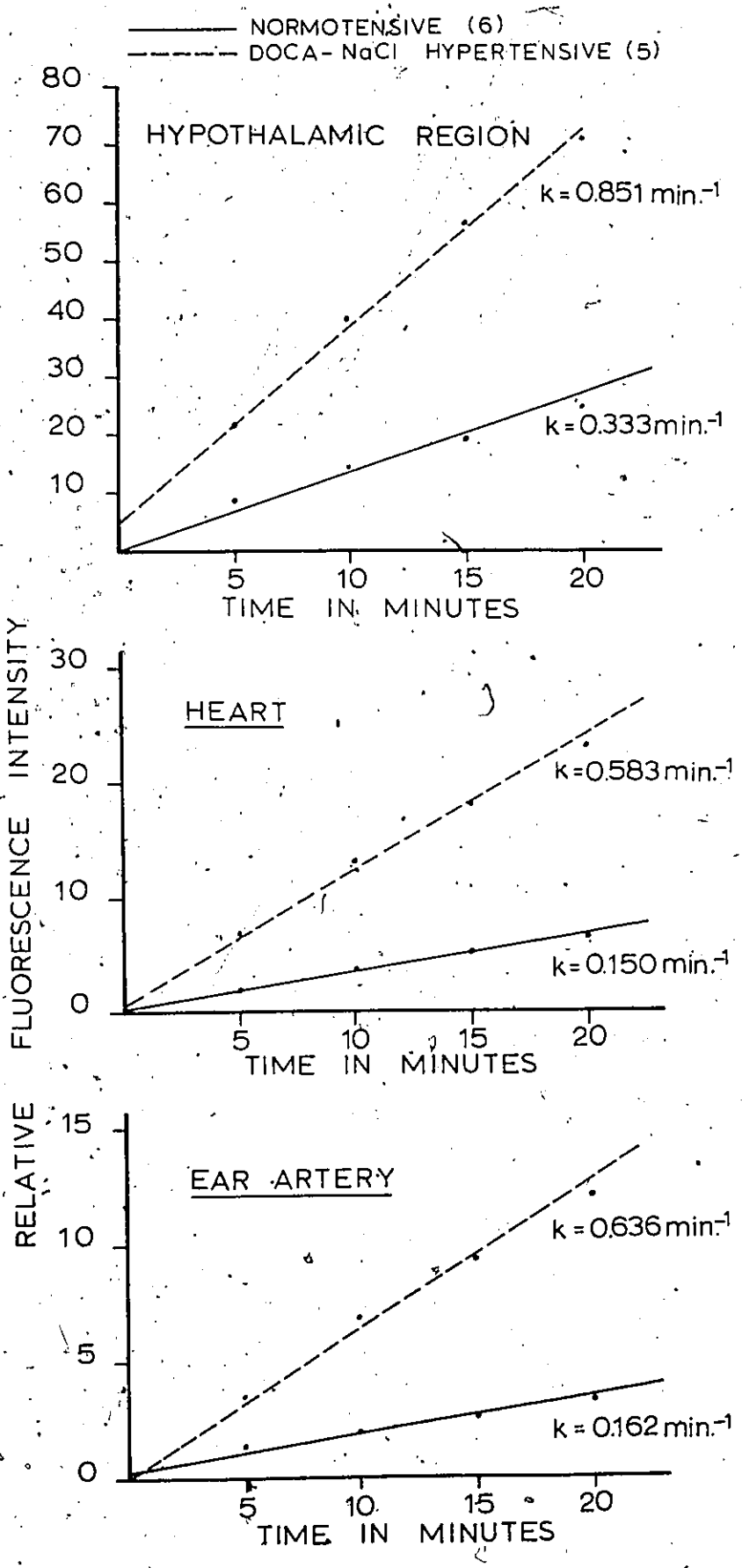


Fig. 22B. Effect of phenoxybenzamine (5×10^{-8} g/ml) on responses of isolated ear arteries from normotensive and DOCA-NaCl (5 weeks) hypertensive rabbits to intraluminal noradrenaline. Vertical bars represent S.E.M. and the numbers in parentheses denote the number of arteries in each group. POB = phenoxybenzamine.

Fig. 23. Monoamine oxidase (MAO) activity in the hypothalamic region, heart and ear artery of DOCA-NaCl (5 weeks) hypertensive rabbits compared to those of normotensive rabbits. Kynuramine was used as substrate for all tissues studied. MAO activity is measured as the increase in relative fluorescence intensity as a function of time due to the formation of 4-hydroxyquinoline during incubation of kynuramine with tissue homogenates. The numbers in parentheses denote the number of values in each group. k represents the rate of reaction.


NORMOTENSIVE (6)
DOCA-NaCl HYPERTENSIVE (5)
HYPOTHALAMIC REGION
k = 0.851 min.-1
k = 0.333 min.-1
80
70
60
50
40
30
20
10
5
10
15
20
TIME IN MINUTES
FLUORESCENCE INTENSITY
HEART
k = 0.583 min.-1
k = 0.150 min.-1
30
20
10
0
5
10
15
20
TIME IN MINUTES
RELATIVE
EAR ARTERY
k = 0.636 min.-1
k = 0.162 min.-1
15
10
5
0
5
10
15
20
TIME IN MINUTES

inhibition of flow in the control vessels was about 4-fold less after iproniazid, it was unchanged in the arteries from hypertensive rabbits.

Effect of Phenoxybenzamine on noradrenaline responses of ear arteries from DOCA-NaCl (5 weeks) hypertensive and normotensive animals

The development of hypersensitivity to noradrenaline in arteries from DOCA-NaCl (5 weeks) hypertensive rabbits might be due either to quantitative or qualitative changes of the alpha-receptors of the vascular smooth muscle. This possibility was tested by studying the effect of blockade of a large proportion of alpha-receptors with a moderate concentration of phenoxybenzamine (5×10^{-8} g/ml) on the responses to extraluminal and intraluminal noradrenaline in arteries from normotensive and hypertensive rabbits. As shown in Figs. 22A and B treatment with phenoxybenzamine appeared to produce equivalent blockade in both groups of arteries.

Monoamine oxidase activity in tissues from DOCA-NaCl (5 weeks) hypertensive rabbits

The depressed responses of arteries from DOCA-NaCl (5 weeks) hypertensive animals to extraluminal tyramine suggests that the endogenous content of noradrenaline is decreased. A factor which is known to be involved in regulating the intraneuronal level of noradrenaline is monoamine oxidase. Consequently, monoamine oxidase activity of tissues from DOCA-NaCl (5 weeks) hypertensive and normotensive rabbits was assayed fluorometrically.

There was a marked increase in monoamine oxidase activity in the heart, ear artery and hypothalamic region of the brain of DOCA-NaCl

(5 weeks) hypertensive rabbits. The rates of reaction calculated from the slopes of the time curves are shown in Fig. 23. Since the rate of reaction is proportional to the enzyme concentration (Neilands and Stumpf, 1958) it appears that the monoamine oxidase concentration of tissues from DOCA-NaCl hypertensive rabbits is higher than in normotensive rabbits. The mean weight of hearts of the hypertensive animals was about 20% lighter than those of the control animals.

Rate of flow of perfusion fluid through isolated arteries from untreated and DOCA-NaCl (5 weeks) hypertensive rabbits

The rate of flow of fluid through isolated perfused ear arteries was estimated by measuring the height of deflection produced by a writing lever on smoked kymograph paper. The lever was attached to an Andrews outflow recorder regulated by a repeating cycle timer operating on a 10 seconds cycle. Each vertical deflection represents total flow over a period of 8 seconds. The recorder was calibrated by displacing a known volume of air from the outflow recorder and determining the consequent lever deflection. A calibration curve of flow volume plotted against height of deflection of the writing lever was thus constructed and used for calculating flow rates.

The results obtained with the arterial segments from untreated and DOCA-NaCl (5 weeks) hypertensive animals are as follows:

FLOW RATES (ml/min)

Animals	Control Artery	Decentralized Artery	Denervated Artery
Untreated	18.9±1.2 (29)	---------	-------
Untreated	19.8±2.0 (7)	18.3±2.5 (7)	-------

Animals	Control Artery	Decentralized Artery	Denervated Artery
Untreated	18.6±4.5(7)	--------	20.7±2.3(7)
Hypertensive	21.0±0.3(33)	20.0±2.0(8)	------

The number of arteries in each group is indicated in parentheses.

There were no significant differences between the flow rates of any of the treated and untreated groups.

RENAL HYPERTENSION

Nerve Stimulation

Frequency-response curves to nerve stimulation of arteries from renal hypertensive rabbits are compared to those from normotensive rabbits in Fig. 24.

There were no significant differences between the responses of arteries with an intact sympathetic innervation from renal hypertensive and from normotensive animals. Decentralized (6 weeks) arteries from renal hypertensive rabbits were, however, significantly less responsive to nerve stimulation. In addition, the maximal response of the decentralized group of arteries was reduced about 50%. In contrast, after a long interval of decentralization (5 weeks) of arteries from normotensive rabbits, their responses to low frequency stimulation (0.5 and 1.0 pulse /sec) were significantly greater than controls($p<0.01$).

Responses to noradrenaline

Responses of arteries with an intact sympathetic innervation from renal hypertensive rabbits to extraluminal and intraluminal nora-

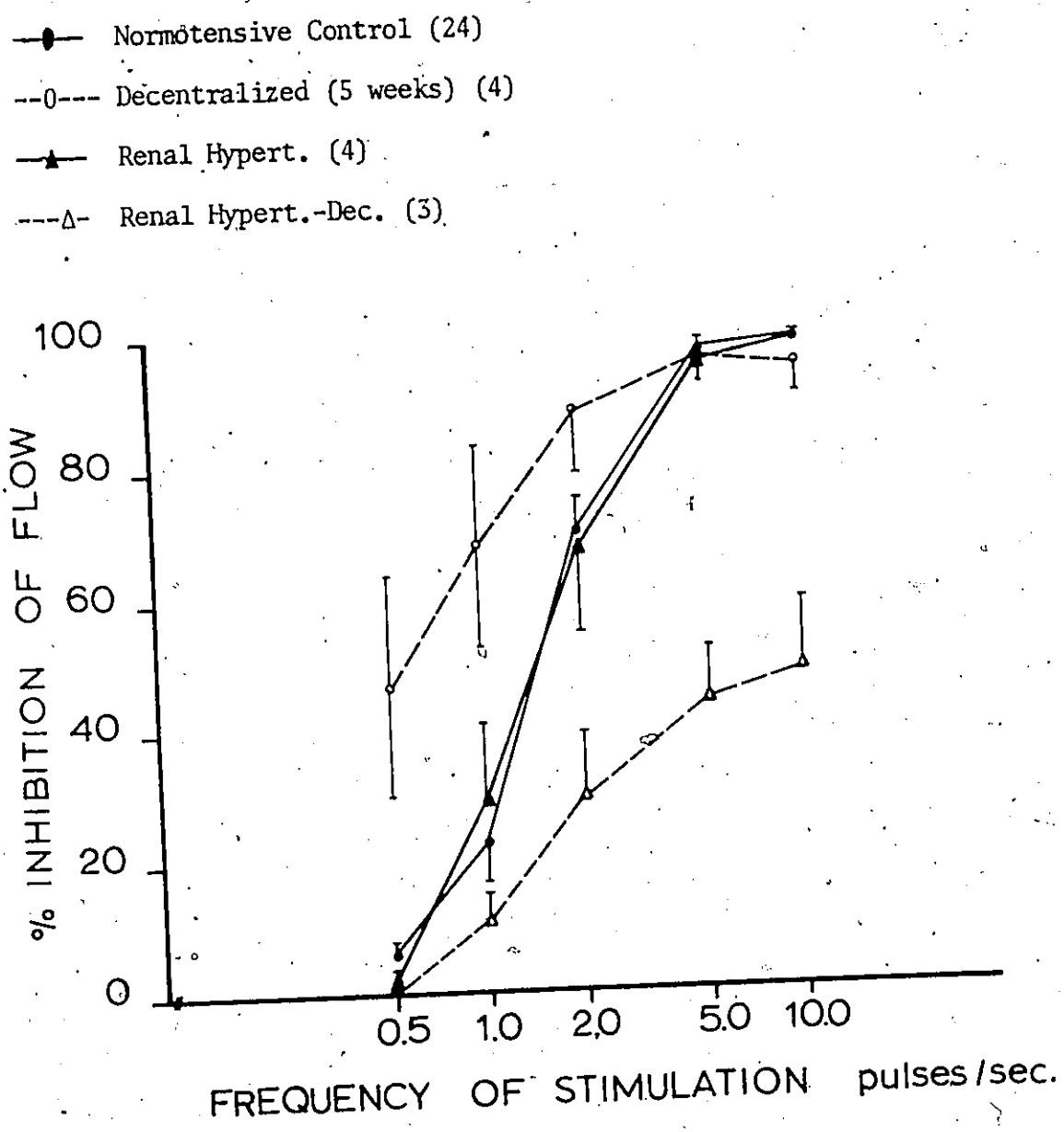


Fig. 24. Responses to periarterial nerve stimulation of decentralized and contralateral control perfused ear arteries from renal hypertensive and from normotensive rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of animals in each group.

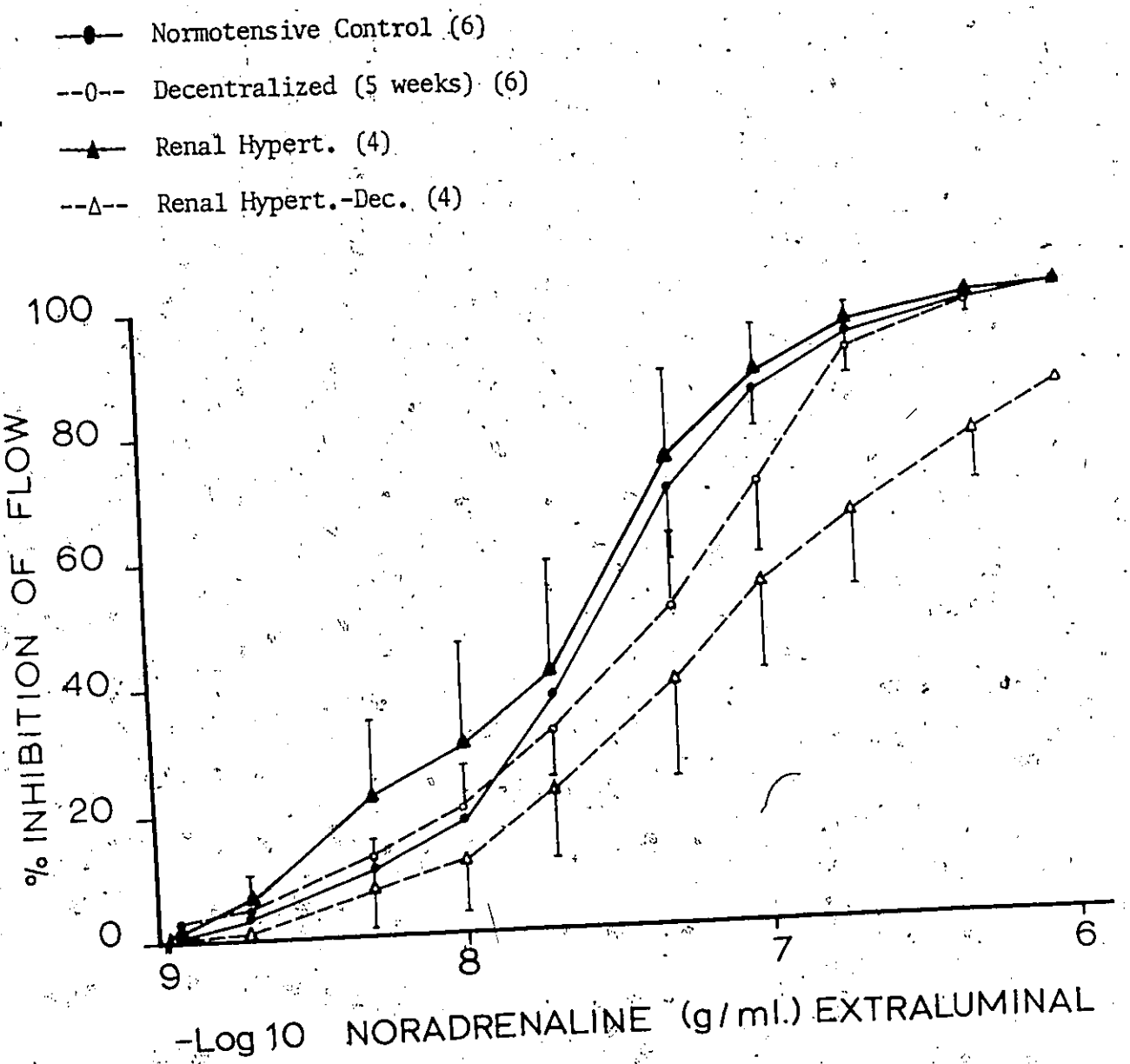


Fig. 25A. Responses to extraluminal noradrenaline of control and decentralized ear arteries from renal hypertensive rabbits compared to those of arteries from normotensive rabbits. Vertical bars represent S.E.M. and the numbers in parentheses denote the number of animals in each group.

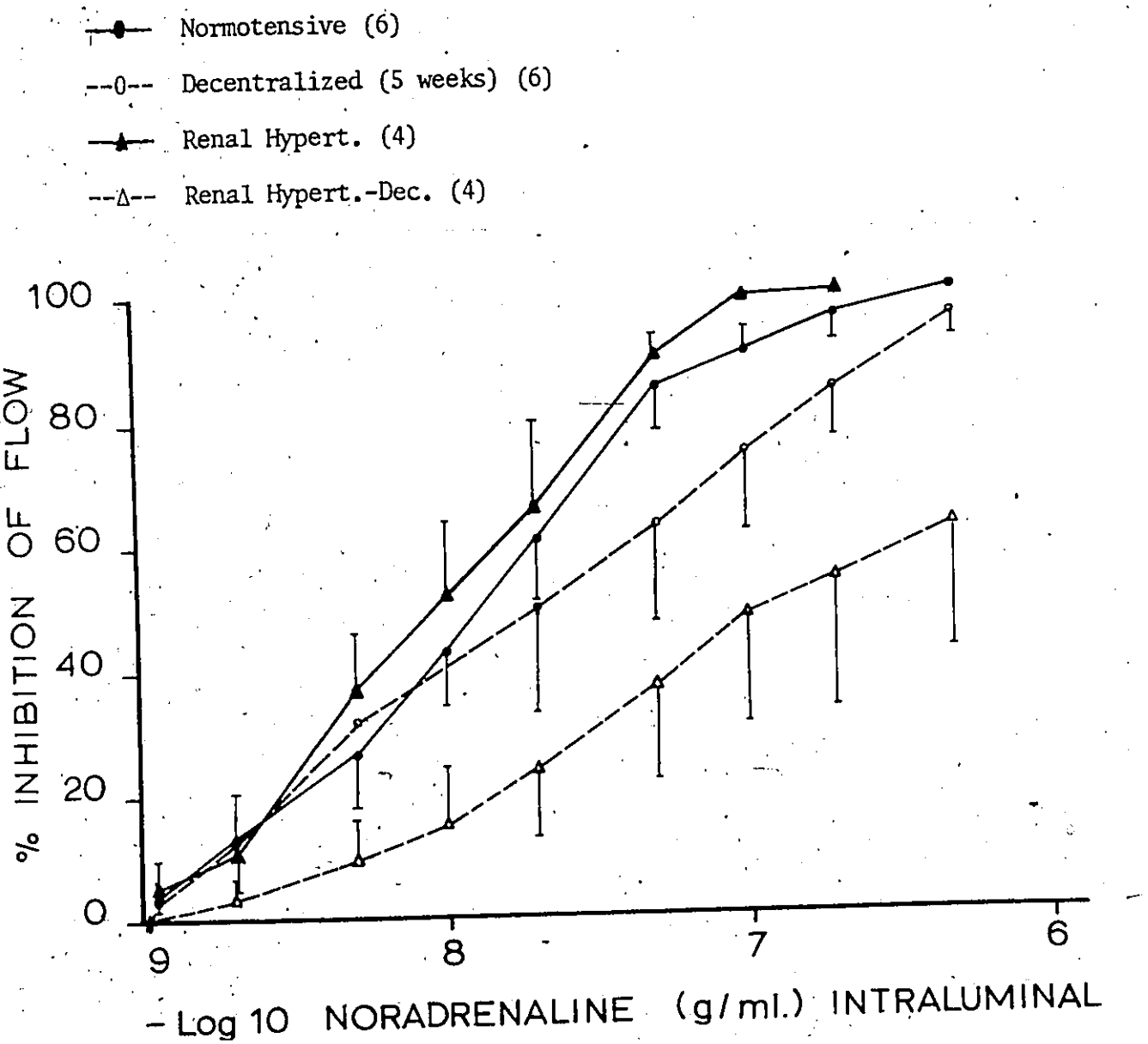


Fig. 25B. Responses to intraluminal noradrenaline of control and decentralized ear arteries from renal hypertensive rabbits compared to those of arteries from normotensive rabbits. Vertical bars represent S.E.M. and the numbers in parentheses denote the number of animals in each group.

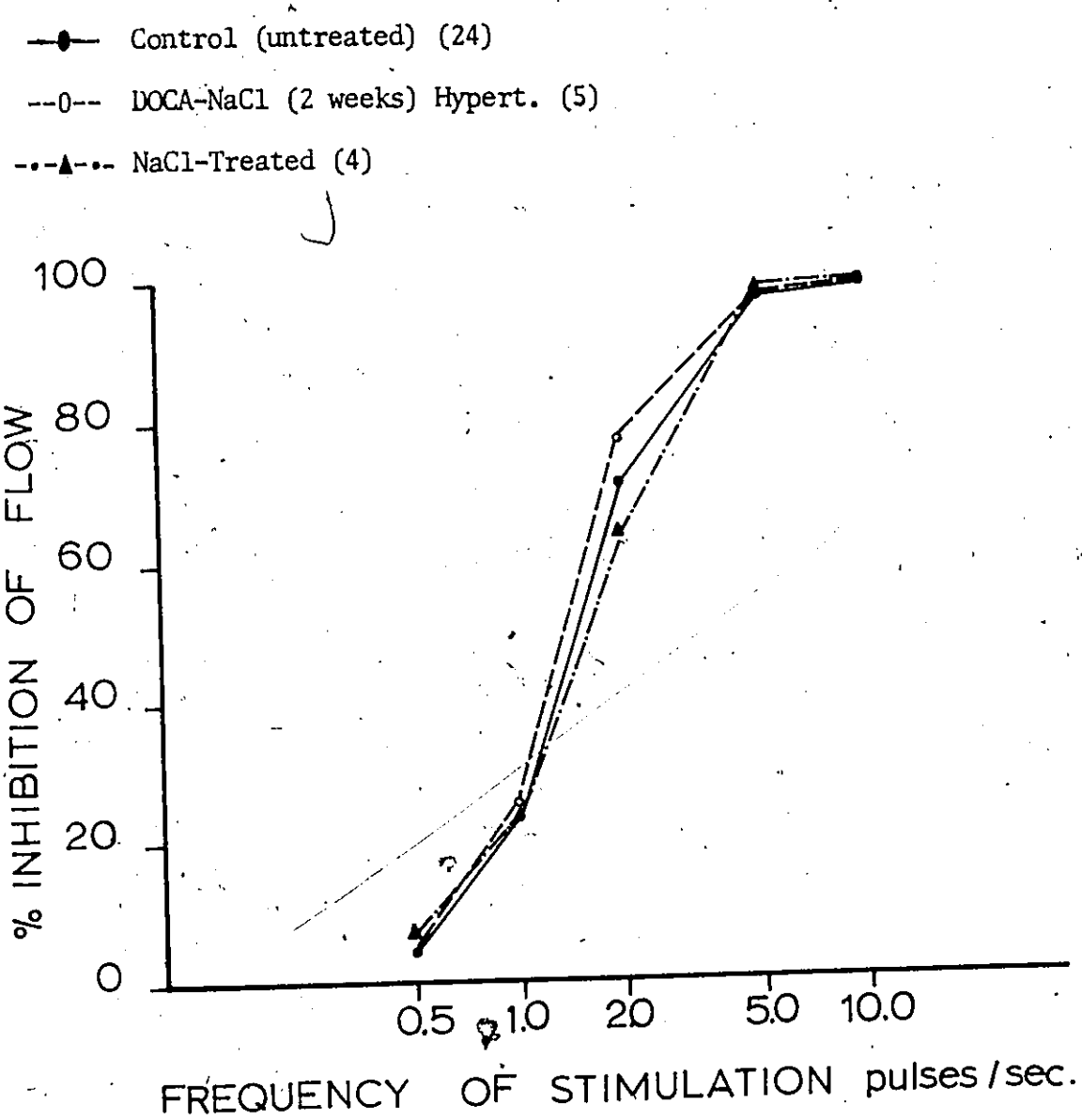


Fig. 26. Frequency-response curves to periarterial nerve stimulation of perfused ear arteries from untreated (control), sodium chloride treated and DOCA-NaCl (2 weeks) hypertensive rabbits. The numbers in parentheses denote the number of arteries in each group.

drenaline were similar to those of arteries from normotensive animals (Figs. 25A and B). Decentralized arteries from renal hypertensive rabbits were markedly less sensitive to extraluminal and intraluminal noradrenaline (at concentrations of 1×10^{-8} to 5×10^{-7} g/ml) compared to control arteries from either renal hypertensive or normotensive rabbits ($p<0.05$ to $p<0.01$). Following a long period of decentralization (5 weeks), arteries from untreated normotensive rabbits showed a tendency, but not significantly so, to be less sensitive to extraluminal and intraluminal noradrenaline compared to control arteries.

EFFECTS OF A CHRONIC HIGH SODIUM CHLORIDE INTAKE ON VASCULAR REACTIVITY

Experiments were performed to study the effects of a chronic high intake of sodium chloride on the reactivity of perfused isolated ear arteries from unilaterally nephrectomized rabbits. Animals were given a 1% solution of sodium chloride to drink instead of tap water for two weeks following unilateral nephrectomy. A comparison was then made between the reactivity of arteries from these animals and those from normotensive and from DOCA-NaCl (2 weeks) hypertensive rabbits.

Nerve Stimulation

Fig. 26 shows the frequency-response curves to nerve stimulation of arteries from sodium chloride treated animals, DOCA-NaCl (2 weeks) hypertensive and untreated normotensive rabbits. There were no significant differences between the frequency-response curves obtained in arteries from the two treated groups and the untreated group.

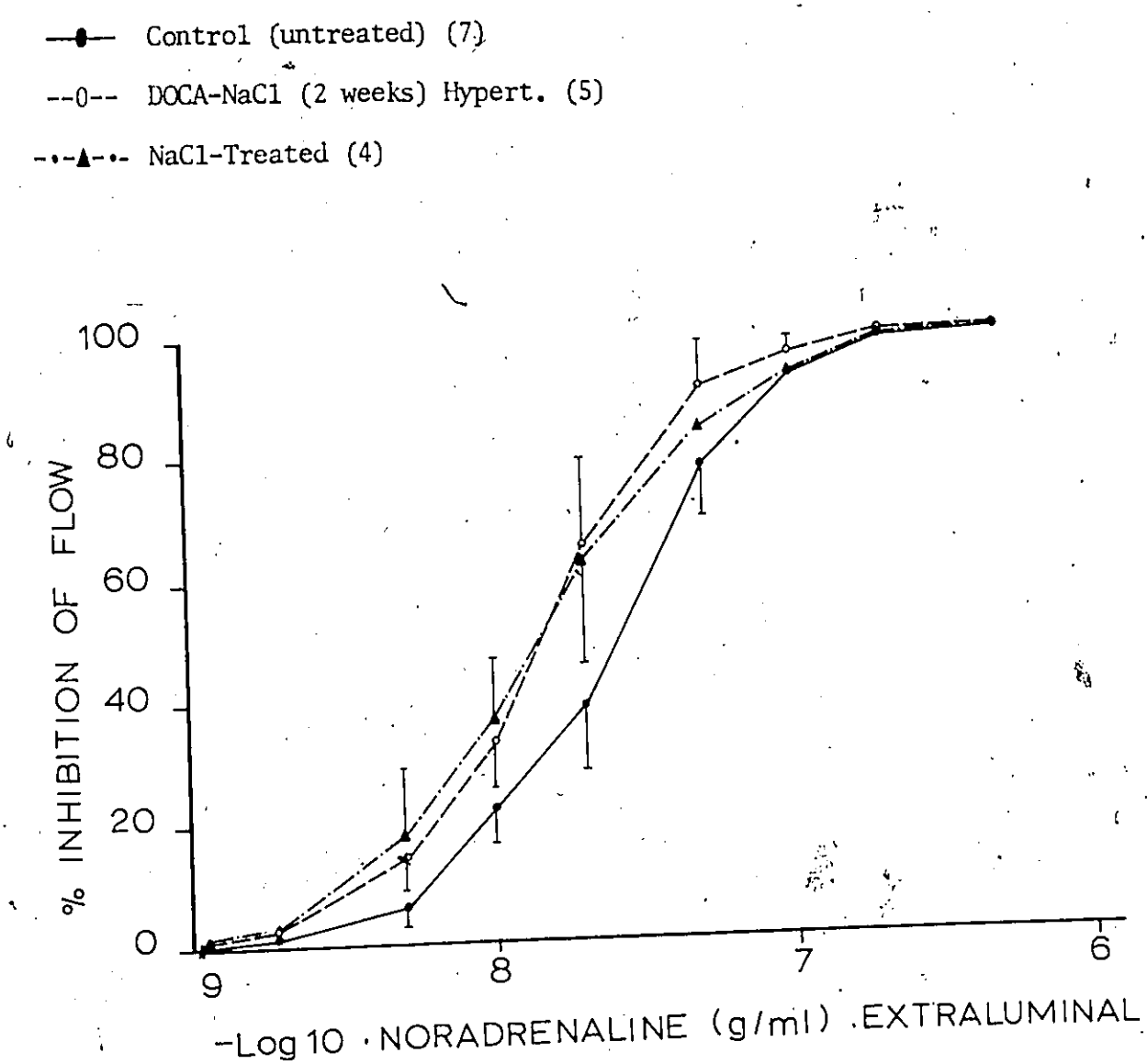


Fig. 27A. Dose-response curves of perfused ear arteries from untreated (control), DOCA-NaCl (2 weeks) hypertensive, and sodium chloride treated rabbits to extraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

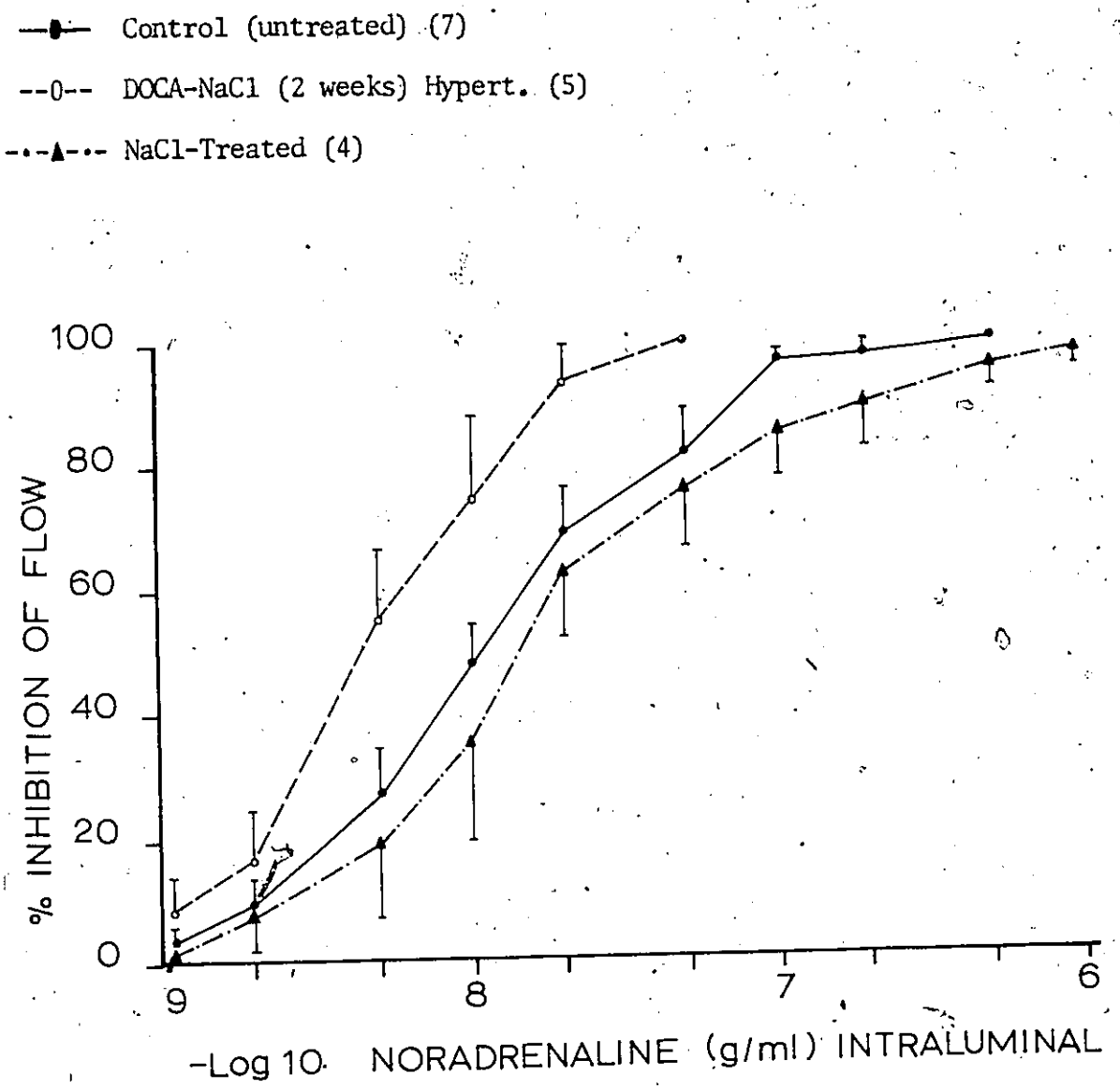


Fig. 27B. Dose-response curves of perfused ear arteries from untreated (control), DOCA-NaCl (2 weeks) hypertensive, and sodium chloride treated rabbits to intraluminal noradrenaline. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

Responses to noradrenaline

The responses to intraluminal noradrenaline of arteries from rabbits on a 1% sodium chloride solution (for two weeks) were similar to those of arteries from untreated animals and responses to extraluminal noradrenaline were enhanced, but not significantly. These results are shown in Figs. 27A and B. In contrast, arteries from DOCA-NaCl (2 weeks) hypertensive animals showed a significant increase in responses to intraluminal noradrenaline ($p<0.05$) as well as enhanced responses to extraluminal noradrenaline. Unilaterally nephrectomized rabbits treated with DOCA and drinking a solution of 1% sodium chloride and 0.5% potassium chloride had a significantly elevated blood pressure by the second week of treatment, while those on a 1% sodium chloride solution but not treated with DOCA had hardly any change in blood pressure over the same time period.

ADRENALECTOMY

It has been shown that there is an increase in both sympathetic nerve activity and in the synthesis rate of noradrenaline after bilateral adrenalectomy in animals (Imms and Jones, 1968; Westfall and Osada, 1969; Bhagat, 1969). To obtain more information on the interaction of the sympathetic nervous system with sodium on vascular reactivity, arteries from bilaterally adrenalectomized rabbits with and without additional treatment with sodium chloride were used for perfusion experiments.

A group of rabbits were bilaterally adrenalectomized and after a period of about two weeks the systolic blood pressure was

TABLE 3

MEAN VALUES (±S.E.M.) OF TOTAL SODIUM AND POTASSIUM CONTENTS OF ARTERIES FROM CONTROL ADRENALECTOMIZED AND SODIUM CHLORIDE TREATED PLUS ADRENALECTOMIZED RABBITS

No. of Values	Tissue and Treatment	Sodium mEq/Kg Wet Weight	P Value	Potassium mEq/Kg Wet Weight	P Value
	1. AORTA				
8	Control	27.2±1.3		11.0±2.3	
4	Adrenalectomized	25.6±1.7		7.5±0.9	
5	Adrenalectomized plus NaCl	37.0±5.1	<0.05	11.1±3.1	
	2. FEMORAL ARTERY				
6	Control	39.3±3.9		17.1±6.3	
4	Adrenalectomized	28.7±4.1		3.7±1.6	<0.05
5	Adrenalectomized plus NaCl	55.1±4.6	<0.05	22.3±4.3	

S.E.M. = Standard Error of the Mean

P Values indicate significant differences between control and treated arteries.

70.5±0.9 mm Hg which did not differ significantly from the pre-operative pressure level of 74.5±1.7 mm Hg. Moreover, another group of bilaterally adrenalectomized rabbits given 1% sodium chloride solution instead of drinking water had, at the end of the treatment, a mean systolic blood pressure of 70.0±2.6 mm Hg which was again not different from the control value, 72.0±3.1 mm Hg.

Electrolyte Distribution in adrenalectomized rabbits

Table 3 shows the electrolyte distribution in the femoral artery and aorta of untreated (control) and bilaterally adrenalectomized rabbits with and without sodium chloride treatment.

Two weeks following adrenalectomy, the sodium and potassium contents of the aorta and the sodium content of the femoral artery did not differ from control values. The potassium content of the femoral artery was significantly decreased compared to the control value. ($p<0.05$).

The sodium content of the aorta and femoral artery of adrenalectomized rabbits fed 1% sodium chloride solution was significantly elevated compared to that of arteries of untreated animals ($p<0.05$). The potassium content, however, was similar to the control values.

Effects of adrenalectomy and adrenalectomy plus high sodium chloride intake on vascular reactivity

Nerve Stimulation

Fig. 28 shows the frequency-response curves to nerve stimulation of arteries from adrenalectomized rabbits (Fig. 28A) and the frequency-response curves of arteries from adrenalectomized rabbits

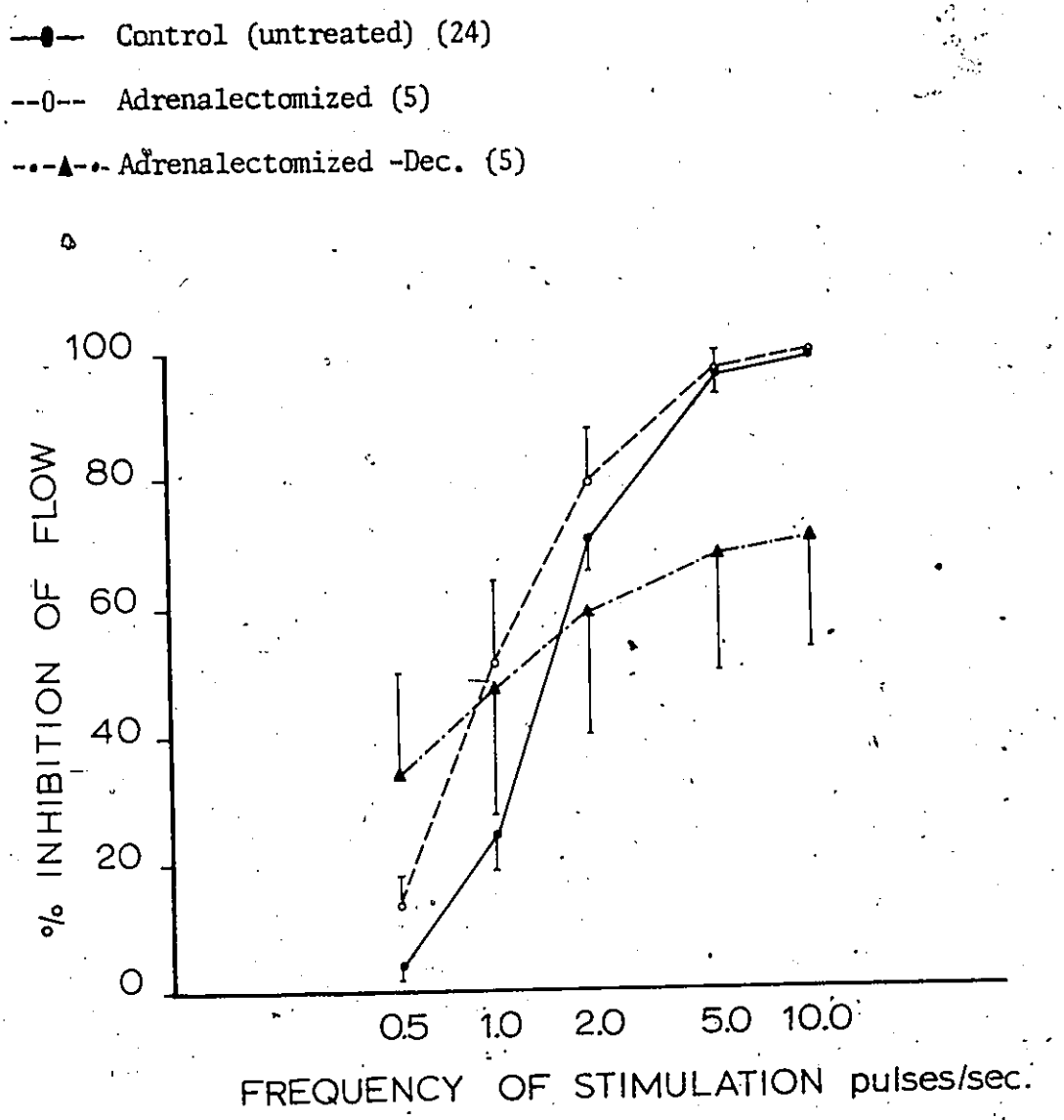


Fig. 28A. Effect of bilateral adrenalectomy on responses of perfused ear arteries from rabbits to periarterial nerve stimulation. The frequency-response curves of decentralized (2 weeks) and control arteries from adrenalectomized rabbits are compared to those of arteries from untreated (control) rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

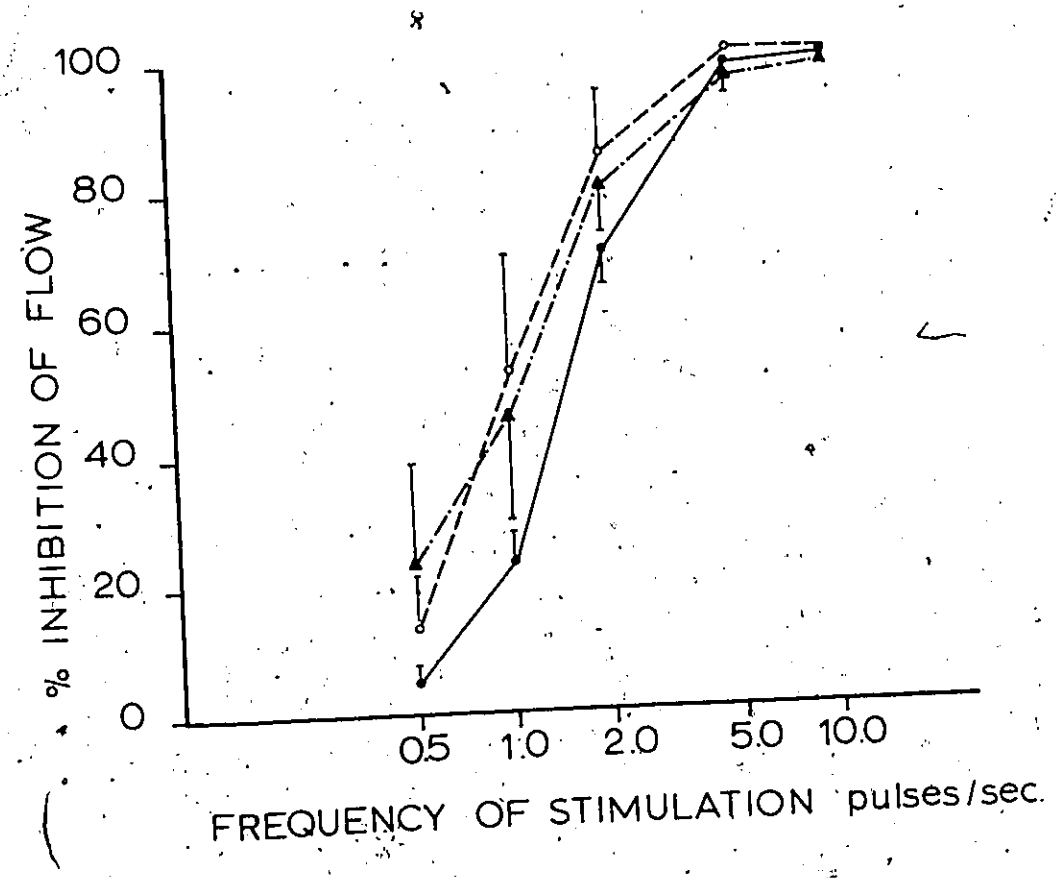


Fig. 28B. Effects of bilateral adrenalectomy with additional high sodium chloride intake on responses of perfused ear arteries from rabbits to perivarterial nerve stimulation. The frequency-response curves of decentralized (2 weeks) and control arteries from adrenalectomized rabbits drinking sodium chloride solution are compared to those of arteries from untreated (control) rabbits. The vertical bars represent the S.E.M. and the numbers in parentheses denote the number of arteries in each group.

given sodium chloride solution instead of drinking water (Fig. 28B). These groups are compared to the untreated group.

Two weeks following adrenalectomy, responses of control arteries to nerve stimulation were enhanced at low frequencies, but not significantly, compared to those of arteries from untreated animals (Fig. 28A). The threshold response of decentralized arteries from adrenalectomized animals to nerve stimulation was significantly greater than that of arteries from untreated animals ($p<0.01$), but the maximal response was significantly decreased ($p<0.01$). However, after giving adrenalectomized rabbits sodium chloride solution instead of drinking water, the decentralized arteries gave responses which were similar to those of the contralateral arteries with an intact sympathetic innervation and slightly but not significantly greater than those of untreated controls. These results are shown in Fig. 28B. The preceding results (Fig. 5) showed that responses of decentralized (2 weeks) arteries from untreated rabbits to nerve stimulation did not differ from control arteries except that the maximal response was significantly decreased ($p<0.01$).

Responses to Noradrenaline

Responses to noradrenaline of arteries from untreated and from adrenalectomized rabbits with and without added dietary sodium chloride are shown in Figs. 29 and 30.

Two weeks following adrenalectomy, responses to extraluminal noradrenaline of arteries with an intact sympathetic innervation did not differ from those of untreated animals, but the decentralized arteries

—●— Control (untreated) (7)

--0-- Adrenalectomized (6)

-•-▲-•- Adrenalectomized -Dec. (6)

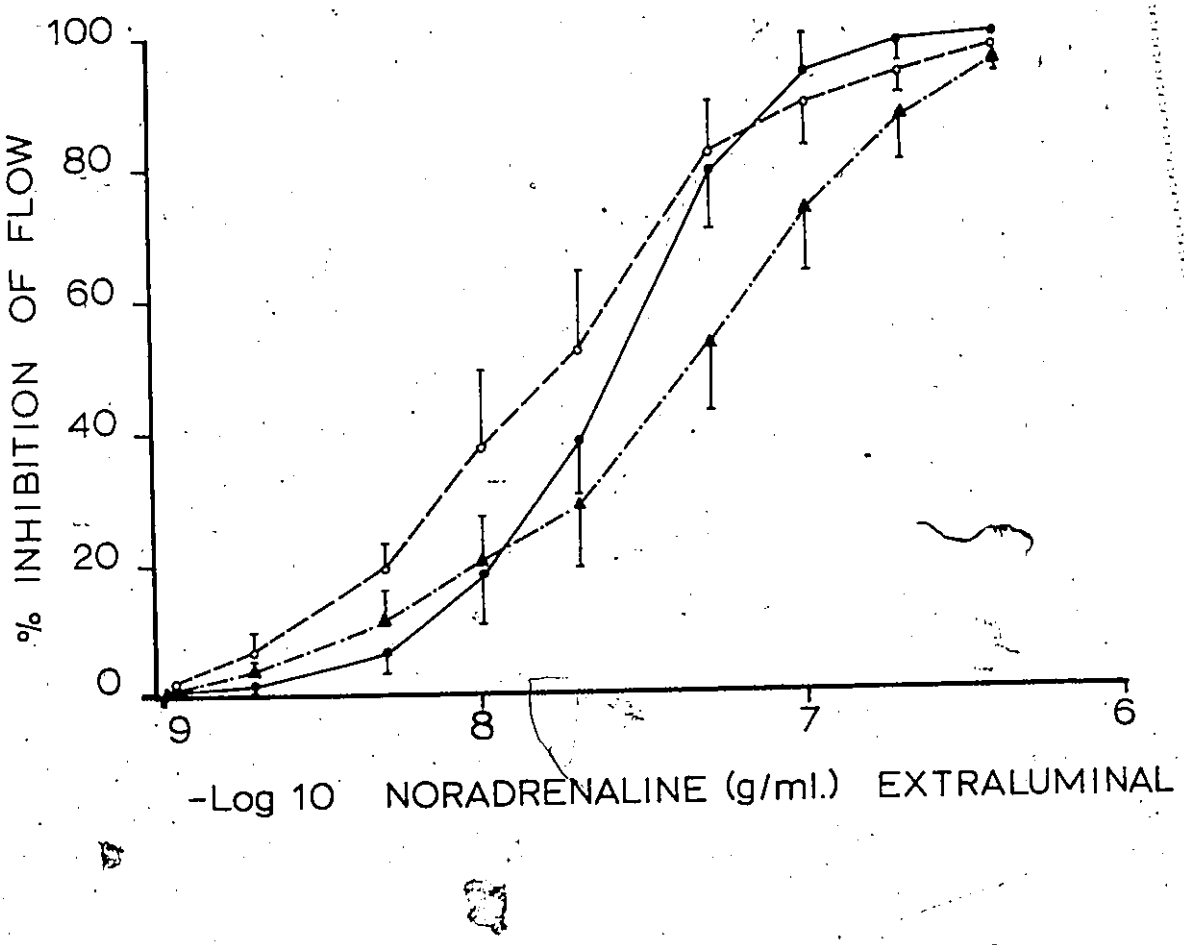


Fig. 29A. Effect of bilateral adrenalectomy on responses of perfused ear arteries from rabbits to extraluminal noradrenaline. The dose-response curves of decentralized (2 weeks) and control arteries from adrenalectomized rabbits are compared to those of arteries from untreated (control) rabbits. The vertical bars represent the S.E.M. and numbers in parentheses denote the number of arteries in each group.

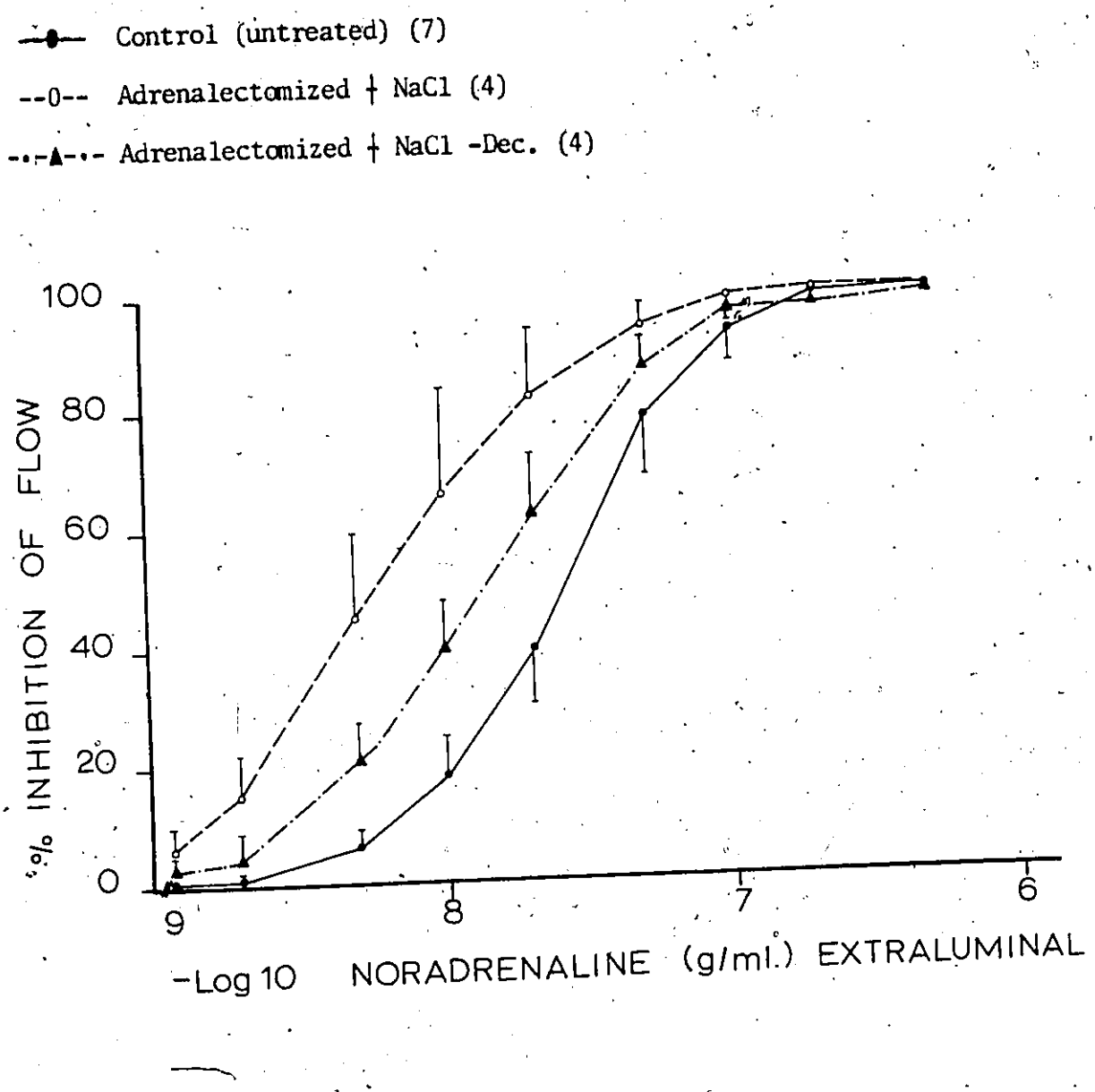


Fig. 29B. Effects of bilateral adrenalectomy with additional high sodium chloride intake on responses of perfused ear arteries from rabbits to extraluminal noradrenaline. The dose-response curves of decentralized (2 weeks) and control arteries from adrenalectomized rabbits drinking sodium chloride solution are compared to those of arteries from untreated (control) rabbits. The vertical bars represent the S.E.M. and numbers in parentheses the number of arteries in each group.

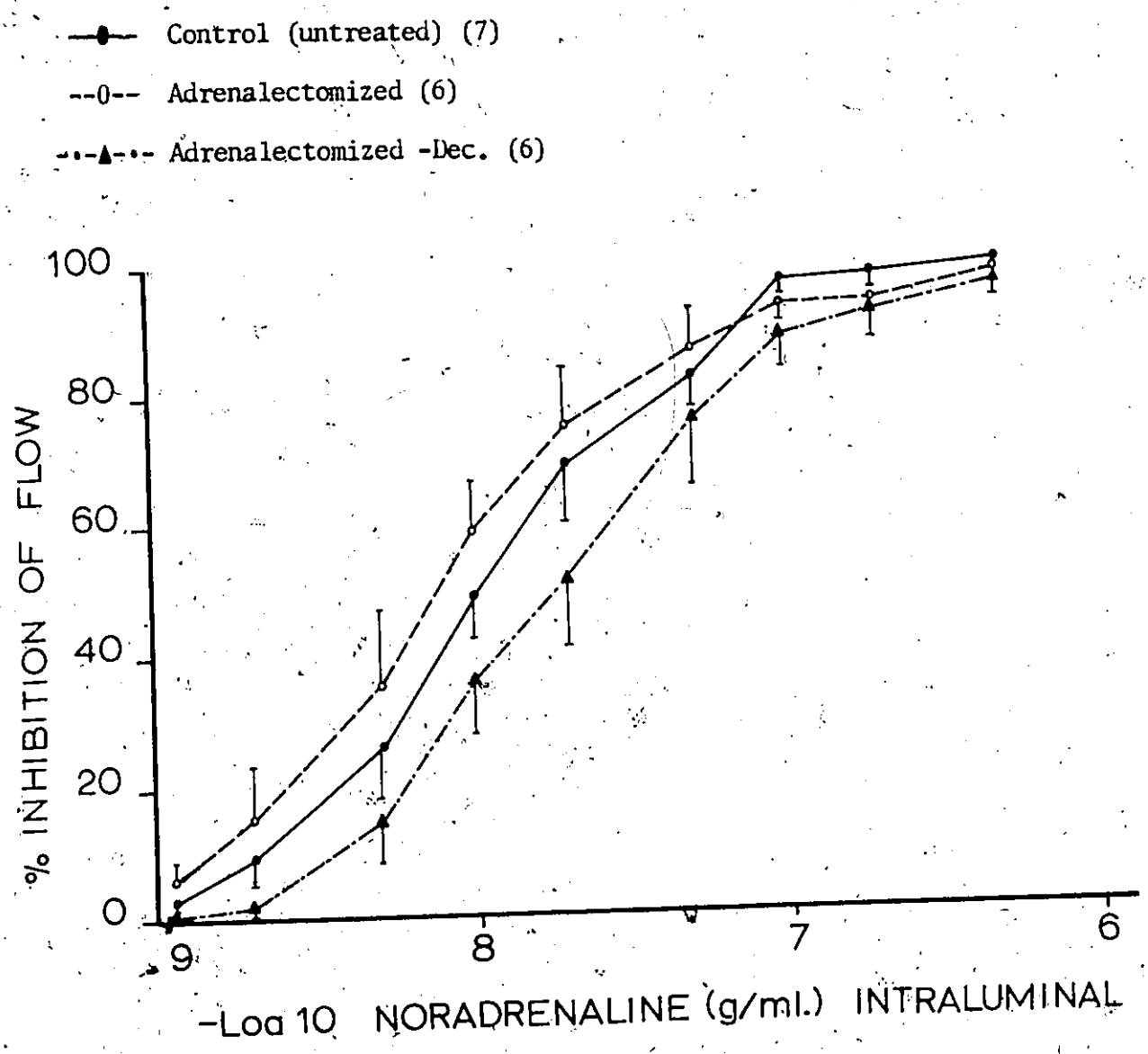


Fig. 30A. Effect of bilateral adrenalectomy on responses of perfused ear arteries from rabbits to intraluminal noradrenaline. The dose-response curves of decentralized (2 weeks) and control arteries from adrenalectomized rabbits are compared to those of arteries from untreated (control) rabbits. The vertical bars represent the S.E.M. and numbers in parentheses denote the number of arteries in each group.

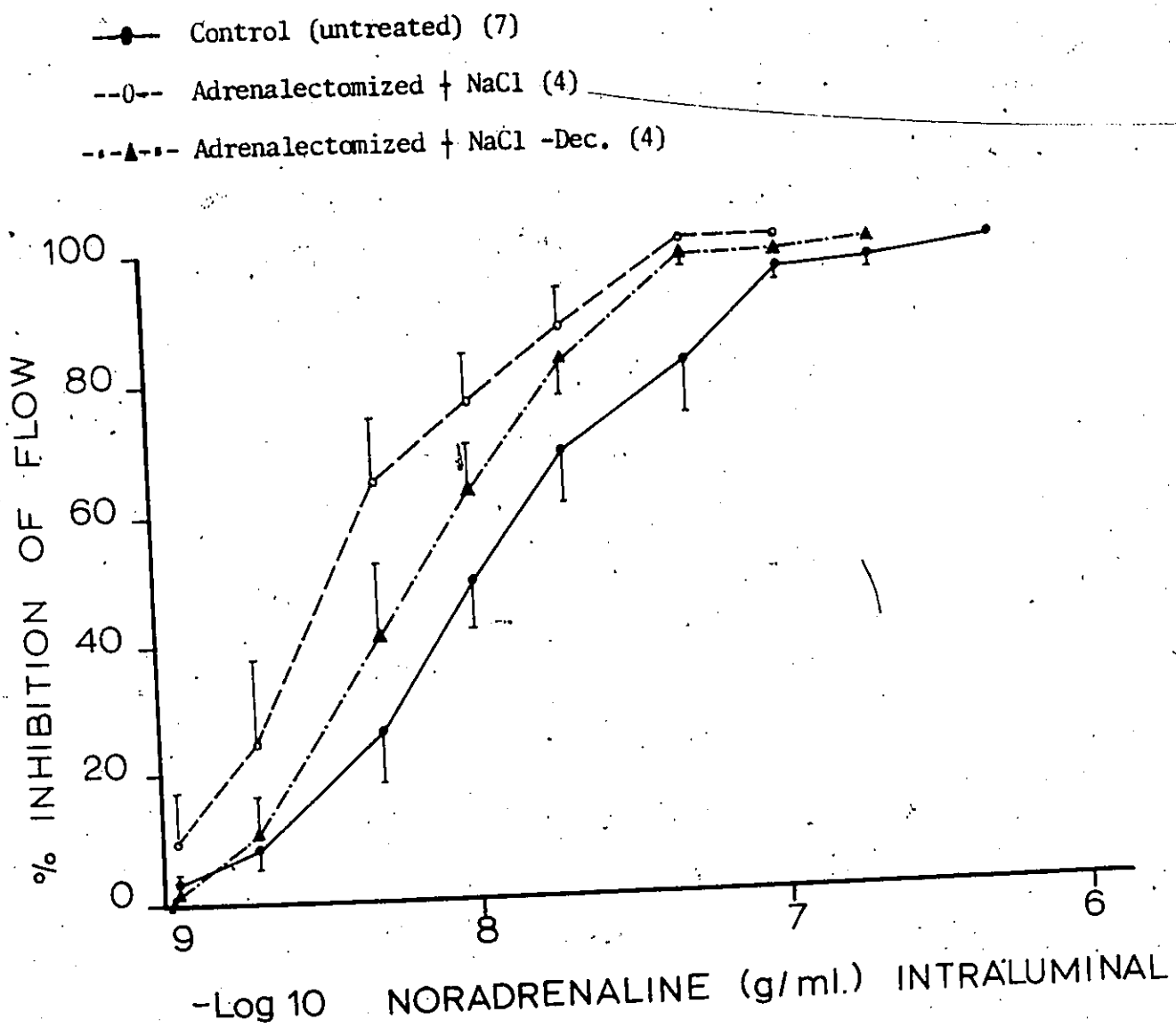


Fig. 30B. Effect of bilateral adrenalectomy with additional high sodium chloride intake on responses of perfused ear arteries from rabbits to intraluminal noradrenaline. The dose-response curves of decentralized (2 weeks) and control arteries from adrenalectomized rabbits drinking sodium chloride solution are compared to those of arteries from untreated (control) rabbits. The vertical bars represent the S.E.M. and numbers in parentheses denote the number of arteries in each group.

from adrenalectomized animals showed a tendency to be less responsive to extraluminal noradrenaline (Fig. 29A). However, after adrenalectomized animals had been given sodium chloride solution instead of drinking water, the control arteries exhibited a significant increase in sensitivity to extraluminal noradrenaline compared to those of arteries from untreated animals ($p<0.05$; $p<0.01$) and the decentralized arteries also showed enhanced responses, but in this case the differences were not statistically significant (Fig. 29B). The same situation held with respect to responses of control and decentralized arteries from adrenalectomized rabbits to intraluminal noradrenaline (Fig. 30A and B).

V. DISCUSSION

Hypertension due to the chronic administration of desoxycorticosterone acetate and sodium chloride (DOCA-NaCl) was produced in rabbits only when potassium chloride was included in the treatment regimen. In preliminary experiments, the omission of potassium chloride resulted in paralysis and death of the rabbits. Masson et al. (1953) treated rabbits with DOCA-NaCl and reported that the animals developed polyuria, muscular weakness and/or paralysis but not hypertension. However, these adverse effects have not been observed in rats and it appears that rabbits are more susceptible to the toxic effects of DOCA-NaCl treatment. It has been claimed that the paralysis which develops after the administration of DOCA-NaCl to rabbits is linked to the replacement of intracellular potassium by sodium in skeletal muscle (Ferrebee et al., 1941). Moreover, severe potassium depletion in animals is known to lead to hypotension and a diminished vascular reactivity to pressor agents (Rosenman et al., 1952). It appears that the maintenance of a critical Na/K ratio in vascular smooth muscle cells is essential during the induction of DOCA-NaCl hypertension in rabbits.

The present results demonstrate a generalized increase in the sodium content of arteries in rabbits with either DOCA-NaCl or renal hypertension. Since small arteries have a greater influence on total peripheral resistance than do large arteries, the elevated sodium content found in the central ear arteries may be particularly pertinent to the development of hypertension. This finding is, in part, consistent with the prevailing view that an elevated sodium content

is associated with both experimentally induced (Tobian and Binion, 1954; Tobian, 1956; Ayitey-Smith and Varma, 1970) and essential hypertension (Tobian, 1952; Ross, 1956).

During the early developmental phase of DOCA-NaCl (2 weeks) hypertension, when the blood pressure was increasing steadily, the arterial sodium was already significantly elevated. In fact, the arterial sodium level in these rabbits was significantly higher than in those treated with DOCA-NaCl for 5 weeks, although the blood pressure was greater in the latter group. This observation suggests that there is no exact correlation between blood pressure and the level of arterial sodium. However, it is clear that the elevation of blood vessel sodium occurs early in the onset of DOCA-NaCl hypertension and that it may be involved in its pathogenesis.

It is interesting that the sodium content of the hypothalamic region but not the cerebral cortex of the rabbit brain was elevated in hypertensive rabbits. The hypothalamus is known to play a key role in the regulation of arterial pressure (Manning and Peiss, 1960) and has been shown to participate in the development of spontaneous hypertension (Yamori and Okamoto, 1969). The present finding may mean that centers in the brain concerned with arterial pressure regulation also participate in the pathogenesis of DOCA-NaCl hypertension.

The present study revealed that the lack of a functional sympathetic innervation (decentralization) did not alter the sodium and potassium contents of the central ear arteries, nor did it modify the marked elevation in tissue content of sodium produced by treat-

ment with DOCA-NaCl. It seems, therefore, that there is no direct relationship between arterial sodium and potassium distribution or the sodium depositing effect of DOCA and the state of activity of the sympathetic nervous system. Several workers have shown that in immunosympathectomized (IMS) rats (Ayitey-Smith and Varma, 1970) or after chemical sympathectomy produced by guanethidine in man (Gill et al., 1964) the administration of DOCA-NaCl resulted in a greater excretion of sodium than in controls and Ayitey-Smith and Varma reported that aortic sodium was not elevated in IMS rats treated with DOCA. This may mean that the influence of the sympathetic nervous system on the sodium retaining effect of DOCA is not at the level of the vascular smooth muscle but exerted indirectly, perhaps through the renal system.

There was no clear correlation between the level of arterial potassium and hypertension. In the present experiments the potassium content of the ear arteries from DOCA-NaCl hypertensive rabbits was increased but no consistent trend was observed in other arteries tested. Moreover, it was found that although sympathetic decentralization did not alter the potassium content of the ear artery of control animals it reduced the elevation in potassium content produced by treatment with DOCA-NaCl. Therefore, the sympathetic nervous system may, in part, influence the effect of DOCA on arterial potassium distribution.

It is well known that section of the postganglionic sympathetic nerve to innervated organs (denervation) depletes noradrenaline stores consequent to nerve degeneration (Kirpekar et al., 1962; Trendelenburg, 1963). Consequently, denervated organs do not respond

to nerve stimulation. The effectiveness of surgical denervation of the central ear artery was confirmed in the present experiments by the lack of vasoconstrictor responses to periarterial nerve stimulation. It has, however, been shown that decentralization has no effect on the noradrenaline stores in innervated organ (Kirpekar et al., 1962; Trendelenburg, 1963). The data from the present study show that two weeks after decentralization, arteries from untreated animals gave responses to nerve stimulation at low frequencies which did not differ from those of contralateral arteries with an intact sympathetic innervation. However, responses to high frequency stimulation were significantly decreased compared to contralateral control arteries. Five weeks following decentralization, responses to nerve stimulation were comparable to those of contralateral control arteries at all frequencies. It seems that responses to nerve stimulation are impaired by short-term (2 weeks) but not by long-term (5 weeks) decentralization.

The present results showed that at an early stage in the development (2 weeks) of DOCA-NaCl hypertension the responses of arteries to nerve stimulation were unchanged. However, at a more advanced stage (5 weeks) the responses of the arteries to nerve stimulation and to the pharmacologic release of noradrenaline by tyramine were significantly reduced. Baum and Shropshire (1967) previously reported that at the second week in the development of renal hypertension in rats the vascular bed of the hindquarters showed increased responses to sympathetic nerve stimulation, however, responses were slightly less than controls by the 12th week. Haeusler and Haefely (1970) also observed reduced

responses to periarterial nerve stimulation in isolated perfused mesenteric arteries from renal hypertensive rats which were also treated with DOCA and sodium chloride for 6 to 8 weeks. These findings might mean that the quantity of noradrenaline released from the sympathetic nerve terminals per nerve impulse decreased with the progression of DOCA-NaCl hypertension. This abnormality might reside at the level of the nerve terminals which are sites of noradrenaline release by conducted impulses and by tyramine (Burn and Rand, 1958; Malmfors, 1964, 1965). Moreover, endogenous noradrenaline stores have been reported to be reduced in the hearts of DOCA-NaCl hypertensive animals (De Champlain et al., 1968).

The depressed responses of arteries from DOCA-NaCl hypertensive rabbits and of decentralized arteries to nerve stimulation from untreated rabbits appear to involve essentially different mechanisms. In the present study the frequency-response curve of arteries from DOCA-NaCl (5 weeks) hypertensive animals showed a parallel shift to the right without any change in slope or maximal response. However, the frequency-response curve of arteries from untreated animals which were decentralized for two weeks were not only shifted to the right but the slope and maximal response were markedly decreased. Furthermore, as discussed below the characteristics of response to exogenous noradrenaline differed in the two groups. It was also observed that although responses of arteries from DOCA-NaCl (5 weeks) hypertensive animals to short periods of stimulation were depressed, during prolonged and continuous nerve stimulation the responses of arteries from DOCA-NaCl (5 weeks) hyper-

tensive animals were sustained almost as well as those of control arteries.

Several workers have presented evidence associating increased vascular reactivity with various forms of experimentally induced hypertension (Sturtevant, 1955, 1956; McQueen, 1956, 1961; Gordon and Nogueira, 1962; Hinke, 1965, 1966 and McGregor and Smirk, 1968) and essential hypertension (Greisman, 1954, 1955; Doyle et al., 1959; Mendlowitz et al., 1962). However reports of other workers are not in agreement (Redleaf and Tobian, 1957, 1958; Mallov, 1959; Zimmerman et al., 1969). The question of increased vascular reactivity in hypertension is presently unresolved.

In the current study a significant increase in vascular reactivity of arteries with an intact sympathetic innervation from DOCA-NaCl treated rabbits was observed at both the early (2 weeks) and advanced (5 weeks) stages of the hypertension. Previous reports have suggested that increased vascular reactivity develops early in essential hypertension (Mendlowitz et al., 1962). It appears that the increased vascular responsiveness, like the electrolyte disturbance, does not occur merely as a result of the elevated blood pressure in DOCA-NaCl hypertensive animals but may be an important factor in the pathogenesis of this form of hypertension. Decentralization of nonvascular tissues (Trendelenburg, 1963; Westfall, 1970) and denervation of the arterial vessel (De la Lande et al., 1967) also lead to increased responsiveness to noradrenaline. Thus decentralization or denervation supersensitivity provides a basis for comparison with DOCA-NaCl hypertension.

De la Lande et al. (1967) proposed that noradrenaline storage

sites (nerve terminals) in the medial adventitial border of the central ear artery of the rabbit influence the sensitivity of the artery to extraluminally applied noradrenaline. According to these workers extraluminally applied noradrenaline diffuses from the adventitia and it is exposed to and taken up by storage sites in the nerve terminals thus reducing its concentration before it enters the smooth muscle layer. On the other hand, the concentration and thus physiological action of intraluminal noradrenaline on the smooth muscle is less affected by uptake because noradrenaline is exposed to storage sites at the nerve terminals only after it has diffused through the smooth muscle layer. Consequently, De la Lande et al. (1967) have attributed the specific supersensitivity to extraluminal noradrenaline but not to intraluminal noradrenaline, which develops after denervation (2 weeks) of the arteries to a defect in nerve uptake.

There is no clear information on the effects of decentralization on the reactivity of the arterial vessel, however, evidence on supersensitivity resulting from decentralization of the nictitating membrane and vas deferens suggests that an alteration at the level of the smooth muscle is involved in this phenomenon (Fleming, 1963; Trendelenburg, 1966; Westfall, 1970).

The data obtained in the present study are not entirely in agreement with the results of De la Lande. It was found that two weeks following either decentralization or denervation, arteries from untreated rabbits demonstrated a significantly increased sensitivity to low concentrations of extraluminal but not to intraluminal noradrenaline.

There was little difference in the sensitization produced by these two procedures. Since it has been shown that uptake proceeds normally in decentralized vessels it appears that denervation as well as decentralization supersensitivity may have a postsynaptic basis in vascular tissue.

After long-term decentralization (5 weeks) the arteries showed a tendency to be less sensitive to both extraluminal and intraluminal noradrenaline. This finding suggests that sympathetic denervation or decentralization does not always result in an increased responsiveness of vascular smooth muscle to noradrenaline. In fact, it seems that in the long-term absence of a tonic or trophic influence of the sympathetic nervous system the vascular tissue tends to lose sensitivity to exogenous noradrenaline.

Contrary to the responses characteristic of decentralized or denervated vessels from untreated rabbits the innervated arteries from DOCA-NaCl hypertensive rabbits demonstrated a significant increase in sensitivity to high concentrations of intraluminally administered noradrenaline. In this case, it appears quite clear that an alteration in the sensitivity of the smooth muscle to the amine is involved. Therefore, it is unlikely that the increased vascular reactivity exhibited by the arteries from DOCA-NaCl hypertensive rabbits was caused by pathological denervation or decentralization of two or five weeks duration. It may, in part, be associated with an alteration in arterial electrolytes since the sodium and potassium contents of the ear arteries were elevated. The supersensitivity of the arteries

from untreated animals following 2 weeks denervation or decentralization appears not to involve an alteration in vascular smooth muscle electrolytes since the sodium and potassium contents were unchanged after sympathetic decentralization. Westfall (1970) also reported that the electrolyte content of vas deferens is unchanged after decentralization.

An increased intracellular sodium is reported to lead to increased vascular reactivity in arterial smooth muscle (Raab, 1952, 1959). According to Raab, the sodium gradient across the cell membrane (the Na_i/Na_o ratio) influences the membrane potential which in turn determines the contractile amplitude of muscle cells upon adequate stimulation. However, other workers relate sodium to contraction in a more indirect way (Somlyo and Somlyo, 1968).

The present observation that in the absence of a functional sympathetic innervation the responses of the ear arteries from DOCA-NaCl (5 weeks) hypertensive animals were markedly decreased rather than increased, although the characteristic increase in arterial sodium still occurred, raises doubt that increased intracellular sodium alone is the cause of the increase in vascular reactivity or the elevation in blood pressure in hypertension. If the increase in arterial sodium content in DOCA-NaCl (5 weeks) treated rabbits accurately reflected the state of intracellular sodium one would anticipate, according to Raab, the same increase in vascular reactivity in arteries with or without an intact sympathetic innervation. These findings suggest the importance of the trophic influence of the sympathetic nervous system on vascular smooth muscle and its relevance to the development of hypertension.

When the blood vessels (ear arteries) of hypertensive rabbits are decentralized so as to eliminate the continuous release of noradrenaline on the muscle cells the changes characteristic of hypertension do not occur. This result calls for a suggestion that vascular contractility in hypertension may not depend simply on an alteration in intracellular sodium concentration per se, but on a more complicated mechanism involving other electrolytes and noradrenaline or a functional sympathetic nervous system. It can be speculated from these findings that if the rabbits are extensively sympathectomized they will not become hypertensive since the entire vascular system may undergo changes similar to those which occurred in decentralized ear arteries. It has been reported by several workers that immunosympathectomized animals failed to develop or sustain hypertension (Willard and Fuller, 1969; Ayitey-Smith and Varma, 1970; Clark, 1971). The present results may explain these findings. It is proposed that the maintenance of the integrity of the vascular smooth muscle and the increase in vascular reactivity in hypertension may be the outcome of the interaction of smooth muscle electrolytes with noradrenaline released locally by the tonic activity of the sympathetic nerves.

It can be recalled that the reactivity of the decentralized ear arteries to noradrenaline was not impaired during the second week of the DOCA-NaCl treatment. It is likely that since the responses of these arteries to nerve stimulation were not markedly depressed that sufficient noradrenaline was still available to maintain the integrity of the vascular smooth muscle.

The observation that pretreatment of DOCA-NaCl hypertensive rabbits with exogenous noradrenaline restored the responsiveness of the depressed decentralized arteries to noradrenaline and to nerve stimulation attests to the need for noradrenaline in the maintenance of the reactivity of vascular smooth muscle in hypertension. Pretreatment with exogenous noradrenaline did not increase the reactivity of decentralized arteries from control rabbits.

Other data obtained in the present study also supported the proposed view that increased vascular reactivity in hypertension is the outcome of the interaction of sodium and possibly other electrolytes, with the neurotransmitter (noradrenaline). It was found that chronic intake of sodium chloride by control rabbits did not alter the reactivity of ear arteries with an intact sympathetic innervation to noradrenaline. However, the same treatment in adrenalectomized rabbits resulted in significantly increased responsiveness to noradrenaline. Bilateral adrenalectomy in animals is known to increase sympathetic nerve activity (Imms and Jones, 1968; Westfall and Osada, 1969) and high dietary sodium elevated the arterial sodium content in adrenalectomized rabbits in the present experiments. Therefore, the increased reactivity observed in these arteries may be associated with the interaction of increased concentrations of sodium and locally released noradrenaline on the smooth muscle cells. In support of this interpretation was the finding that in the absence of a functional sympathetic innervation (following decentralization) sodium chloride treatment did not significantly increase the reactivity of the ear arteries from adrenalectomized rabbits.

One possible explanation for increased vascular reactivity in hypertension is to postulate an increase in the population of functional alpha receptors. An increase in the receptor area for the neurotransmitter acetylcholine has been reported by Axelsson and Thesleff (1959) after denervation of skeletal muscle. If increased vascular reactivity in DOCA-NaCl hypertension is due to an increase in the number of receptors, the dose-response curves to noradrenaline should remain significantly different in preparations from untreated and DOCA-NaCl treated rabbits after partial alpha receptor blockade. However, after treatment with a moderate concentration of phenoxybenzamine the responses of arteries from normotensive and DOCA-NaCl hypertensive animals were both shifted markedly to the right and there were no significant differences between them. It appears that the population of alpha receptors does not differ in normotension and hypertension.

It is recognized that the basic defect in hypertension is an increased peripheral resistance due to a diminished internal calibre of arterial vessels, especially in small arteries and arterioles (Pickering, 1955; Folkow, et al., 1958). It should be pointed out that in the present experiments, in conscious hypertensive animals the innervated central ear arteries were markedly constricted while the contralateral arteries which were decentralized at the time of the induction of the hypertension were dilated. This narrowing in the vessels with an intact sympathetic innervation could be due to a number of factors such as muscular hypertrophy, swelling of the vascular walls

as a result of waterlogging or to increased muscular activity. Increased muscular activity in turn could result from excessive sympathetic nerve activity, elevated concentrations of circulating pressor substances or increased responsivity of smooth muscle cells to a given concentration of pressor agent (Mendlowitz and Naftchi, 1958; Fitz and Armstrong, 1964). Evidence for the latter possibility has been presented above as well as has evidence for its dependency on a functional sympathetic innervation.

Data were presented in the present study which indicates that the in vitro rate of flow of fluid through the lumen of control and decentralized arteries from DOCA-NaCl hypertensive animals did not differ from that through the lumen of control and decentralized or denervated vessels from untreated rabbits. Since rate of flow through a vessel is directly related to the diameter of the vessel, the results appear to indicate that the arterial walls of vessels from DOCA-NaCl treated hypertensive rabbits did not undergo any structural change resulting in narrowing of the lumen of the arteries. It should be emphasized that the situation with respect to arterioles cannot be extrapolated with safety from the present data obtained on a small artery.

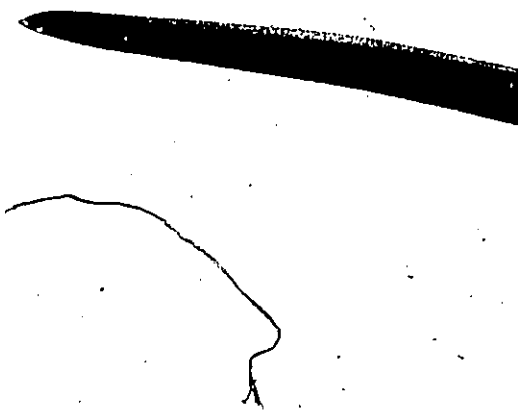
Recently, De Champlain et al., (1968) have demonstrated a pronounced increase in monoamine oxidase (MAO) activity in the hearts of DOCA-NaCl and renal hypertensive rats. This increase in enzyme activity was attributed to cardiac hypertrophy which occurred during the development of hypertension and not to DOCA or sodium. In the present

study it was found that MAO activity was significantly increased in the ear artery, heart and hypothalamus of DOCA-NaCl (5 weeks) hypertensive rabbits. This increase in MAO activity appears to be associated with hypertension rather than cardiac hypertrophy since the mean weight of the hearts from the hypertensive animals was 20% less than the control value and the phenomenon was also observed in tissues other than the heart. It is interesting that the hypothalamic region of the brain had both elevated sodium content and also increased MAO activity.

In renal hypertension, innervated arteries responded normally to nerve stimulation and to noradrenaline. In this form of hypertension, unlike DOCA-NaCl hypertension, angiotensin has been shown to elevate the blood pressure (Laragh, 1967; Dickinson and Lawrence, 1963). Angiotensin is also known to influence sympathetic function in various ways: thus it has been shown to stimulate ganglia (Farr and Grupp, 1967; Aiken and Reit, 1968; Lewis and Reit, 1968), to increase biosynthesis of noradrenaline (Boadle et al., 1969), to potentiate responses to sympathetic nerve stimulation (Benelli et al., 1964; Zimmerman and Gomez, 1965) and to enhance the release of noradrenaline from the nerve endings (Hughes and Roth, 1969). All these pharmacological actions of angiotensin could contribute to the pathogenesis of renal hypertension in animals with a functional sympathetic nervous system. Therefore, it appears that in this form of hypertension increased vascular reactivity may not be fundamentally essential for the induction of hypertension. However, contralateral decentralized arteries from these animals showed profound impairment to nerve stimulation and markedly reduced responses to

exogenous noradrenaline. It seems that the integrity of vascular smooth muscle which is determined by a functional sympathetic nervous system and electrolytes, interacting at the level of smooth muscle cells, is essential in the pathogenesis of this form of hypertension as it was shown to be for DOCA-NaCl hypertension.

In conclusion, the pertinent findings in this study show that the integrated role of the vascular system and electrolytes, particularly sodium ions under the influence of adrenal hormones, and noradrenaline released by sympathetic nerve impulses are essential for the development and maintenance of hypertension.



VI. SUMMARY AND CONCLUSION

The relationships of the sympathetic nervous system, electrolyte metabolism and vascular reactivity in the development and maintenance of experimental hypertension has been investigated using, as a model, the central ear artery of DOCA-NaCl and renal hypertensive rabbits. The results obtained are summarized as follows:

1. Hypertension due to the chronic administration of DOCA-NaCl was produced in unilaterally nephrectomized rabbits only when potassium chloride was included in the treatment regimen. In preliminary experiments, the omission of potassium chloride resulted in paralysis and death of the rabbits.

2. There was a generalized increase in sodium content of the arteries of rabbits with either DOCA-NaCl or renal hypertension. This increase in arterial sodium was observed as early as two weeks after the initiation of the DOCA-NaCl treatment and at a stage when arterial pressure was gradually increasing.

3. Although there was a significant increase in potassium content of the ear artery in DOCA-NaCl hypertension, this was not consistent with the potassium changes in other arteries.

4. The hypothalamus of the hypertensive rabbits was found to contain a greater concentration of sodium and the cerebral cortex a lower concentration of potassium than in control animals.

5. Chronic sympathetic decentralization of the central ear artery did not alter the sodium and potassium contents of the artery

from untreated rabbits, nor did it modify the marked elevation in tissue sodium content produced by treatment with DOCA-NaCl. However, the elevated potassium content of the ear artery produced by DOCA-NaCl treatment was significantly less after decentralization although still above the control values.

6. Surgical denervation (2 weeks) of the ear artery from untreated animals abolished completely responses to periarterial sympathetic nerve stimulation.

7. Two weeks after decentralization, arteries from untreated animals gave responses to low frequency sympathetic nerve stimulation which did not differ from those of contralateral arteries with an intact sympathetic innervation. However, responses of these arteries to high frequency stimulation were significantly decreased. Five weeks following decentralization responses of the arteries to nerve stimulation were comparable to those of contralateral innervated arteries at all test frequencies.

8. Two weeks following decentralization or denervation, arteries from untreated rabbits exhibited a significantly increased responsiveness to low concentrations of extraluminal but not to intraluminal noradrenaline. However, after five weeks decentralization the arteries showed a tendency to be less responsive to both extra- and intraluminal noradrenaline.

9. Two weeks following the initiation of DOCA-NaCl hypertension the responses of innervated arteries to nerve stimulation were unchanged. However, after five weeks and when the hypertension ap-

peared to be fully developed the responses of the arteries to nerve stimulation and to the pharmacologic release of noradrenaline by tyramine were significantly reduced.

10. Two and five weeks following the initiation of DOCA-NaCl hypertension, central ear arteries with an intact sympathetic innervation from these hypertensive rabbits exhibited a significant increase in responsiveness to intraluminal noradrenaline. However, after sympathetic decentralization responses to both extra- and intraluminal noradrenaline of arteries from DOCA-NaCl (5 weeks) but not from DOCA-NaCl (2 weeks) hypertensive rabbits were markedly depressed.

11. Pretreatment of DOCA-NaCl hypertensive rabbits with exogenous noradrenaline (1 mg NA per rabbit for 10 days) restored the depressed responses of the decentralized arteries to nerve stimulation and to noradrenaline. However, the same pretreatment in normotensive rabbits did not increase the responsiveness of the decentralized arteries to noradrenaline. The mechanism underlying these changes is discussed in relation to other results.

12. Monoamine oxidase activity was significantly increased in the ear artery, heart and hypothalamus of DOCA-NaCl hypertensive rabbits. This increase may be relevant to the development of the hypertension.

13. In renal hypertension, innervated arteries responded normally to nerve stimulation and to exogenous noradrenaline. However, responses to nerve stimulation and to noradrenaline were markedly depressed in the contralateral decentralized arteries.

14. The reactivity to noradrenaline of innervated central ear arteries was not influenced by the chronic intake of a high sodium chloride diet. However, the innervated arteries of rabbits on a high sodium chloride diet after adrenalectomy exhibited a significant increase in responsiveness to noradrenaline. It was observed that responses to noradrenaline were not significantly increased in the contralateral decentralized arteries from sodium chloride treated adrenalectomized rabbits.

From the present data the following conclusions were drawn:

1. The elevation of arterial sodium in DOCA-NaCl and renal hypertension and the elevation of hypothalamic sodium in DOCA-NaCl hypertension may, in part, be involved in the pathogenesis of the disease.

2. There is no simple direct relationship between arterial sodium and potassium distribution or the sodium depositing effect of DOCA and the state of activity of the sympathetic nervous system or vascular reactivity.

3. The increased vascular reactivity exhibited by the innervated arteries from DOCA-NaCl hypertensive rabbits cannot be attributed to pathological denervation or decentralization of two or five weeks duration. It may, in part, be associated with an alteration in arterial electrolytes since the sodium and potassium contents of the ear artery were elevated. However, it appears quite clear that an alteration in the reactivity of the smooth muscle to biogenic amines is involved.

4. The increased vascular reactivity may have an etiological role in DOCA-NaCl hypertension although it may not be an essential factor in renal hypertension.

5. In the long-term absence of a tonic or trophic influence of the sympathetic nervous system the vascular smooth muscle tends to lose sensitivity to exogenous noradrenaline in untreated animals and undergoes a marked reduction in responsiveness to noradrenaline in the course of the development of hypertension without concomittant reduction in sodium or potassium content. Thus, those changes in responsiveness to noradrenaline which are characteristic of the central ear artery in hypertension do not occur when the artery is deprived of its sympathetic innervation during the induction of the hypertension. Further support for this conclusion was the finding that the decreased responsiveness to noradrenaline in decentralized arteries from hypertensive rabbits was reversed by depot injections of exogenous noradrenaline.

It appears that the integrated role of the vascular system and electrolytes, particularly sodium ions under the influence of adrenal hormones, and the noradrenaline released locally on the vascular smooth muscle by sympathetic nerve impulses is essential for the development and maintenance of the hypertension.

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