Pharmacological studies of isolated turkey abdominal aorta

by

Jenn Huei Ueng

A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Department: Veterinary Physiology and Pharmacology
Major: Physiology (Pharmacology)

Iowa State University
Ames, Iowa

1986

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ED50 drug concentration resulting in 50% of the maximal response.

ES electrical stimulation.

ESR electrical stimulation response.

NE norepinephrine.

K_B dissociation constant of a receptor for a specified antagonist .

pA_2 negative logarithm of the concentration of antagonist required to give a twofold shift (a dose ratio of 2) in the dose response curve.

I. INTRODUCTION

As mammals are the dominant vertebrates of the land, birds have so claimed the sky. They have adjusted themselves to severe environmental stresses such as humidity, temperature, and altitude and have body temperatures (~41° C) which are usually higher than the other vertebrates. These adjustments are all linked with a functional cardiovascular system. Investigators have shown a tremendous difference among avian blood vessels, such as ultrastructure, enzyme content, permeability, innervation, and myogenic activity. While information on these has been published, virtually none of the recent observations has been confirmed or contradicted by a second report. The bulk of it stands as a series of isolated observations. In view of its relative neglect in the past and its growing economic importance, the opportunity for research in domestic fowl is much greater than in almost any other species (Akester, 1971).

Turkeys, for example, have an arterial blood pressure which is among the highest of the vertebrates. Table 1 shows the blood pressures of some vertebrates. Whether one goes from small animals (rat, chicken) to large animals (lion) or from lower vertebrates (fish, frog) to higher vertebrates (monkey), the mean arterial blood pressure is around 100 mmHg. But for those of turkeys, their systolic levels may reach 300-400 mmHg (Ringer and Rood, 1959; Speckmann and Ringer, 1963). The arterial blood pressure of the turkey is not only unusual for that of birds, but also unique in comparison to other vertebrates (for more information, see Sturkie, 1976; Geddes, 1970). Yet the real reason for this high blood pressure in the turkey remains an unknown.

It is generally believed that the factor responsible for high blood pressure in established hypertension is increased vascular resistance caused primarily by an increased contractile response of vascular smooth muscle to vasocontrictors (Folkow et al., 1973;

Table 1. Examples of blood pressures in vertebrates (Sturkie, 1976; Geddes, 1970)

Species	Age (wt)	Artery	Blood Pressure (mmHg)		
			Systolic	Mean	Diastolic
shark	100 lbs	brachial	-	32 (59-22)	-
grass snake and viper	100-200 grams	ventricle	55	-	0
frog	?	carotid	43 (36-50)	-	31 (24-44)
rat	?	carotid	187 (180-195)	-	138 (135-140)
African lion	60 kg (37-121)	carotid	130	-	90
Rhesus monkey	3-12 kg	carotid	103-132	-	78-101
chicken (w. leg)	7 wks male	carotid	151	-	128
duck	12-13 mos. adult male	carotid	179	-	134
pigeon	adult	brachial	135	-	105
turkey (bronze)	22 wks male	carotid	297	-	222

Bohr and Berecek, 1976). Increased blood pressure with age is also known to be associated with increased peripheral vascular resistance and elevations in circulating catecholamines (Ziegler et al., 1976; Lake et al., 1977). Functional changes of arteries which can be a cause of increased vascular resistance have been reported to occur prior to the development of hypertension (Jones and Hart, 1975; Hansen and Bohr, 1975). Changes in structure,

contractile responses, and catecholamine content in various vascular smooth muscle, including aorta, from hypertensive rats have been well studied (Head et al., 1985; Lee, 1985; Pang and Scott, 1985), but not for those from the turkey.

This high blood pressure and the tendency toward formation of atheromatous plaques in turkey (Carnaghan, 1955; Grollman et al., 1963; Krista et al., 1970) are also probably the most important underlying causes of death by aortic rupture (Jones and Johansen, 1972). Aortic rupture is an important cause of mortality in turkeys, especially of large tom turkeys, and is characterized by massive internal hemorrhage from a ruptured aneurysm. Aortic rupture is also called dissecting aneurysm. It occurs in the heaviest, fastest growing birds, especially in the tom turkey, whenever rapid gains in weight are being pushed (Whiteman and Bickford, 1983). In an economic sense, these losses can be very extensive and harmful to the turkey industry.

Given the above as a very brief introduction, I decided to begin studies on turkey vascular smooth muscle for the purpose of contributing to the pool of information which some day may permit a better understanding of blood pressure mechanism(s), especially in the turkey.

II. LITERATURE REVIEW

A. Anatomy and Histology

The anatomical positions and histological structures of blood vessels may play an important role in their responses to various drugs. There are entire books on each of these areas (Robinson, 1970; McLeod, 1964; Andrew, 1959; and Hodges, 1974). The following review is closely related to my research. Since there is poor documentation of turkey vascular smooth muscle, some information will be extrapolated from the chicken, other vertebrates, or mammals. The terminology used in avian anatomy and histology differs somewhat from that used in the mammalian literature.

1. Anatomy of aorta

The avian aorta, like the mammalian aorta, arises from the upper and cranial part of the left ventricle. From its origin, it curves forward, upward, and then backward to the lower part of the ventral spine of the thoracic vertebrae. Once it leaves the thoracic cavity, through the hiatus aorticus, and enters the abdominal cavity, it is called the abdominal (dorsal) aorta. The abdominal aorta follows the mid-dorsal line of the body and maintains its relation to the vertebrae. The size of the aorta abruptly becomes smaller as it reaches the origin of the sciatic (ischiatic) arteries. Eventually, it ends ventral to the caudal part of the lumbosacral bones by dividing into paired internal iliac (hypogastric) arteries and a single middle sacral (caudal) artery (McLeod, 1964; Robinson, 1970).

2. Branches of abdominal aorta

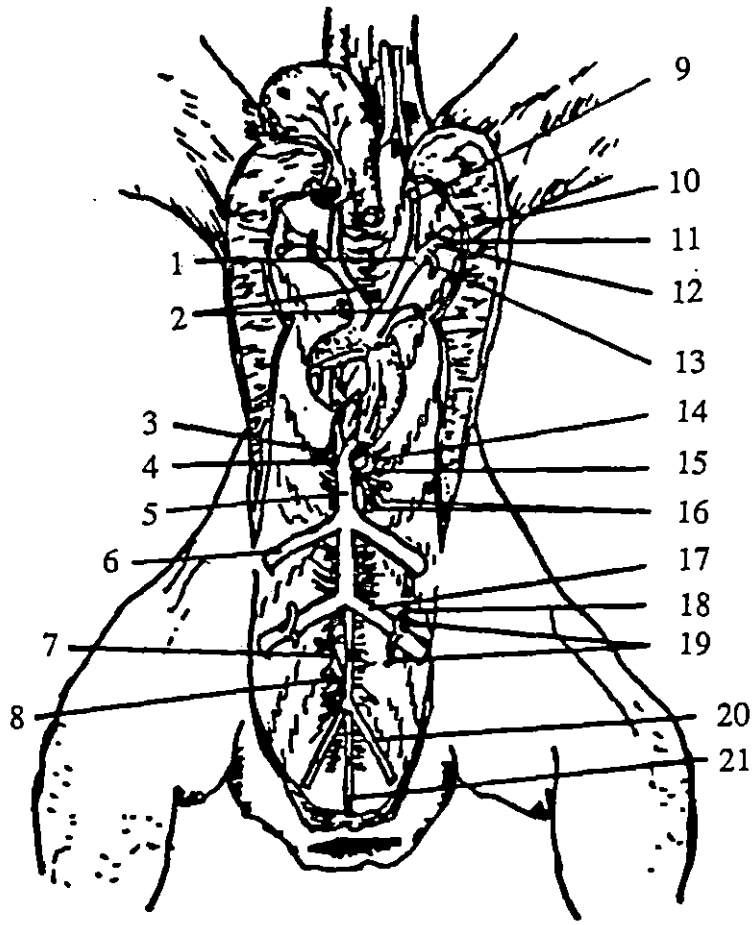
There are 8 arteries originating from the abdominal aorta. From the anterior site to the posterior site they are: 1) coeliac artery (coeliac axis), 2) superior (anterior) mesenteric artery, 3) superior renal (renal) artery, 4) testicular artery, 5) external iliac artery, 6) ischiatic

(sciatic or external ischiatic) artery, 7) posterior mesenteric artery, and 8) internal iliac artery. After the internal iliac the dorsal aorta continues into the tail portion of the body and is called the sacral (caudal) artery, see Figure 1.

3. Histology of avian blood vessels

a. Types of avian arteries In birds the separation of the arterial wall into layers is similar to that in mammals. The larger arteries show a predominantly elastic structure, whereas the medium-sized arteries show a predominantly muscular structure (Andrew, 1959). As in mammals, the arteries can be divided into three types according to structure and size: 1) small arteries and arterioles, 2) medium size or muscular arteries, and 3) large or elastic arteries. The mammalian aorta is classified as the latter type (Bloom and Fawcett, 1962). However, Hughes (1943) considers that in fowl there are three basic types of medium-sized and large arteries, instead of two. According to this classification, type I and type II are elastic arteries but differ so much in structure that they need a separate classification. The thoracic and upper abdominal aorta are representatives of type II arteries. The rest of the abdominal aorta and all of its major branches are muscular in nature and belong to the type III classification (Hodges, 1974).

b. Histological structures There are three basic structural layers in the wall of any avian artery. These are passing from the lumen outwards, the tunica intima (or interna), the tunica media and the tunica adventitia (Hodges, 1974). The tunica intima is the single layer of endothelial cells which lines the lumen of the entire cardiovascular system, together with the adjacent elastica interna or inner elastic membrane. The tunica media consists of smooth muscle cells arranged in a circular manner around the lumen and is normally the thickest portion of the arterial wall. The tunica adventitia is mainly composed of loose connective tissue in which lie nerve fibers and blood vessels (vasa vasorum).



1	Subclavian	9	Carotid	17	Sciatic
2	Innominate	10	Brachial	18	Oviductal
3	Coeliac axis	11	Pectoral	19	Inferior renal
4	Superior mesenteric	12	Axillary	20	Internal iliac
5	Dorsal aorta	13	Thoracic	21	Caudal
6	External iliac	14	Suprarenal		
7	Superior hemorroidal	15	Superior renal		
8	Posterior mesenteric	16	Testicular		

Figure 1. Anatomy of avian aorta (Robinson, 1970)

4. Innervation of blood vessels

The existence of postganglionic constrictor sympathetic nerve fibers in blood vessels has been well-documented in various tissues such as rabbit pulmonary artery (Bevan and Su, 1964) and rabbit aorta (Paterson, 1965; Yates and Gillis, 1963). In contrast, Akester (1971) indicated that the great majority of blood vessels in the fowl are dually innervated, i.e., by both adrenergic and cholinergic (acetylcholinesterase-positive nerve) fibers in approximately equal numbers. The richness of the innervation may vary considerably between individual vessels and also between different areas of a single vessel. These nerve fibers are usually restricted to the media-adventitia boundary (Hodges, 1974).

B. Review of Methodology

1. Important studies from the literature

For many years, strips of arteries have been used to study the physiological and pharmacological responses of vascular smooth muscle. In 1952, Furchgott used isolated spirally-cut strips of rabbit aorta to study this tissue. He reported that application of an electrical stimulus to these tissues for about 10 seconds causes a relatively fast contraction during current flow, and after cessation of current an additional slower contraction followed. He suggested that the secondary contraction after current flow was probably due to the liberation of an epinephrine-like substance since dibenamine, an α-adrenergic receptor antagonist, could abolish this response. This suggestion was strengthened by many other investigators. Gillis and Yates (1960) found that reserpine, which lowers the catecholamine content of rabbit aorta (Burn and Rand, 1958), abolished that secondary contraction of rabbit aorta and carotid artery, but not the primary contraction (during current flow). Similar antagonism of responses to electrical stimulation (ES) was found using other adrenergic receptor or neuronal blocking drugs such as phentolamine, azapetine, piperoxan, bretylium,

guanethidine, and bethanidine for rabbit aortic and carotid strips. However, ganglionic blocking agents such as hexamethonium and pentolinium had no effect on electrical stimulation. It was concluded that the secondary response to electrical stimulation was the result of excitation of post-ganglionic adrenergic axons in the walls of the arterial preparations (Paterson, 1965; Yates and Gillis, 1963). The epinephrine-like substance, originally proposed by Furchgott to be released by electrical stimulation, was found to be norepinephrine (NE).

Paton (1954) introduced the method of transmural stimulation to stimulate nerves in isolated smooth muscle preparations. This technique permitted a better and more uniform excitation to be applied over the whole length of the tissue. This method of electrical field stimulation has been used for many years to enhance our comprehension of vascular neuroeffector mechanisms. The utility of this approach depends on the assumption that by properly choosing the right stimulation parameters, intramural nerves can be activated without directly stimulating vascular smooth muscle. To differentiate between direct muscle stimulation and neuronal-mediated stimulation, the adrenergic blocking agents described above and other nerve-blocking drugs such as tetrodotoxin are often used. The puffer fish poison, tetrodotoxin, appears to have no effect on direct acting agents and blocks responses only to electrical stimulation (Kao, 1966). This assumption was confirmed by Duckles and Silverman (1980). By using a coupling device, which provided a very low source of impedance, they demonstrated that in rabbit ear artery, it was possible to establish appropriate stimulation parameters to selectively activate nerves without stimulating smooth muscle directly. They suggested that responses to lower voltages (up to a plateau) were due to stimulation of intramural nerves, while responses to higher voltages, which were not blocked by phentolamine, tetrodotoxin, or guanethidine reflect a direct excitation of smooth muscle.

In other smooth muscle preparations such as the vas deferens from guinea pig, rat, or rabbit, a biphasic type of contractions has been observed to transmural stimulation. The initial phase was selectively reduced by arylazido aminopropionyl ATP ($ANAPP_3$), a P_2-purinoceptor antagonist, while the second phase was selectively antagonized by prazosin, an α_1-adrenoceptor blocker (Sneddon et al., 1984). These investigators proposed that ATP and NE acted as cotransmitters in the vas deferens of several species. As to the response for the rabbit aorta mentioned above, it is probably another example of a different biphasic type response. Since α-adrenergic receptor antagonists, reserpine, and other neuronal blockers could block only the second phase, the primary contraction is probably due to the direct stimulation of smooth muscle.

2. Methods used to study vascular smooth muscle

Many investigators use the spirally-cut strip preparation for studying agonist-induced postsynaptic responses. This preparation permits good flexibility when investigating drug concentration-effect relationships. They are easy to prepare and can give very good reproducible response to drugs. However, it suffers from the disadvantage of being less physiological than other preparations (such as ring preparations) as the muscle, nerves and endothelium may be cut or damaged in making the preparations. Another potential problem is the angle of the spiral-cut; for instance, in a canine femoral artery, the response to NE can be varied from contraction to relaxation. The responses are dependent upon the pitch of the angle of orientation of the helix from the transverse axis of the vessel (Ohhashi and Azuma, 1980).

A convenient alternative to the helically-cut preparation is the use of rings of vascular smooth muscle mounted on opposing stainless steel hooks. This method probably yields a more physiological type of response since there is minimal variation in smooth muscle cell

orientation, minimal damage to the intimal surface of the vessel, and less tissue damage produced by cutting. The cutting of blood vessels has been shown to have a significant effect on the functional properties of both the muscle layer and its innervation (Owen et al., 1983; Cowen et al., 1982). The endothelial cells on the intimal surface have been shown to be involved in various physiological or pharmacological responses of the vascular smooth muscle such as vasodilation induced by acetylcholine (Furchgott, 1983), release of endothelium-derived relaxing factor mediated by serotonin (Vanhoutte, 1983), and inhibition of B-HT 920 (an α_2-adrenergic agonist)-induced contractions (Miller et al., 1984). However, the disadvantage of ring preparation is that more accurate micrometers and transducers may be needed to measure the responses (Moulds, 1983).

Perfusion and superfusion technique can also be used to study electrical stimulation and presynaptic mechanisms. Superfusion techniques are good for bioassy techniques where very small volumes of biological fluids are to be assayed. Perfused vessels, notably the rabbit ear artery, are useful in that they allow for the study of agonists exclusively applied to the intimal or advential side of the blood vessel (De la Lande, 1975).

3. Sources of variations in isolated tissue experiments

Many factors such as: animal age, sex, weight, and strain; operating temperature and ions in the bathing solution; mechanism of agonist uptake and metabolism; chemical degradation of drugs; time course of the experiment; receptor distribution; equilibrium conditions; and differences in the efficiency of stimulus-response mechanisms can alter the sensitivity of tissues to agonists (Kenakin, 1984). Age, for example, has been found to affect the receptor density, distribution, and the reactivity of various isolated tissues. Relaxation in response to β-adrenoceptor agonists is less in aorta and mesenteric arterioles of aged rats than it is in younger animals (Fleisch et al., 1970). Also, an increase in age has

been shown to increase the contraction of rat aortae to norepinephrine and serotonin (Cohen and Berkowitz, 1974). Altura (1975) reported that mesenteric arterioles from female rats showed greater responses to vasopressin and oxytocin than do the corresponding vessels from male animals. Nonvascular smooth muscle such as rat uterus has a completely different response to NE (excitation or inhibition) which is dependent on the hormonal status (days of pregnancy) (Osa and Ogasawara, 1984). The possible nature of heterogeneous receptor distribution should also be considered. It has been shown that rabbit aorta is not uniformly sensitive to β-adrenoceptor agonists (Altura and Altura, 1970). Another possible concern in analyzing tissue receptors is that the use of an agonist with mixed receptor stimulating properties may give variable responses. For instance, in rat veins, β-adrenoceptor antagonists potentiate the vasoconstriction response to mixed α-, β-adrenergic agonists (Cohen and Wiley, 1977).

Generally speaking, since the intrinsic contractile behavior of the smooth muscle is adapted to the function of the vessel, it is unlikely that comparable blood vessels will show much variation between species. In contrast, the variation of pharmacological responses from functionally different blood vessels of the same species can thus reasonably be much greater than those from strictly comparable vessels of different species (Robinson, 1983).

It is obvious from the above description of sources of variation that one must be very careful when interpreting experimental results obtained by using any particular method when studying isolated smooth muscle.

C. Release and Disposition of Adrenergic Transmitters

The basic information about synthesis, release, and disposition of adrenergic transmitters is well described in recent text books (Euler, U.S.V., 1972; Kopin, 1972;

Weiner and Taylor, 1985). Here I will limit myself to the areas closely related to my research.

1. Uptake of adrenergic transmitters

Functional uptake of catecholamines was demonstrated as early as 1933 by Burn who observed a modification of the effect of nerve stimulation after adding epinephrine to the perfusion fluid used for the hind leg of the dog. Using highly radioactive catecholamines, Axelrod et al. (1959a) were able to study the dynamics of the distribution and metabolism of epinephrine after administration in physiological doses. They concluded that those tissues in which the concentration of endogenous catecholamines was high were also those which showed the highest degree of uptake of administered H^3 epinephrine.

Now there is considerable evidence to confirm this existence of uptake of catecholamine into post-ganglionic sympathetic neurons (Iversen, 1967; Axelrod, 1973). The adrenergic transmitter, namely norepinephrine (NE), released by exocytosis of storage vesicles in response to adrenergic nerve impulses, can be recaptured by active transport back into the presynaptic nerve terminal. This process is called neuronal uptake or $uptake_1$. The rest of the transmitters in the synaptic cleft then either diffuses out via the circulation or is taken up by the extraneuronal compartment. This latter process is called extraneuronal uptake or $uptake_2$ (Iversen, 1974; Paton, 1976).

Cocaine, has been shown to delay the disappearance from the blood of injected NE (Trendelenburg, 1959). Macmillan (1959), suggested that cocaine, by preventing the uptake of NE into tissue stores, increased the amount available for reacting with the receptors and thus potentiated NE's effect. In rat vas deferens, cocaine greatly increased the maximal responses to NE (Ursillo and Jacobson, 1965) but not those to methoxamine (Kenakin, 1980), suggesting that methoxamine probably did not have an uptake process in this tissue.

Also, Leighton (1982) has shown that a potency ratio of 50:1 for methoxamine over NE in rat anococcygeus muscle was changed to 0.2:1 after cocaine.

Cocaine has been shown to block neuronal uptake, but not to affect extraneuronal accumulation (Iversen, 1965; Eisenfeld et al., 1967; Simmonds and Gillis, 1968). On the other hand, normetanephrine and metanephrine have been shown to block this extraneuronal process, but to have no effect on neuronal uptake (Iversen, 1965). For example, it has been shown that the uptakes$_2$ mechanism is the only important process for removing isoproterenol in guinea pig isolated trachea. Sensitivity to this catecholamine could be increased over 10-fold with normetanephrine, but not at all with cocaine. In the same tissue, both uptake$_1$ and uptake$_2$ were found to be active in the removal of norepinephrine, with uptake$_1$ being dominant (Foster, 1967, 1968, and 1969).

The uptake$_2$ mechanism was thought to be of relatively little physiological importance unless the neuronal uptake mechanism was blocked or was otherwise not functional (Iversen, 1975; Trendelenburg, 1980). In isolated rabbit aorta, both the uptake$_1$ and uptake$_2$ mechanisms have been suggested to be energy-dependent (Abrahamsen and Nedergaard, 1985).

2. Metabolism of adrenergic transmitters

In contrast to most cholinergic junctions, which possess the powerful enzymatic mechanism of acetylcholinesterase for degrading acetylcholine, such a rapid enzyme system is absent from the adrenergic nervous system. Instead, the catecholamines in the adrenergic nerve endings and extraneuronal tissues are metabolized by monoamine oxidase (MAO) and catechol-o-methyltransferase (COMT). Both of these enzymes are widely distributed in the body and appear to be located in both non-neuronal tissues and sympathetic neurons (Blaschko, 1974; Guldberg and Marsden, 1975). But the available evidence obtained from

vascular tissues (Levin, 1974; Paiva and Guimaraes, 1978) and many other tissues (Kopin, 1972) indicates that the principal neuronal enzyme is MAO, while the chief extraneuronal enzyme is COMT.

The role of MAO inhibitors has been discussed ever since the work on iproniazid by Zeller et al. (1952). It was demonstrated that pretreatment with a MAO inhibitor increased brain concentrations of endogenous monoamines in rat (Green and Erickson, 1960; Crout et al. 1961). Schayer et al. (1953 and 1955) showed that the half of the N-methyl-C^{14} epinephrine, which was normally lost, could be recovered from animals which had been pretreated with a MAO inhibitor. This important observation provided convincing evidence that this enzyme was responsible for removal of the N-methyl group.

Axelrod (1957) showed that o-methylation was also a route of metabolism for epinephrine and norepinephrine. COMT, the enzyme which transfers the methyl group from s-adenosylmethionine to a wide variety of catechols (Axelrod, 1959); was found to be widely distributed in many tissues from a variety of species (Axelrod and Tomchick, 1958; Axelrod et al., 1959b). Inhibitors of COMT such as pyrogallol and tropolone were known to potentiate the effects of administered catecholamines (Axelrod and Laroche, 1959; Belleau and Burba, 1961). However, pyrogallol and tropolone were thought to be not as potent and selective as MAO inhibitors (Marley and Stephenson, 1972), and produced only slight enhancement of the actions for catecholamines (Weiner and Taylor, 1985).

3. Adrenergic receptors

As pointed out by Langer (1974), the adrenergic transmitters released form presynaptic ending can be recaptured, metabolized, or they can act on adrenergic receptors to exert their physiological effects. There are two distinct type of adrenotropic receptors as originally described by Ahlquist (1948): the alpha adrenotropic receptor is associated with

most of the excitatory functions (e.g. vasoconstriction and stimulation of the uterus, nictitating membrane, ureter and dilator pupillae) and one inhibitory function (i.e., intestinal relaxation). The beta adrenotropic receptor is associated with most of the inhibitory functions (vasodilation, and inhibition of the uterine and bronchial musculature), and one excitatory function (myocardial stimulation). Subsequently, the development of selective agonists and antagonists that act at adrenergic receptors has allowed these receptors to be subclassified. Beta-receptors were categorized as β_1 (in cardiac tissues) or β_2 (in smooth muscle and gland cells) (Lands et al., 1967a, 1967b). Alpha receptors also appear to be heterogeneous. Those designated α_1 predominate at postsynaptic effector sites of vascular smooth muscle and are responsible for the vasoconstriction. On the other hand, α_2-receptors, which are proposed to exist on nerve terminals, are believed to mediate the presynaptic feed-back mechanism (Langer, 1974). Alpha$_2$-adrenergic receptors are also present at postjunctional sites in several tissues including certain blood vessels.

The presynaptic action of norepinephrine indicates that once the norepinephrine released by nerve stimulation reaches a threshold concentration in the synaptic gap, it activates presynaptic α_2-adrenergic receptors, thereby triggering a negative feedback mechanism that inhibits further release of the transmitter (Starke, 1972 and 1981). This proposal was based on the finding that NE and some other adrenoceptor agonists inhibit stimulation-induced efflux of ^{3}H-labeled transmitter whereas alpha adrenoceptor antagonists (e.g., yohimbine) enhance efflux. This hypothesis is now widely accepted but is not without its critics. Much of this criticism comes from the work of Kalsner and co-workers (Kalsner, 1982, 1983a,b; Kalsner and Quillan, 1984). They found that the neuronal effect of adrenergic agents are due to presynaptic interactions which are independent of the ambient levels of transmitter. An alternative explanation was thus proposed: yohimbine and other presynaptic antagonists may prolong the period of potassium efflux from nerve varicosities,

and by this means prolong depolarization and the associated period of transmitter release rather than disrupting an ongoing system of sensing and responding to fluctuations in extracellular transmitter levels.

This alternative proposal is strengthened by the finding that 1) in guinea pig atria, yohimbine prolonged the period of potassium efflux from nerve terminal during the action potential, 2) tetraethylammonium, an inhibitor of stimulation-induced potassium efflux from nerves had an effect on transmitter efflux in rat spleen essentially like that of α_2-antagonists.

4. Other findings regarding the release of adrenergic transmitters

Clonidne (α_2-agonist) has been shown to inhibit the electrically evoked tritium overflow in human vasa deferentia, and this effect was blocked by phentolamine, however, phentolamine or yohimbine given alone did not enhance transmitter release (Belis et al., 1982). Inhibitory muscarinic receptors and facilitatory β-adrenoceptors have been found on the adrenergic presynaptic sites and are thought to modulate the release of transmitters (Langer, 1980; Muscholl, 1980; Starke, 1981). Acetylcholine caused an inhibition of the electrically evoked tritium overflow in human saphenous veins, and this was antagonized by atropine. Isoproterenol and salbutanol (β_2-receptor agonists) enhanced the electrically evoked tritium overflow in human digital arteries and metatarsal veins, and this was blocked by propranolol. Atropine and propranolol given along had no effect on the release of transmitters (Moulds, 1983; Stevens et al., 1982).

Thus, presynaptic receptor agonists may have either inhibitory or stimulatory effects on the release of transmitter, whereas its antagonist usually blocks this agonist's effect. Antagonist alone may or may not exert an effect.

In rabbit pulmonary arteries, peptide hormones such as angiotensin II and ACTH may also be capable of modulating NE release via specific presynaptic receptors. The

involvement of adenylate cyclase in the regulation of stimulation-evoked exocytosis of NE has been reported by Göthert (1984). In isolated guinea pig vas deferens and rat spleen, membrane permeable analogues of cAMP and phosphodiesterase (PDE) inhibitors facilitated the release of NE (Wooten et al., 1973; Cebeddu et al., 1975). These investigators suggested that cAMP is involved in the regulation of adrenergic transmitter release.

5. Avian adrenergic receptors

In domestic fowl, two types of adrenergic receptors (i.e., α and β) are also considered to be associated with vascular smooth muscle. These receptors are similar to those found in other higher vertebrates (Akester, 1971). The inhibitory effect of β-receptor stimulation on α-receptor responses has been shown using helically-cut strips contracting in response to NE. When propranolol (β-blocker) was used in these preparations, the maximal isotonic contraction was considerably greater than control (Somlyo and Woo, 1967). They concluded that both α- and β-adrenergic receptors are present throughout the muscular blood vessels in the fowl, and the existence of β-receptors could be demonstrated by using β-antagonists. These antagonists potentiated the contractile response to mixed alpha-beta adrenergic receptor agonists. However, this is not the case in the superior mesenteric artery of the chicken. Low concentration of catecholamine caused relaxation instead of contraction. The relaxation was unaffected by α-blockade (phentolamine), but it was abolished by β-blockade (propranolol) (Bolton, 1968). It seems that in this particular tissue, the β-receptors are dominant rather than α-receptors. This is the opposite of what is usually found in vascular smooth muscle of other higher vertebrates. Another example of avian vascular smooth muscle which has been studied is the isolated renal portal valves of the turkey. This tissue contracts under the influence of acetylcholine and histamine, whereas NE and epinephrine caused the renal portal valves to relax (Rennick and Gandia, 1954).

The number and depth of investigations into adrenergic receptors in avian vasculature leaves a lot to be desired. Clearly, there is a need for work in this area.

6. Modulators of adrenergic transmitter release

a. Serotonin (5-hydroxytryptamine, 5-HT) Serotonin has several effects on vascular smooth muscle through different mechanisms. It can act on specific membrane receptors to modulate contractile activity in smooth muscle. The response to 5-HT depends on the species, receptor type and distribution, and type of vascular smooth muscle (e.g., artery, vein, large vessels, or small vessels). Virtually every major artery responds to serotonin with constrictions (Vanhoutte, 1982), whereas the direct relaxant effects to serotonin are usually more prominent in arterioles and large veins. This monoamine in threshold concentration can also amplify the response to neurohumoral mediators, such as angiotensin II, NE (exogenous or endogenous source), histamine, and prostaglandins (Van Nueten, 1983). Its vasoconstriction action may be mediated by displacement of the NE from the storage site of adrenergic nerve terminals (Nishino et al., 1970; Vanhoutte, 1983) or directly by stimulation of postsynaptic receptors which can be brought about by activating classical 5-HT receptors (e.g., in rabbit thoracic aorta), or alpha adrenoceptors (e.g., rabbit ear artery) (Purdy et al., 1981). Serotonin-induced vasodilation may be caused by activating the release of endothelium-derived relaxing factor, reducing extracellular calcium influx, increasing cellular levels of cyclic AMP, or by stimulating the synthesis of prostacyclin (De Clerck and Vanhoutte, 1982; Webb and Vanhoutte, 1985).

b. Angiotensin II Angiotensin II is a peptide which has various effects. It has been shown to enhance NE release in experimental hypertensive rats (Kawasaki et al., 1982; Westfall et al., 1984 and 1985). This phenomenon was further confirmed in rat portal vein from several normotensive strains as well as hypertensive animals (Westfall et al., 1985)

when electrical field stimulation was applied on these preparations. Angiotensin II is also one of the most potent physiological pressor agents known, being 4-8 times as active as NE on a weight basis in normal individuals (Ganong, 1985). However, Webb (1982) found that the magnitude of contraction it produced in isolated blood vessel segments was generally less than that produced by alpha-adrenergic agonists. Furthermore, its ability to cause contraction was not at all uniform for isolated vascular smooth muscle from different anatomical sites. In isolated dog renal vein, or perfused rabbit kidney and mesentery, vasodilatory responses to angiotensin II rather than vasoconstriction occurred. The vasodilation effect was inhibited by indomethacin (a cyclooxygenase inhibitor) suggesting that it was mediated by prostaglandins (Blumberg et al., 1977a; Blumberg et al., 1977b; Pure and Needleman, 1979). In turkey aorta, angiotensin II (10^{-5}M) produced little or no contraction (Dyer and Badr, 1985).

III. STATEMENT OF THE PROBLEM

The turkey's high blood pressure and the lack of information about factors influencing vascular smooth muscle in the turkey prompted this study. The purpose of this research was to begin the process of developing information which may help to elucidate the possible factors controlling turkey vascular smooth muscle tone. The assumption is that this will, at some future date help explain the mechanism(s) in turkey hypertension. For this purpose, the following objectives were defined:

1) Develop the methodology to study drug effects on isolated abdominal aorta by using isometric tension monitoring procedures and electrical field stimulation.
2) Ascertain if modulators of adrenergic neurotransmission such as angiotensin II and serotonin influence electrical field stimulation and/or have a direct effect.
3) Ascertain the importance of possible sites of loss (termination mechanisms) of the transmitter released by field stimulation by the use of antagonists for each termination mechanism (e.g., $uptake_1$, $uptake_2$, monoamine oxidase, and catechol-o-methyltransferase).
4) Ascertain the affinity of adrenergic receptors of the turkey abdominal aorta for selected adrenergic antagonists.

IV. MATERIALS AND METHODS

A. Experimental Procedures

Male turkeys weighing 7-15 lb from a commercial turkey farm (Louis Rich, Ellsworth, Iowa) were purchased in groups of 10-12 birds. Turkeys were killed by decapitation (guillotine) without anesthesia. The abdominal cavity was opened and the abdominal aorta was separated from surrounding tissues and all of its branches. We removed the segment of abdominal aorta from the branch of the coeliac axis to the branch of sciatic artery (Figure 1) and placed the tissue in a modified Krebs-Henseleit (Krebs) solution (see Appendix for chemicals). The tissues were used fresh or stored in the refrigerator overnight and used the next day. The surrounding connective tissue was removed and the blood vessel was cut into six pieces (4-6 mm in length/ each piece) and numbered from the proximal end to the distal end. For instance, the most proximal piece of the tissue was numbered as tissue 1 and the most distal piece of the tissue was numbered as tissue 6. These 6 pieces of tissues were then put into 6 isolated tissue baths, one by one in order. Tissue 1 was always put in the first bath and tissue 6 was always placed in the last bath. Each of the 6 rings was mounted on a tissue holder between one fixed stainless steel rod and one moveable stainless triangle. A silk thread was used to connect the triangle to the force-displacement transducer (FT03C, Grass, Quincy, MA). The tissues were placed in 50 ml isolated organ baths (Metro Scientific, Farmingdale, NY) containing Krebs solution at 39° C and oxygenated with 95% O_2–5% CO_2. The isometric tension developed by the smooth muscle was recorded on a 6-channel R-611 Dynograph Recorder (Beckman Instruments, Schiller Park, IL). Each tissue was located between two platinum electrodes and stimulated by a Grass SD 9B (Quincy, MA) stimulator. In most cases, the following parameters were

used to stimulate the tissues: voltage, 8-10 v; duration = 1 millisecond; current, about .5 milliamperes; frequency, 4-32 hertz; stimulation time, 20 seconds. After stimulation when the tension returned to near the original level (base line) the tissue was ready to be stimulated again.

B. Experimental Design

Each experiment was begun by permitting the tissue to equilibrate for 2-4 hours under one gram tension. Periodic adjustments were made as the tissue changed tone during the equilibration period in order to maintain approximately the original one gram tension, which was used as a base line for the entire experiment. Potassium chloride 120 mM was added periodically to the Krebs solution in the organ bath to ascertain if the contractility of the smooth muscle had stabilized. In pilot experiments KCl 120 mM was established as the concentration for the approximate maximal response, since increasing the concentration of KCl to more than 120 Mm did not significantly increase the contractile response of the tissues. At the beginning of each experiment, a control response (either to an agonist or to electrical stimulation) was obtained for each of 6 tissues. These responses obtained from this initial control period were referred to as round 1 for all of the tissues. After round 1, only some tissues (referred to as treated tissues) were treated with one concentration of durg (one drug per tissue), while one or two tissue(s) (control tissues) received no drug. The tissues were then subjected to the initial form of stimulation. The responses from this period were referred to as round 2. Round 3 and round 4 were used when different concentrations of drug treatment were employed. Agonists were added to the bath in a cumulative manner by micropipette. Typically the bath concentration of the agonist (NE) ranged from below the threshold level to 10^{-6} M. The response to each new concentration of agonist was permitted to plateau before adding the next dose. At the concentration of 10^{-6} M, the dose response to

NE usually reached a plateau. Increasing the concentration to 10^{-5} M or 10^{-4} M, usually did not increase the contractile response of the tissues. The maximal response obtained during this initial control period for NE, referred to as round 1, was arbitrarily established as the 100% response. All subsequent responses (round 2, 3, or 4) to agonists of each tissue were then compared to this initial (100%) response. Frequency-response curves were generated from 4 to 32 hertz. Prior to the initial frequency-response study, the tissues were maximally contracted with KCl 120 mM to establish the contractility of the tissues. The maximal contraction to KCl 120 mm was arbitrarily set as 100% contraction for the field stimulation set of experiments. The subsequent initial response to electrical stimulation is referred as round 1 and designated as ——— A ——— in the figures. The magnitude of contraction to electrical stimulation is given as a percentage of the contraction to KCl. All subsequent frequency-responses, namely, rounds 2, 3, or 4 are designated as -----B-----, — - — C — - —, or — — D — — in the figures, respectively.

Following each round of stimulation (via agonist or electrical) the bath fluid was repeatedly changed with fresh Krebs solution and the tissue permitted to relax. Once a stable baseline was achieved, the tissue was prepared for the subsequent round of stimulation by adding the appropriate drug (treatment). All drugs (such as receptor antagonists, nerve conduction blockers, or enzyme inhibitors, etc.) were allowed to equilibrate with the tissues for a specified period and remained in the bath while repeating the initial form of stimulation.

C. Statistical Analysis

The effects of drugs were estimated by the following mathematical analysis: Type I: drug effect on NE dose response curves. The student t-test for ED50 of the NE dose response curve was examined for this purpose. ED50 values were calculated as followed:

1. The maximal response for each individual curve was set at 100%. All points between 15 and 85% were used for regression lines.

2. In those situations where a point was just under the 15% or over the 85% level, the following rule was used to ascertain if that point should be used in calculating the regression line: if the difference in percent response for a dose was greater than 10% to the nearest point within 15-85% range, then this point was kept and used for linear regression. Thus, at a maximum only two points outside the 15-85% range were used, one above 85% and one below 15%.

3. The ED50 was calculated from the above regression line using both procedure 1 and 2.

The shifts of these ED50s and slopes by the drug effect in the treated tissue were then corrected in a ratio manner by the control tissue to eliminate any "time factor" changes in sensitivity during the course of the experiment. Student t-test was computed by these values from several turkeys using the same treatment to see if these differences were significant.

Type I analysis is to estimate the drug effect on the linear portion of NE dose response curve. Another way to estimate the drug effect is to look at the contractile response to each individual dose (or hertz). This mathematical analysis is referred to as Type II analysis and is especially more appropriate when examining the drug effects on contractile responses to electrical stimulation (ES) than isType I analysis. It may not be appropriate to estimate ED50 from these "hertz response curves" since the linear portion in these curves is not clear.

Type II analysis evaluates the vertical response of a treated and a control tissue to a specific dose of agonist or frequency of stimulation. For instance, suppose the percent contraction for a certain dose or hertz (e.g., 1×10^{-8} M of NE, or 4 hertz of electrical stimulus) in the first round of the treated tissue = a, and that in the n-th (n=2,3,4) round of

the treated tissues = b, in the same manner, if the percent contraction for the first round of the control tissue = c, for the n-th (n=2, 3, or 4) round = d, then:

Distant difference (D) = (b-a) - (d-c)

Ratio difference (R) = b - a × (d/c)

In the equations, a and c are the control responses for treated and control tissues, respectively. The above D or R values from several different turkeys (using the same treatment) were then used to compute a student t-value. In some cases, distant difference (D) seems to be more appropriate for statistical analysis, while in other cases, ratio difference seems to be more appropriate. For this reason, I used both of these ways for statistical analysis. See Appendix C for clarification.

D. Calculation of pA_2 Values

The characterization of alpha adrenergic receptors was accomplished in the past by determining the pA_2 value for selected adrenergic antagonists. This method was introduced first by Schild (1947) and now has become a popular way to express the potency of a competitive antagonist and a useful tool to study drug receptor interaction. The pA_2 value is the negative logarithm of the molar concentration of an antagonist that produces a two-fold (concentration basis) shift to the right in the agonist dose-response curve. In other words, the concentration of the agonist has to be doubled to maintain the same responses. The formula used to calculate the pA_2 value is indicated below:

$$\frac{[A']}{[A]} - 1 = \frac{[B]}{KB} \qquad \text{(Eq. 1; Furchgott, 1972)}$$

where [A']/[A] is the ratio of the concentration of agonist giving an equal response in the presence ([A']) and in the absence of the antagonist ([A]), respectively. This ratio is referred to as the dose ratio (DR). [B] is the concentration of antagonist in equilibrium with the receptor. KB is the dissociation constant of the receptor-antagonist complex. The equation

above is usually put in the logarithmic form for calculation and plotting (Furchgott, 1972): log (DR - 1) = log (B) - log KB. When Dose ratio = 2, then -log[B] = pA_2 = -log KB.

Therefore, the pA_2 value is an important tool to quantitatively estimate drug-receptor interaction (from KB).

The optimal conditions (Furchgott, 1972) and experimental designs (Ott et al., 1981) for determining pA_2 values are well-described in the literature. My experiments were carried out with these precautions in mind.

Methoxamine was used as the α_1-adrenergic agonist (Nelson et al., 1977), since it is 1) not a candidate for $uptake_1$, (Trendelenburg et al., 1970) 2) not metabolized by monoamine oxidase or catechol-o-methyl transferase, and 3) is a rather "pure" agonist for the α_1-adrenergic receptor (Katzung, 1984). The experimental determination of the pA_2 value was accomplished as described below:

A dose response relationship to methoxamine was determined on five rings cut from the same aorta. After washing out and re-establishment of the original baseline, an antagonist was added to each of four baths using a different concentration for each bath. The antagonist (prazosin or yohimbine) was allowed to equilibrate with the tissues for 40 minutes. The fifth tissue received no antagonist and was used to correct for changes in sensitivity to methoxamine which occurred during the course of the experiment. At the end of the equilibrium period of the antagonist with the tissue (the antagonist remaining in the bath), a cumulative dose response relationship to methoxamine was obtained. The shift of the dose ratio produced by the antagonist was taken at the ED50 for calculation purposes. Then these dose ratios and the concentrations of the antagonist were used in the Schild plot. The regression line ($y = mx+b$) for these points (x_i,y_i) in the Schild plot was calculated by the equations below:

$$m = \frac{\Sigma x_i y_i - n\bar{x}\bar{y}}{\Sigma x_i^2 - n\bar{x}^2} \qquad \text{(Eq. 2)}$$

$$b = \bar{y} - m\bar{x} \qquad \text{(Eq. 3)}$$

Here $x_i = \log [B]$, $y_i = \log (DR - 1)$.

In the regression line, $y = mx + b$, pA_2 value is the x value when $y = 0$, since $\log (2-1) = 0$, thus $pA_2 = -\log [B] = -(-b/m) = (b/m)$.

E. Description of the Figures

Each point in Figures 3-31 is the mean of the number of observations, and the vertical bar is the standard error of the mean (SEM). The standard error bars are distinguished from the various rounds in each experiment by varying the horizontal length of each bar's cap, from example: A (first round), B (second round), C (third round), D (fourth round), etc. The following symbols are designated for those points with statistical meaning of significance (see discussion in statistical analysis): for distant difference, *=$p < 0.05$, **=$p < 0.01$; for ratio difference, #=$p < 0.05$, ##=$p < 0.01$.

V. RESULTS

A. General Description

During the equilibration period, spontaneous contractile activity was observed in most of the tissues. This activity was abolished by adding KCl (120 mM) to the tissue bath several times followed by washing out each dose of KCl with fresh Krebs solution. After a stable base line was obtained, each experiment was initiated.

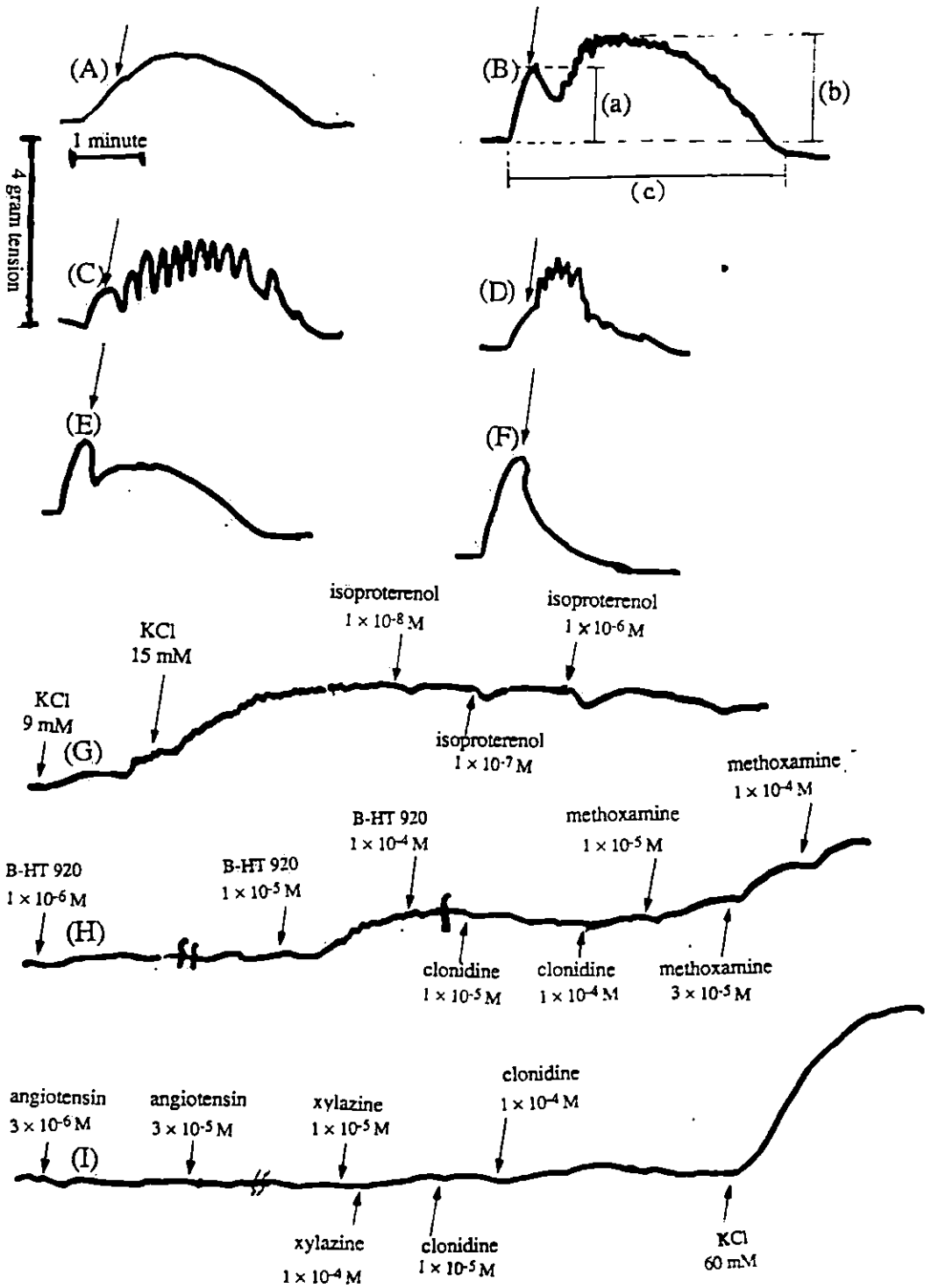
During electrical field stimulation, a rapid contractile response was observed in most turkey aorta preparations. After cessation of the electrical current, a slower and more prolonged contraction continued. This two-phased response to ES will be referred to as ESR (electrical stimulation response). The initial contractile response during electrical current will be called phase 1, and the secondary contraction which occurred after cessation of the electrical current will be called phase 2. The maximal response (grams of tension developed) to each phase of contraction was obtained by subtracting the base tension from the peak tension developed. Figure 2 (A)-(F) shows several different types of ESR. Among them, (A) and (B) were the most common occurring types of contractions.

B. Drugs Acting on the Electrical Stimulation Response (ESR)

1. Adrenergic neuronal blockers

a. Bretylium Bretylium was applied to isolated aorta from 5 different turkeys. Statistical analysis (described in materials and methods) shows that bretylium had no significant effect on phase 1 when compared to control. However, bretylium did have a significant ($p < 0.05$) inhibitory effect on phase 2 at 8 and 32 hertz. The effect of bretylium

Figure 2. Tracings (A)-(F) are responses to electrical stimulation. Arrows on these tracings indicated when the stimulator was turned off. In tracing (B), lines labeled (a) and (b) indicate the measurement of the response to phase 1 and phase 2, respectively Line (c) is for the measurement of duration. Tracing (G) indicates the response to isoproterenol in a preparation in which tone was induced by adding KCl (15mM) to the bath. Tracing (H),(I) are the comparisons of the contractile response to several alpha agonists


(A)
1 minute
4 gram tension
(B)
(a)
(b)
(c)
(C)
(D)
(E)
(F)
KCl
9 mM
(G)
KCl
15 mM
isoproterenol
1 × 10-8 M
isoproterenol
1 × 10-7 M
isoproterenol
1 × 10-6 M
B-HT 920
1 × 10-6 M
(H)
B-HT 920
1 × 10-5 M
B-HT 920
1 × 10-4 M
clonidine
1 × 10-5 M
methoxamine
1 × 10-5 M
clonidine
1 × 10-4 M
methoxamine
1 × 10-4 M
methoxamine
3 × 10-5 M
angiotensin
3 × 10-6 M
(I)
angiotensin
3 × 10-5 M
xylazine
1 × 10-5 M
xylazine
1 × 10-4 M
clonidine
1 × 10-5 M
clonidine
1 × 10-4 M
KCl
60 mM

on the phase 1 (Figures 3a and 3b) and on the phase 2 (Figures 4a and 4b) responses to ES is presented in the figures indicated.

b. Guanethidine After control responses to electrical stimulation were obtained, guanethidine (10^{-6} M) was added to the treated tissue baths, and the control tissue bath did not receive any drug. This is referred to as round 2 in Figures 5a and 5b (phase 1), and in Figures 6a and 6b (phase 2). Following the determination of the tissue response to varying the frequency of stimulation in round 2. The guanethidine in the bath was increased to 10^{-5} M. After an additional 30 minute equilibration, another frequency-response relationship was obtained and this is referred to as round 3. At this point, the bath fluid was repeatedly changed with fresh Krebs solution to remove the guanethidine. After a 30 minute period, a frequency-response relationship was again obtained (round 4). We can see from the figures that phase 2 seems to be much more sensitive to guanethidine than phase 1, and that guanethidine 10^{-5} M (round 3) had a better inhibitory effect than guanethidine 10^{-6} M (round 2). After washing out the treatment (round 4) 30 minutes, the responses did not recover to the control levels. Statistical analysis shows that round 3 and round 4 are significantly ($p < 0.05$) different from the control period (round 1) at 4 hertz and 32 hertz (Figures 5a and 5b), respectively.

2. Neuronal conduction blocker: tetrodotoxin

Tetrodotoxin (TTX), blocked phase 2 (Figures 8a and 8b) rather than phase 1 (Figures 7a and 7b) of the ESR. As would be expected, a concentration of 10^{-6} M inhibited the response to ES greater than did 10^{-7} M. In contrast to guanethidine, the inhibitory effect of TTX on the ESR dissipated when fresh Kreb solution was added to the bath and the 4th frequency-response relationship (round 4) was similar to the control (Figures 8a and 8b). The first phase was not impressively blocked by any of the above neuronal blockers.

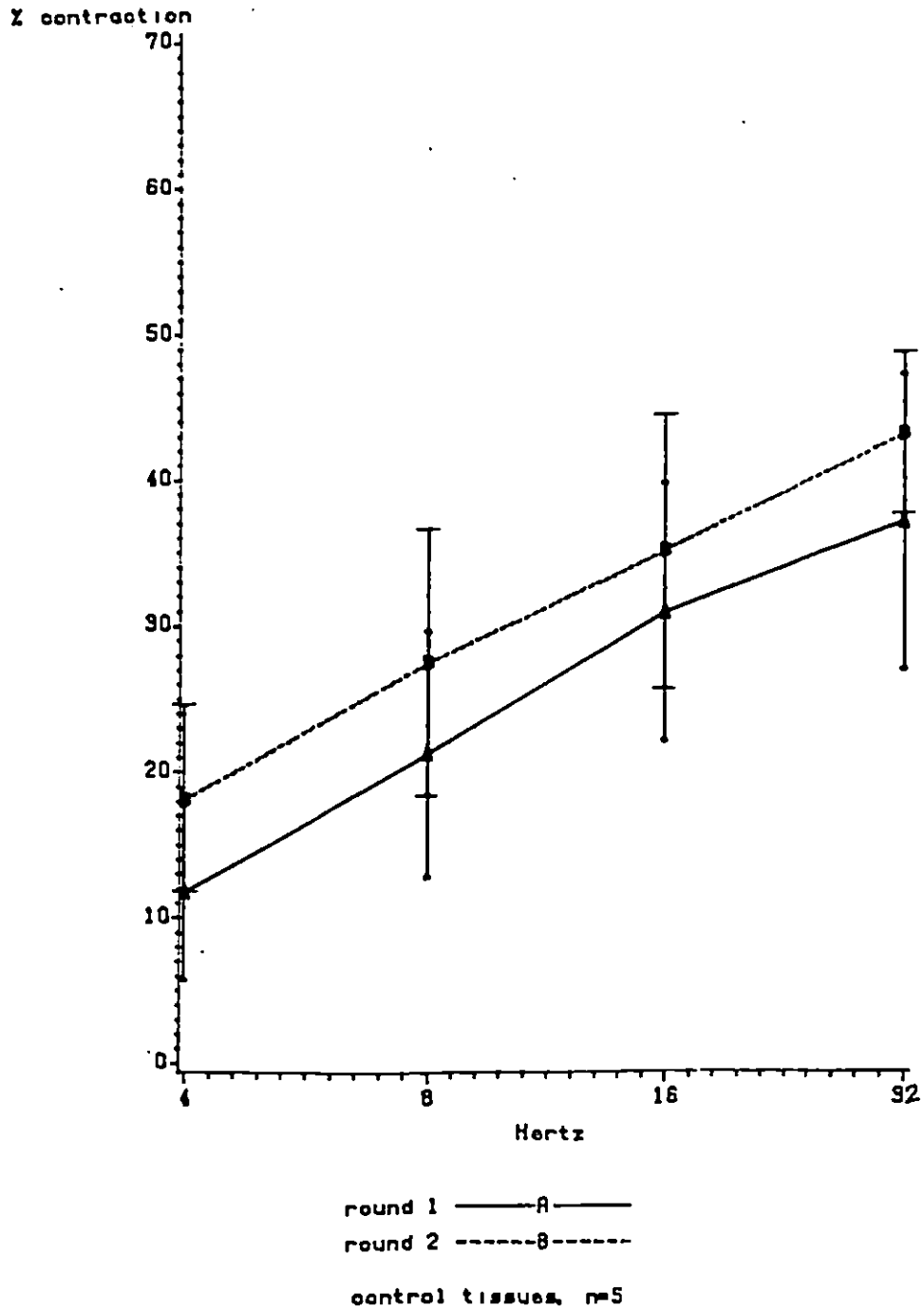


Figure 3a. Control of Figure 3b

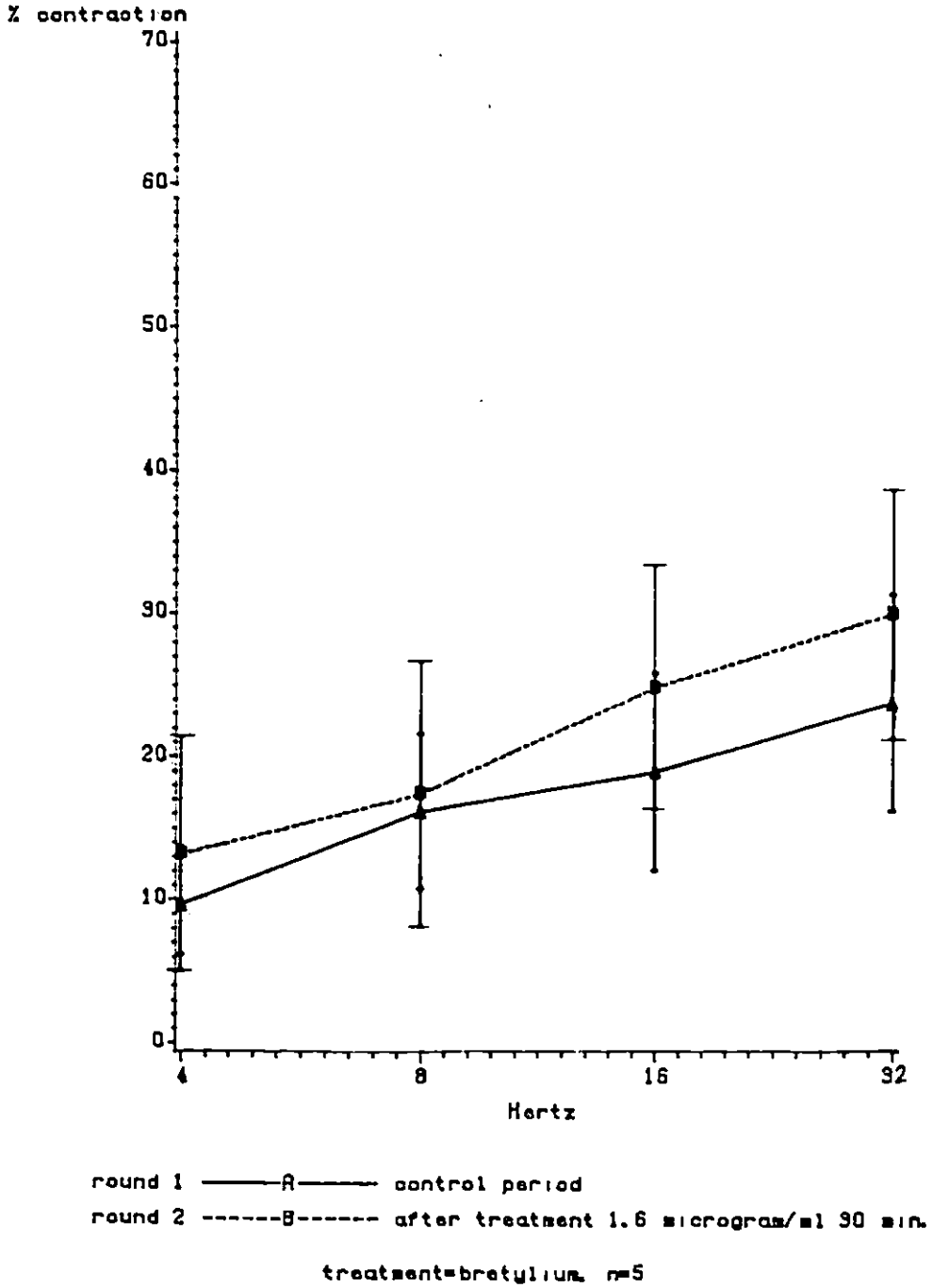


Figure 3b. Effect of bretylium on phase 1 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 3a

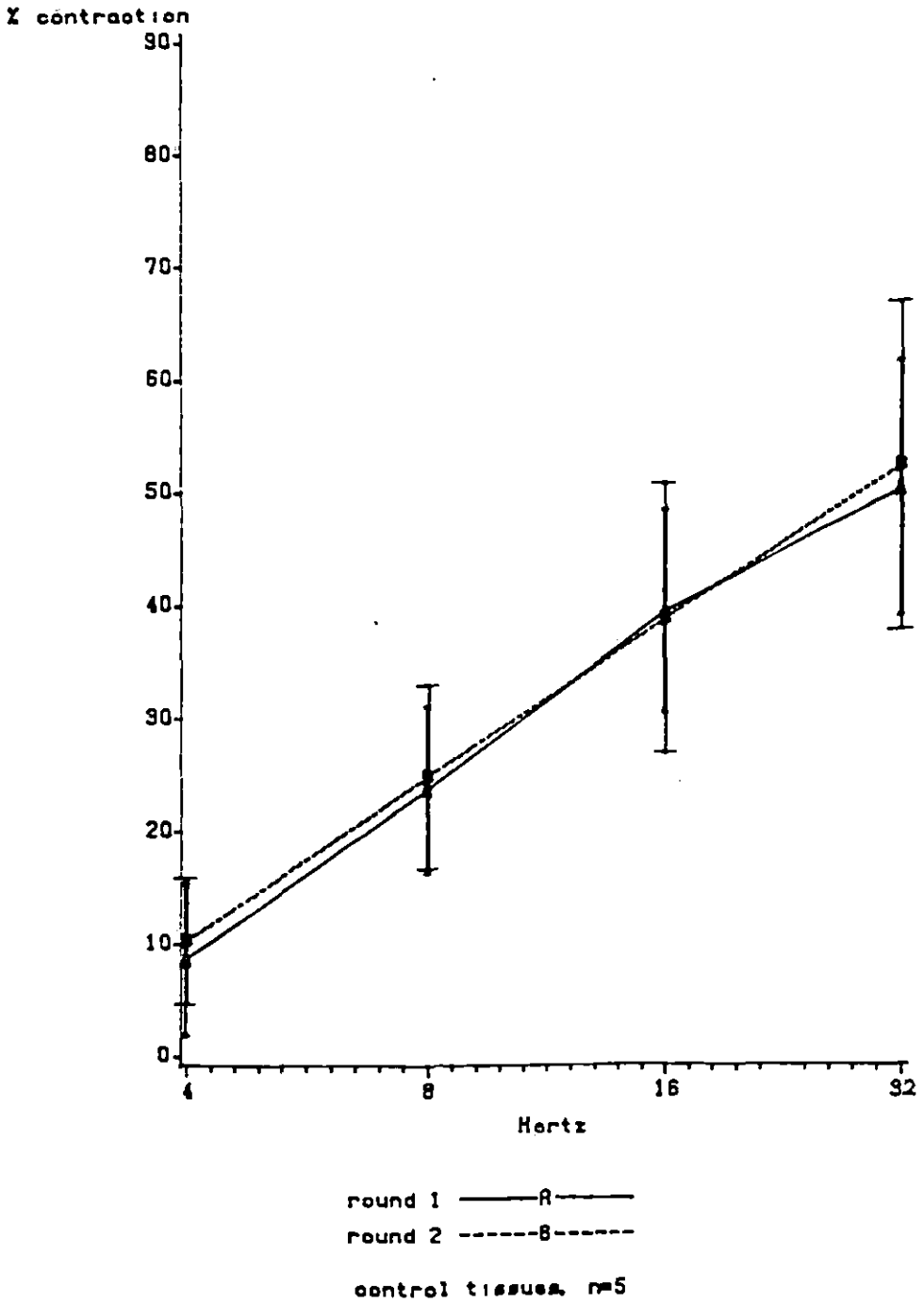


Figure 4a. Control of Figure 4b

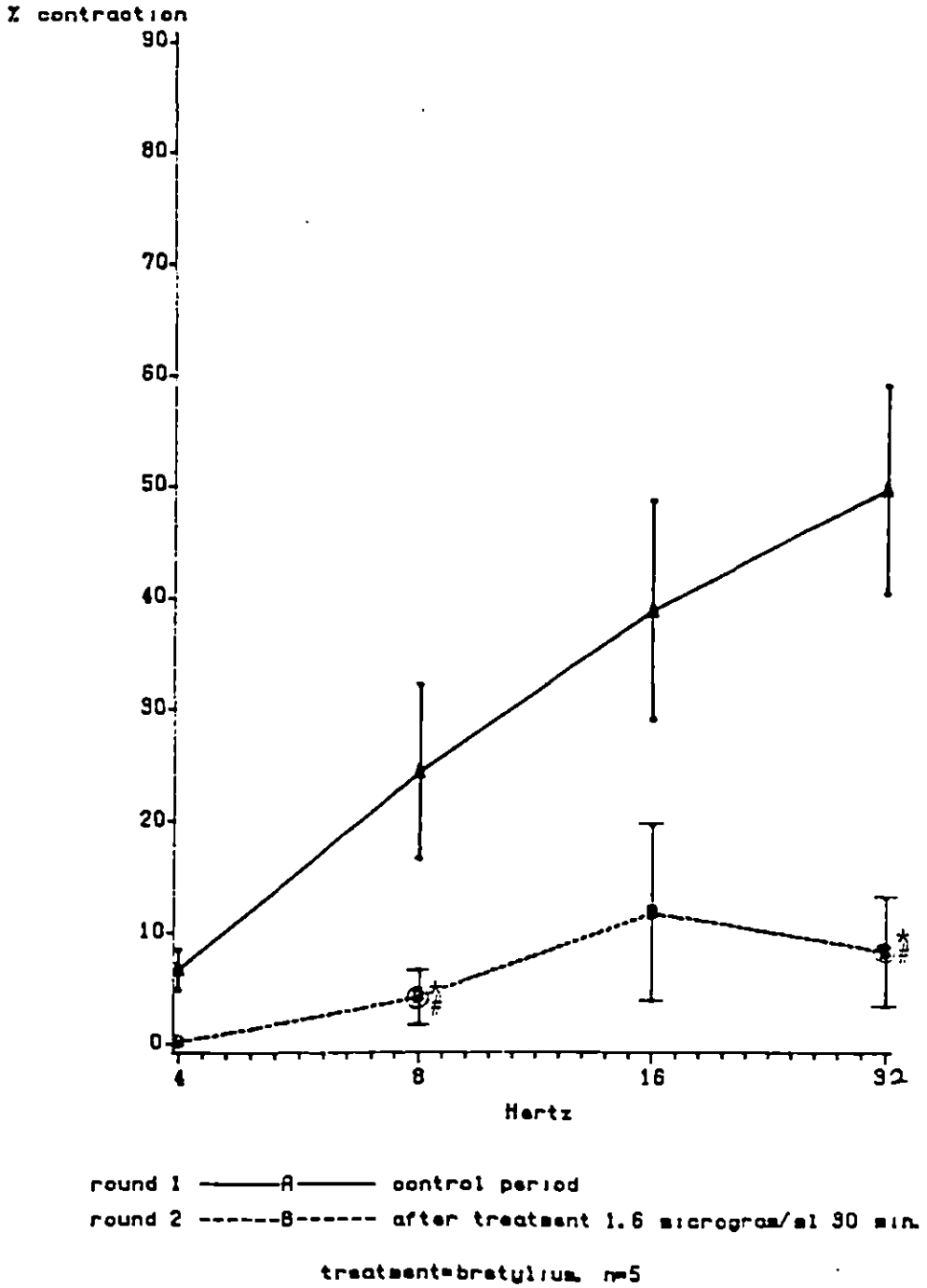


Figure 4b. Effect of bretylium on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 4a

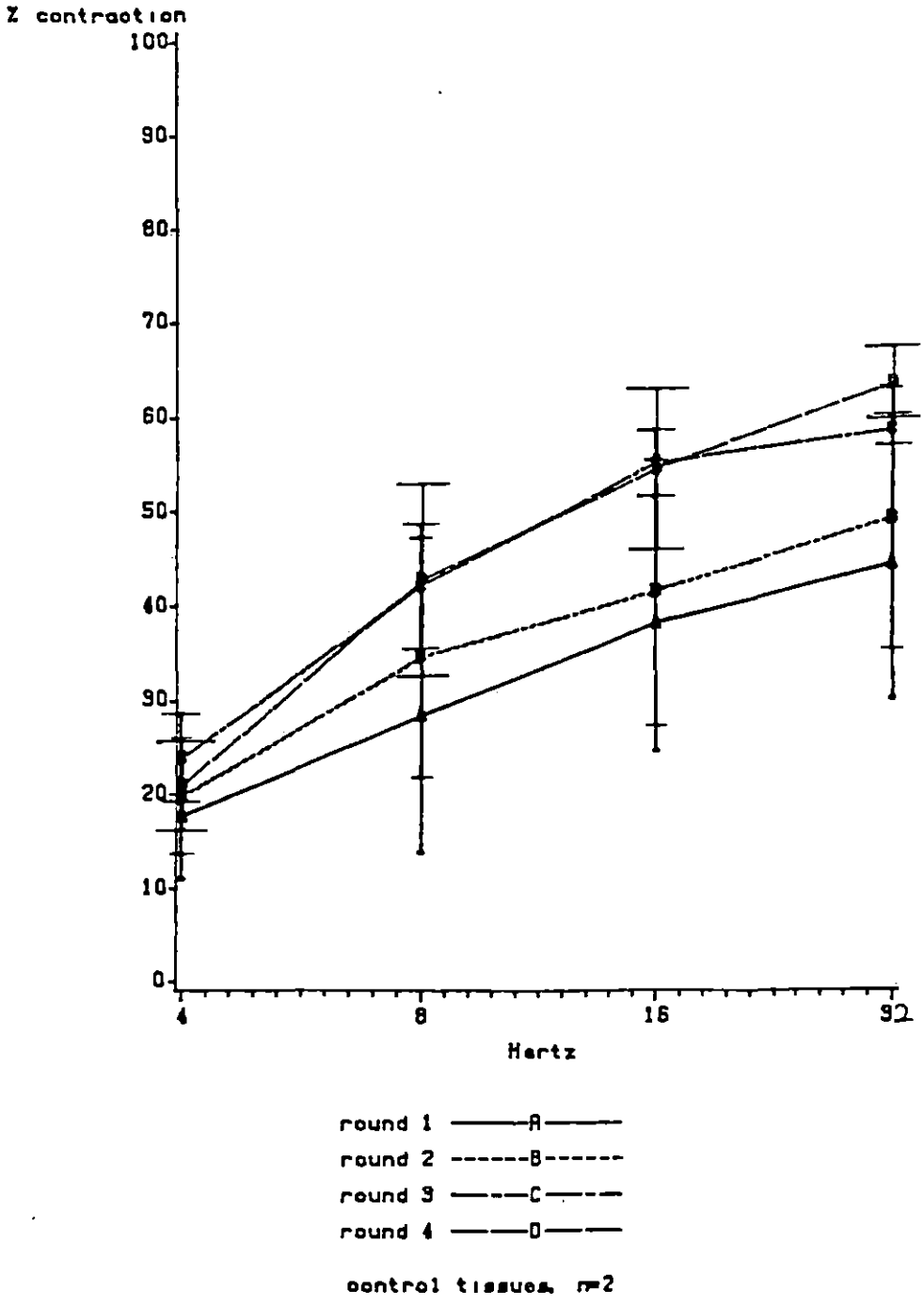


Figure 5a. Control of Figure 5b

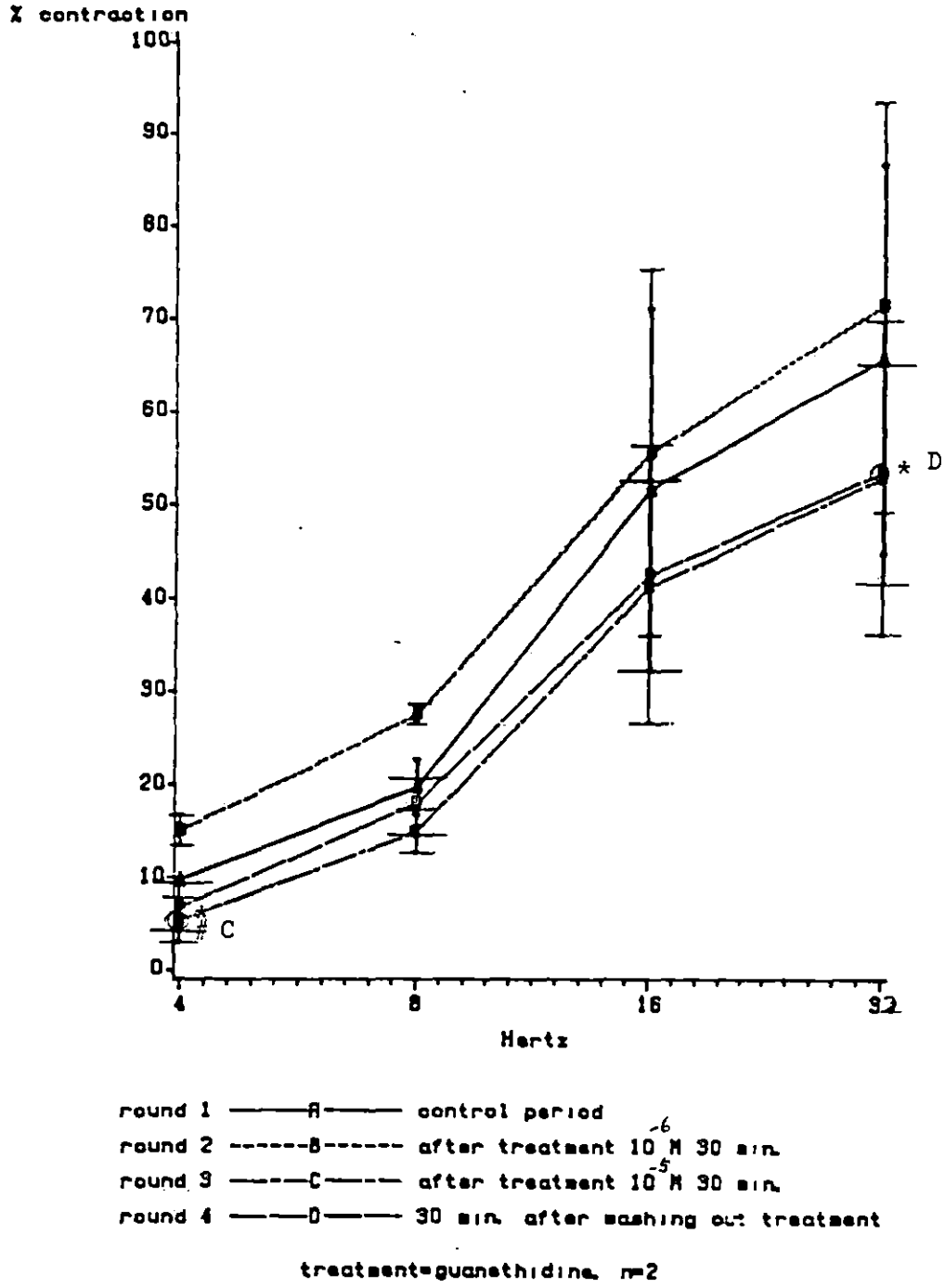


Figure 5b. Effect of guanethidine on phase 1 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 5a

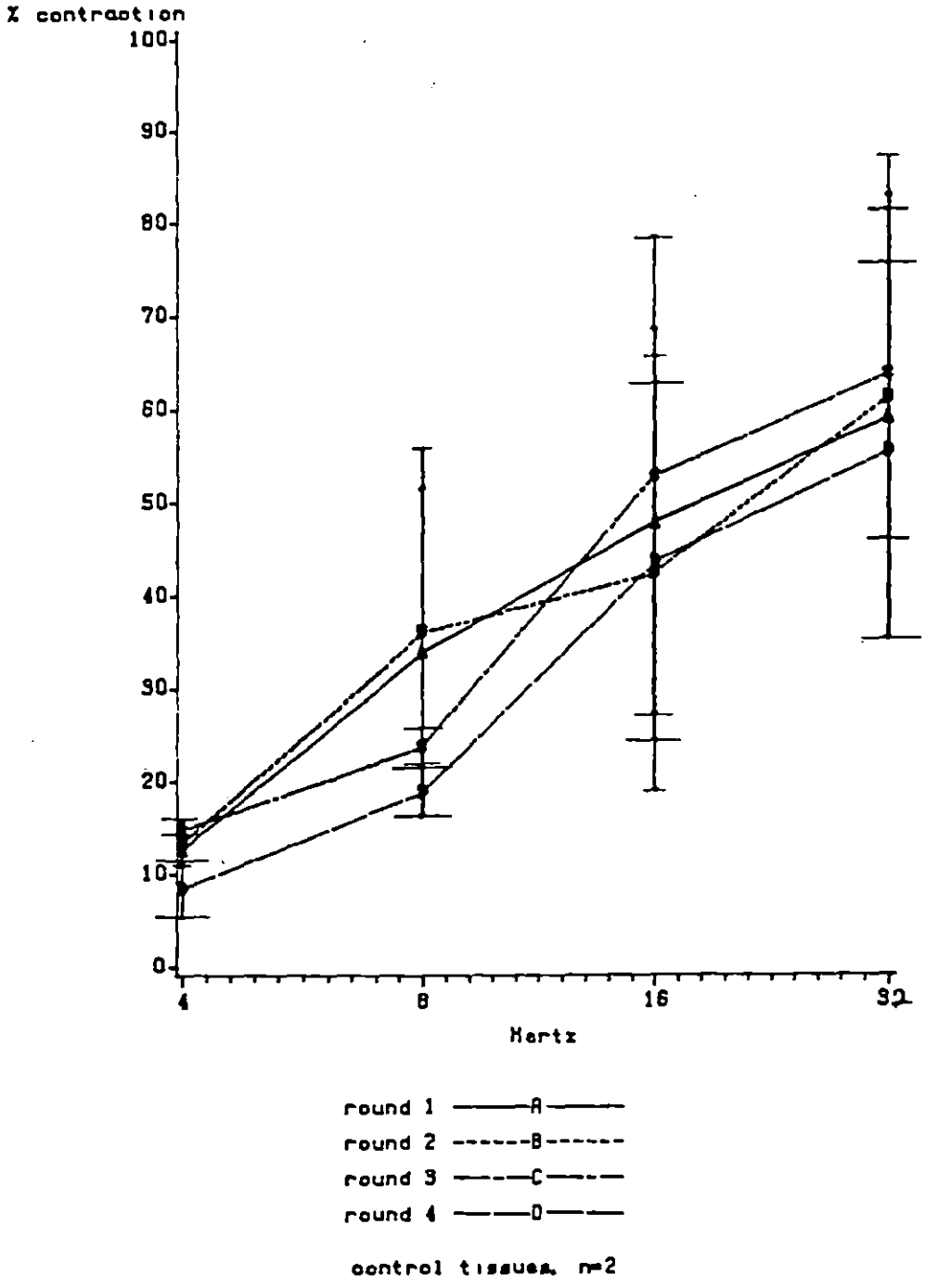


Figure 6a. Control of Figure 6b

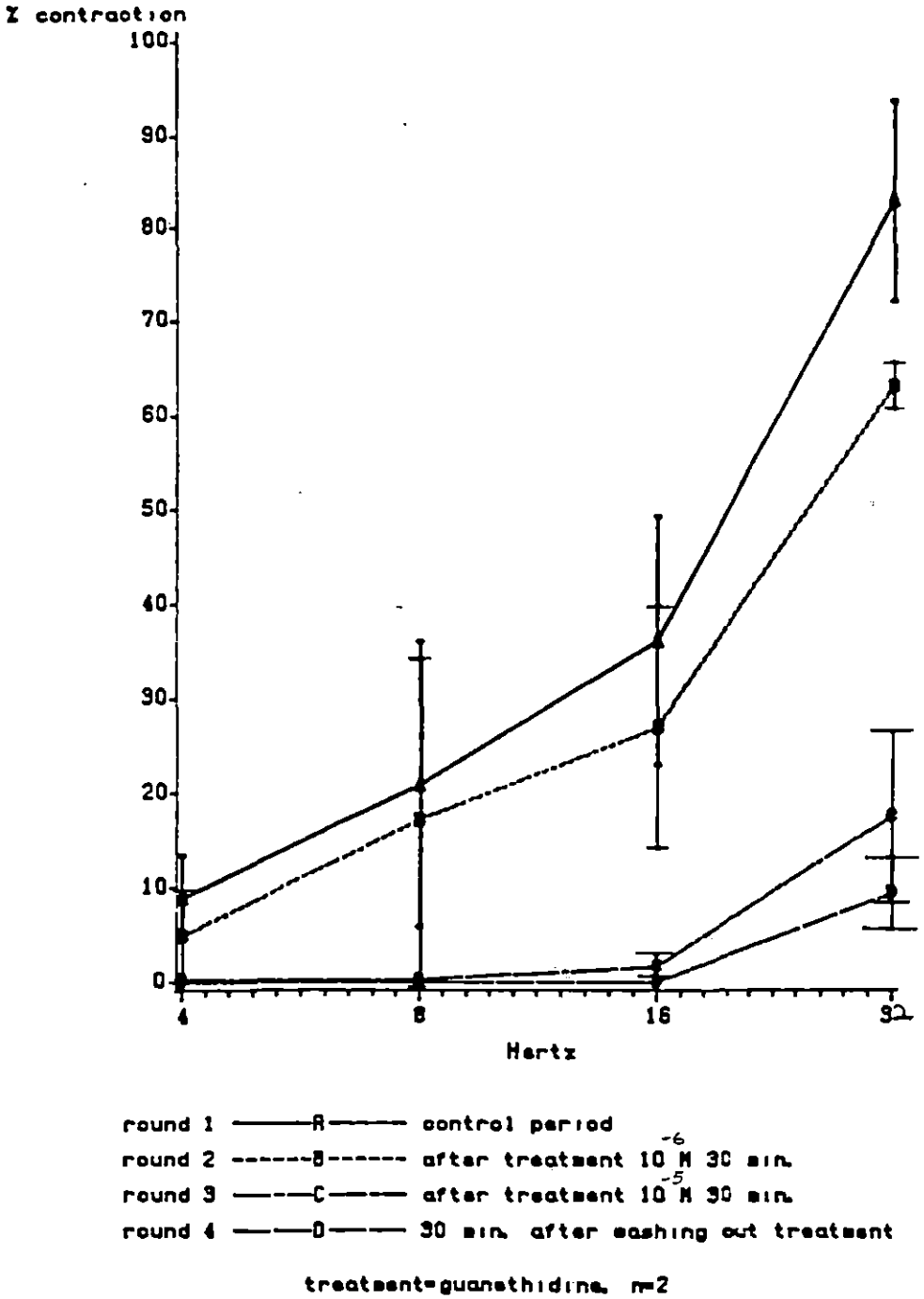


Figure 6b. Effect of guanethidine on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 6a

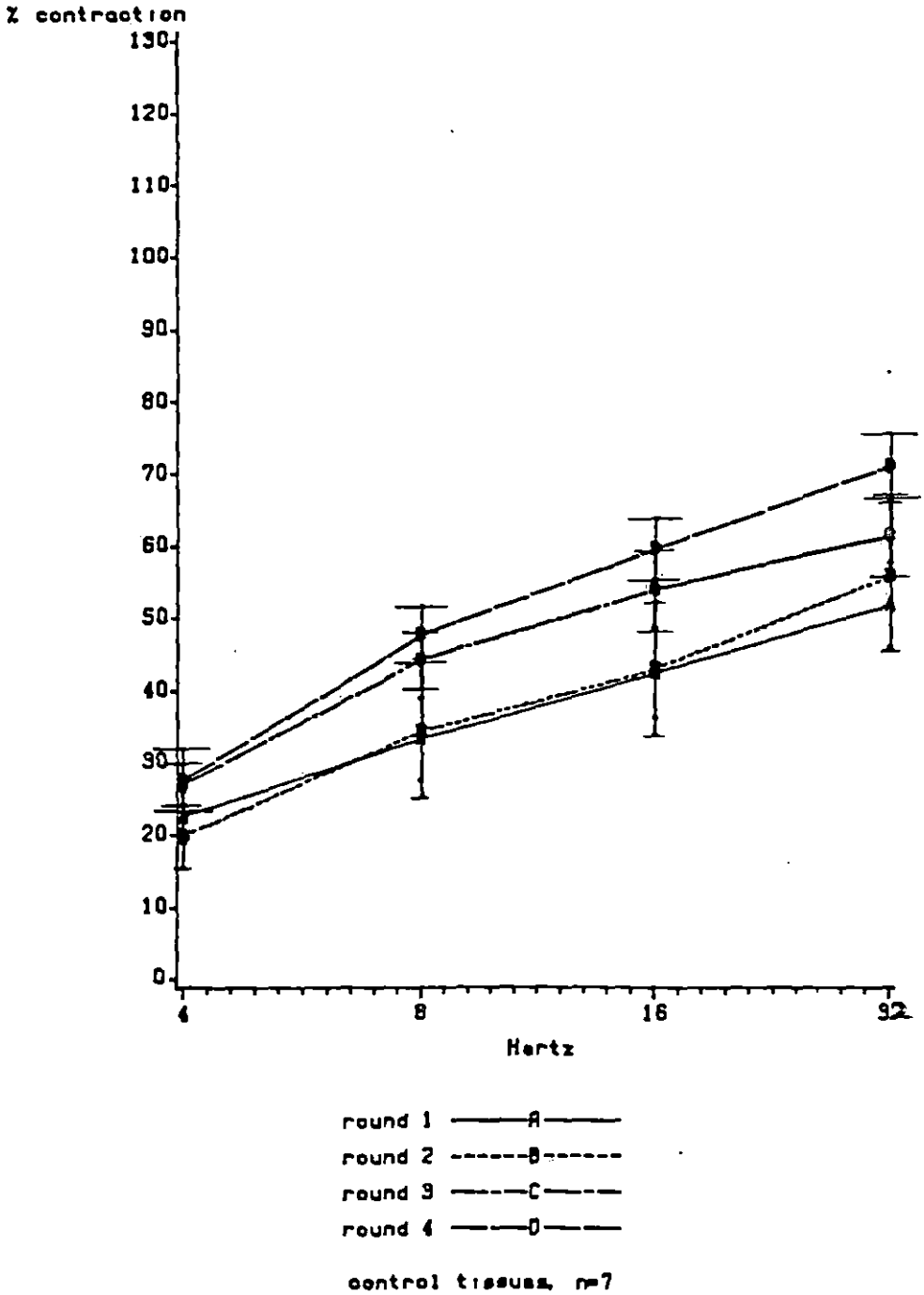


Figure 7a. Control of Figure 7b

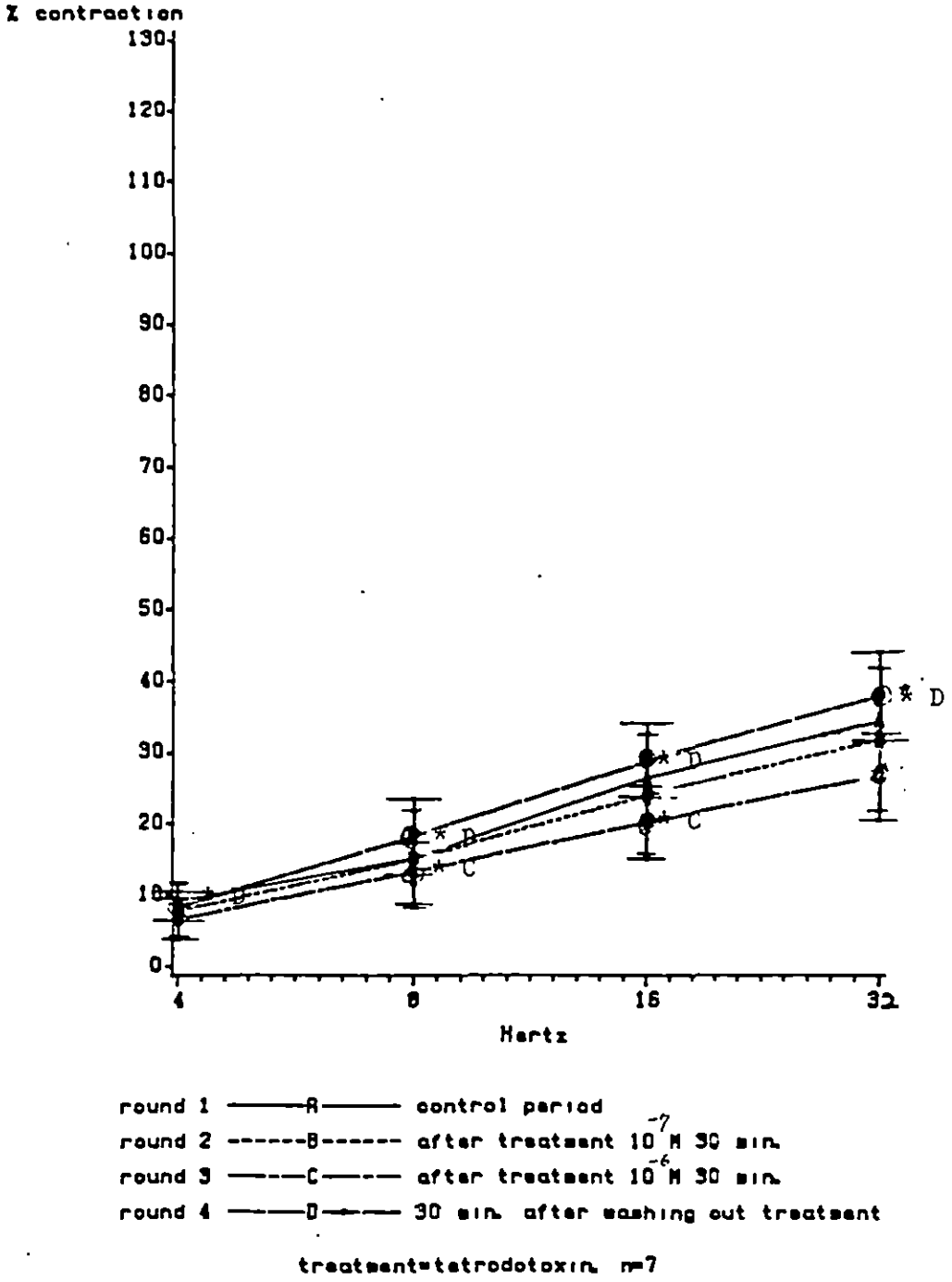


Figure 7b. Effect of tetrodotoxin on phase 1 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 7a

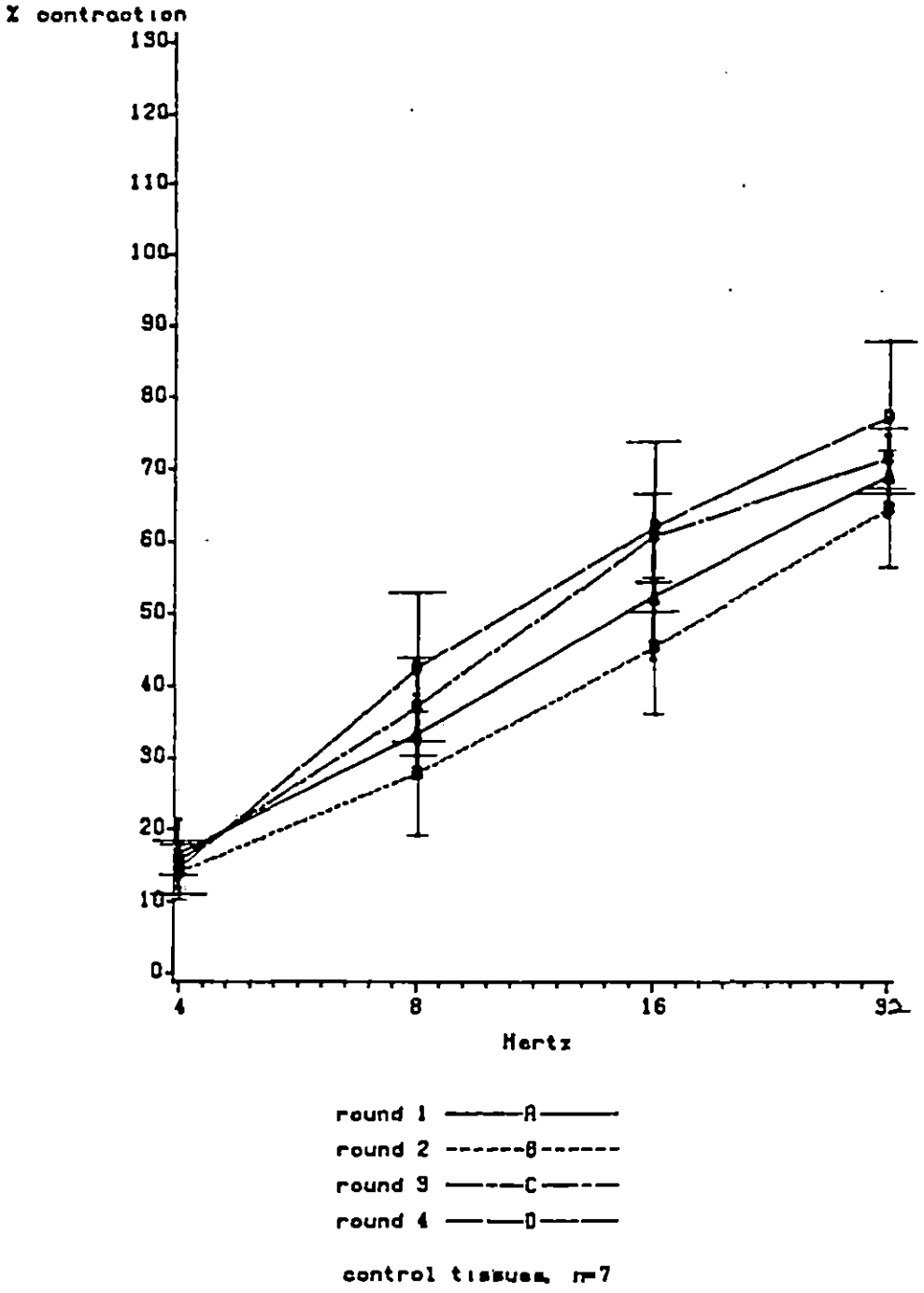


Figure 8a. Control of Figure 8b

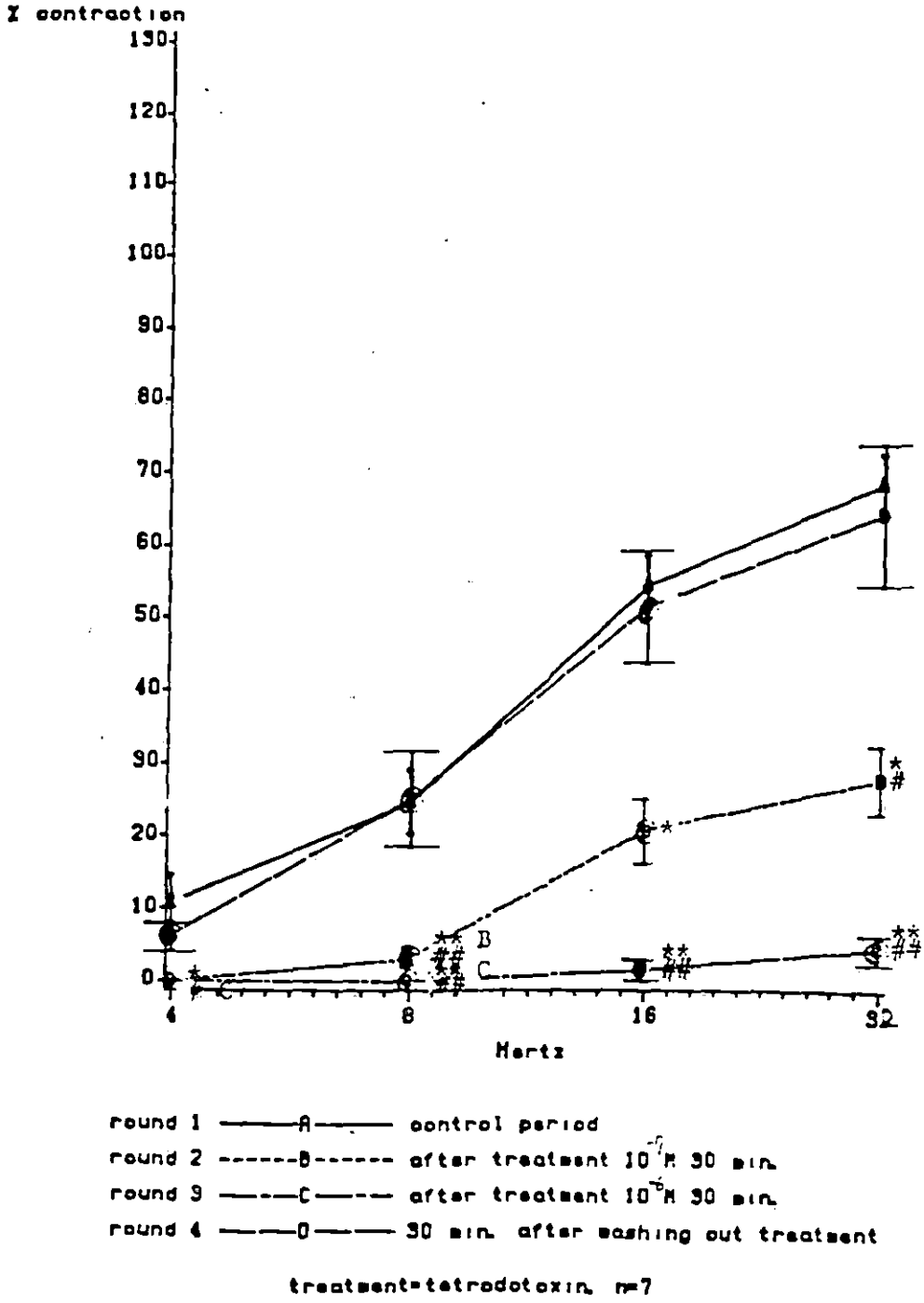


Figure 8b. Effect of tetrodotoxin on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 8a

3. Other tissues from turkeys and other animals

To further establish our methodology, other tissues from turkeys and other animals were studied. A large artery originating from the beginning of the abdominal aorta (coeliac artery) was used from 5 turkeys. The data presented here shows that both phase 1 (Figures 9a and 9b) and phase 2 responses (Figures 10a and 10b) in this tissue could be as well blocked by TTX (except at 32 hertz) as those from phase 2 (Figures 8a and 8b) of the turkey aorta. This blockade is statistically significant at 16 hertz (Figures 9b and 10b). Again, the inhibitory effect of TTX on the ESR dissipated when fresh Kreb solution was added to the bath (round 3).

Vas deferens from rat (n=6) and guinea pig (n=3) were also studied. The contractile responses to ES in these tissues were all one-phased type, namely, the contractile response occurred only during the flow of electrical current, and these responses could be completely blocked by all of the above neuronal blockers even at high frequency stimulation (> 30 hertz). In rabbit uterus (n=3), the contractile response to ES was also one-phased typed, and these ESR were completely blocked at low frequency stimulation (< 16 hertz). In the case of rabbit (n=3) and rat aorta (n=4), the ESR are of the two-phased type, like turkey aorta, and only the secondary phase of ESR could be well-blocked by neuronal blockers. Detailed data are not presented for these experiments.

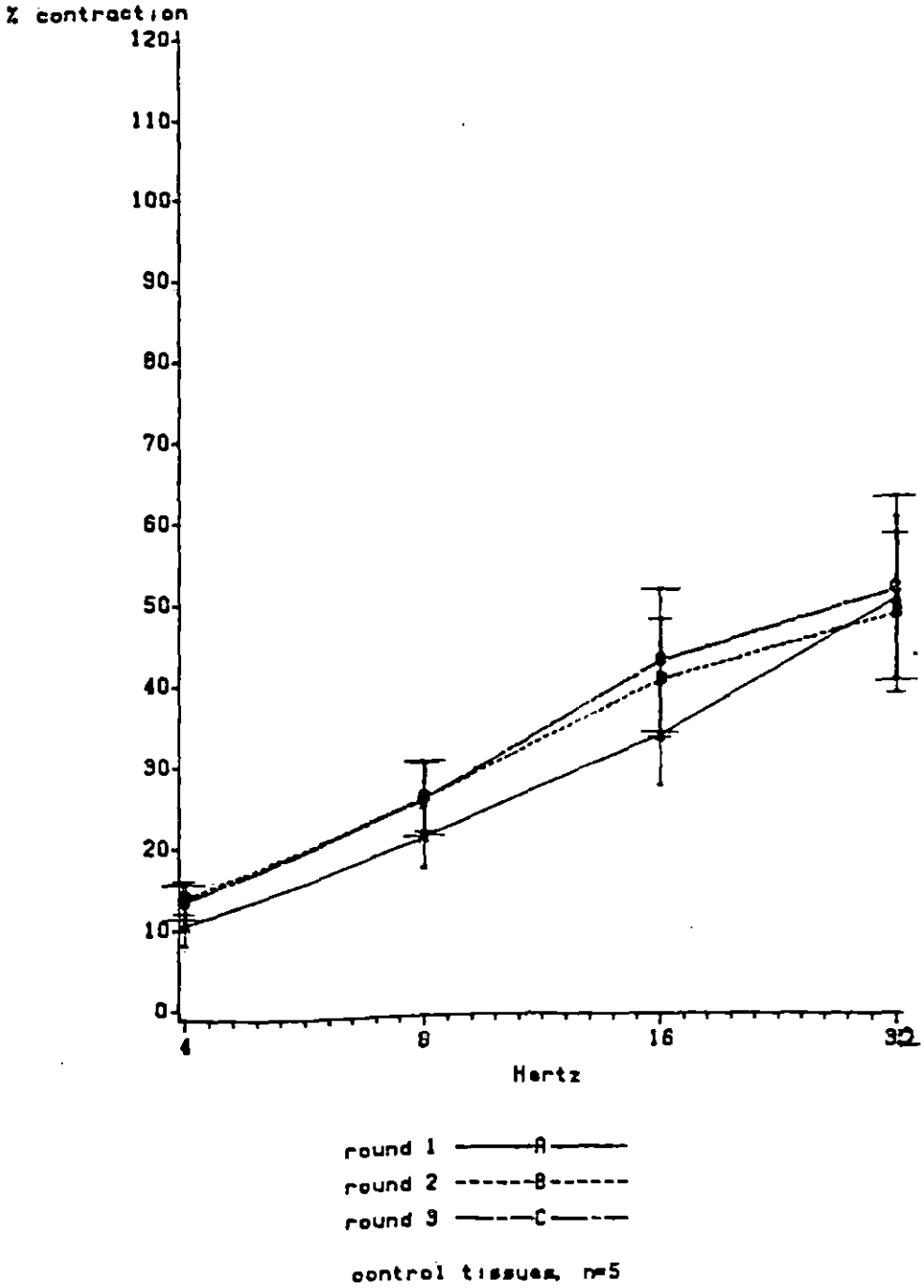


Figure 9a. Control of Figure 9b

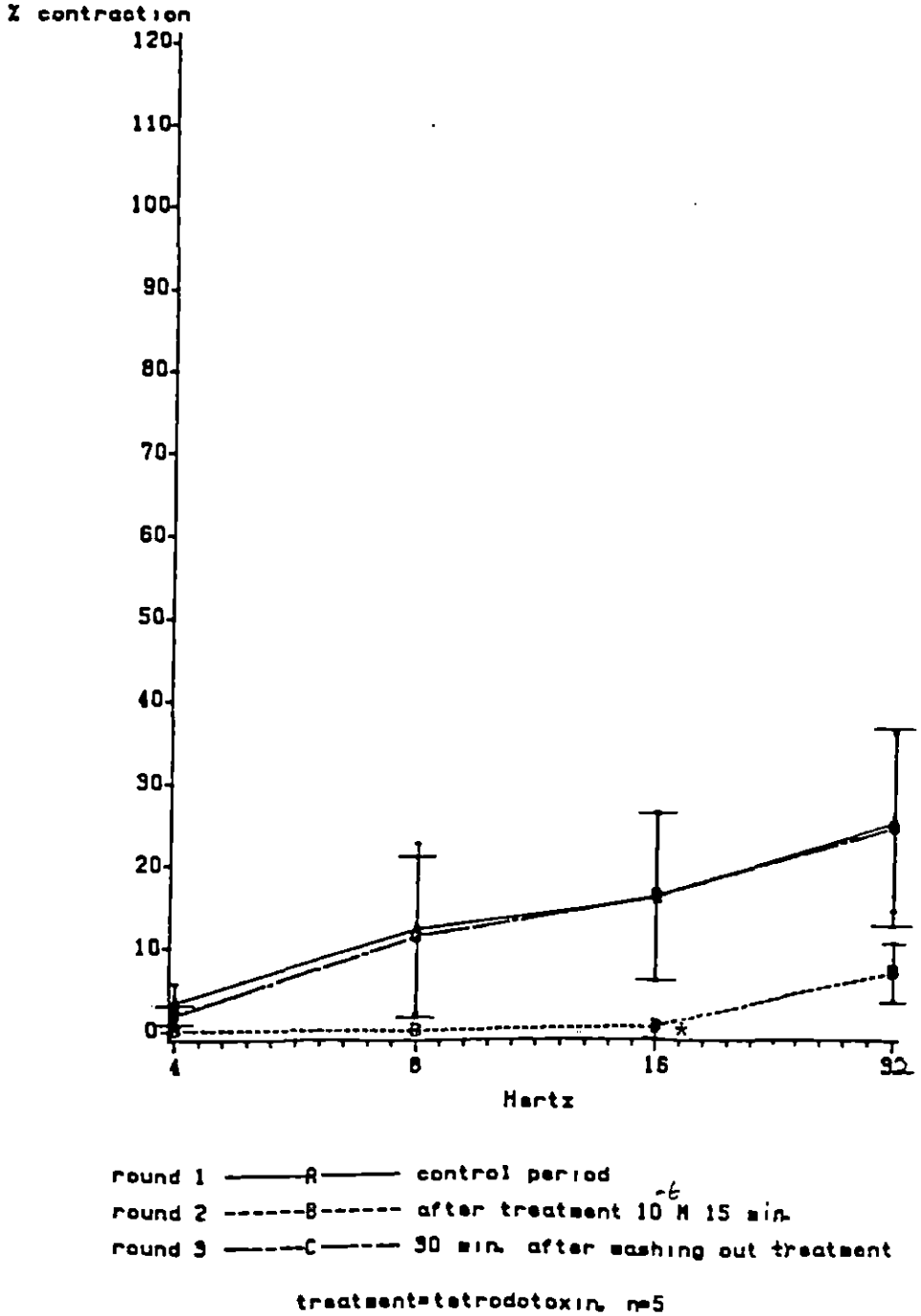


Figure 9b. Effect of tetrodotoxin on phase 1 of the electrical stimulation response (ESR) in turkey coeliac artery. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 9a

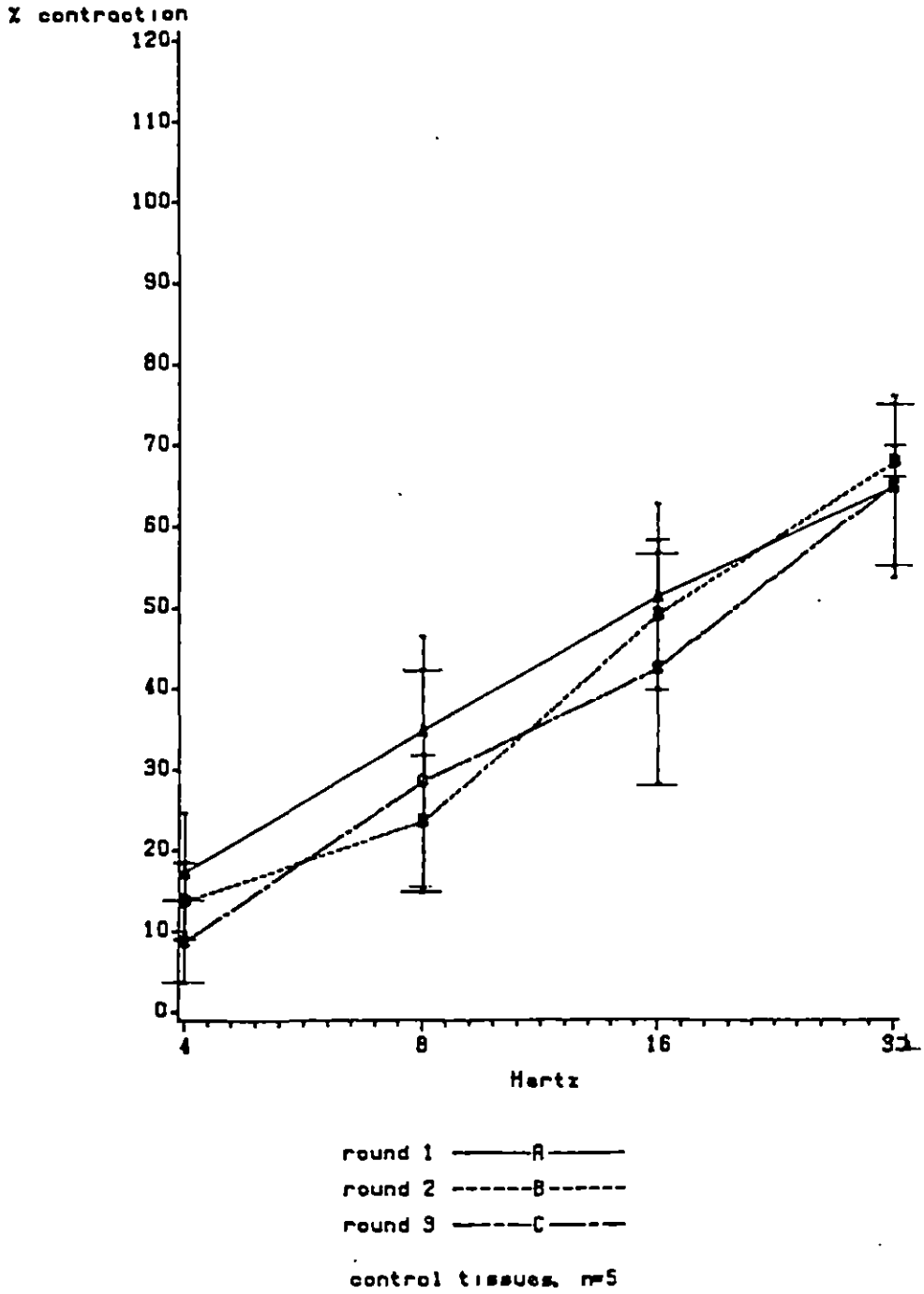


Figure 10a. Control of Figure 10b

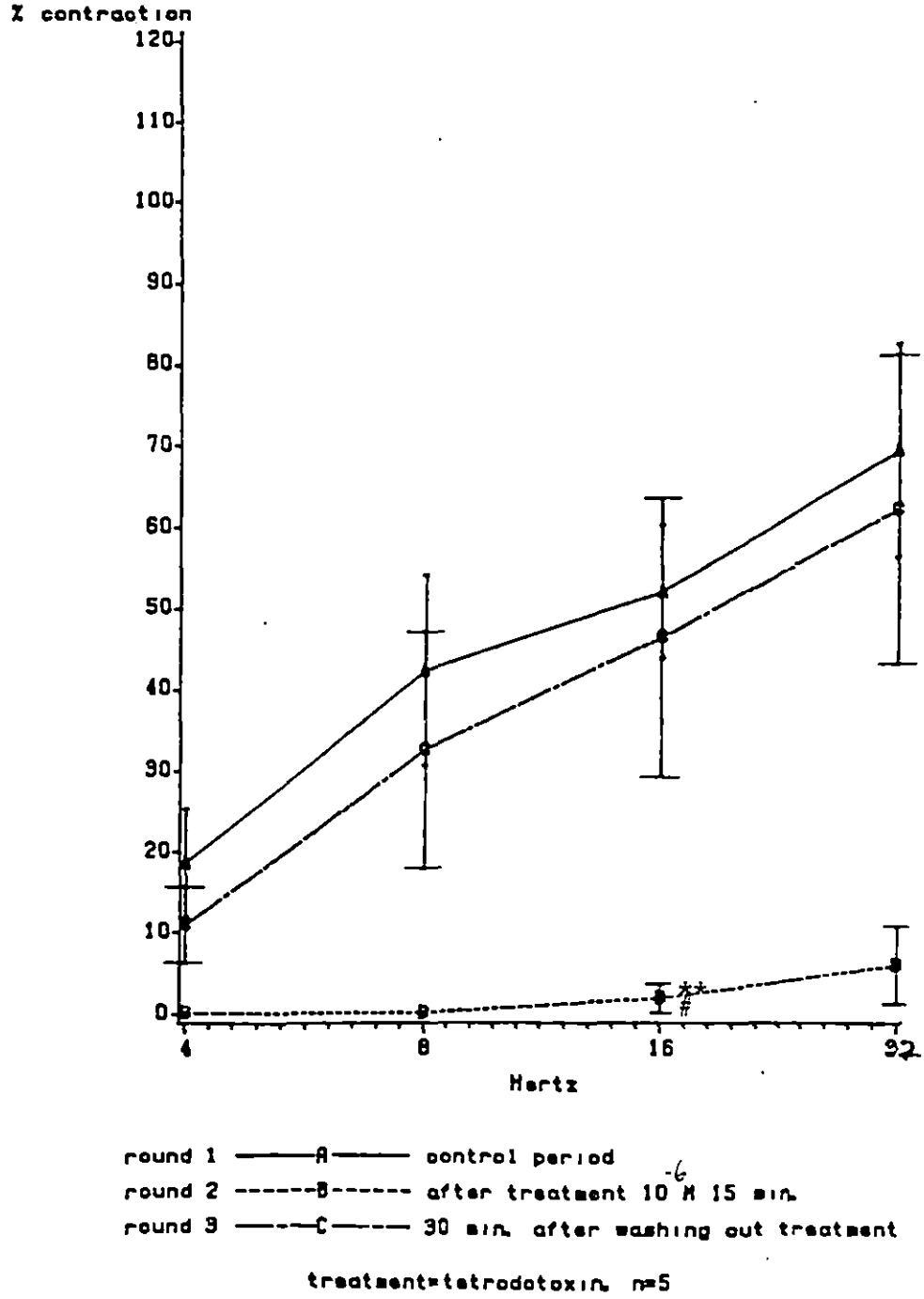


Figure 10b. Effect of tetrodotoxin on phase 2 of the electrical stimulation response (ESR) in turkey coeliac artery. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 10a

C. Modulators of Adrenergic Transmission

1. Serotonin

Serotonin alone caused smooth muscle contraction. Concentrations of serotonin from 2×10^{-7} - 10^{-6} M evoked large contractions. The maximal response was similar to that produced to KCl 120 mM. To test the effect of serotonin on responses to ES and NE we used subthreshold concentrations (10^{-8} M and 10^{-7} M). Figures 11a and 11b show that serotonin (10^{-8} M) and (10^{-7} M), exert a significant ($p < 0.05$) inhibitory effect on the ESR at 16 hertz and 32 hertz. Serotonin (10^{-7} M) exerted a significant ($p < 0.05$) inhibitory effect on the slope of the responses to NE (Table 2, and also Figures 12a and 12b). Serotonin did not significantly alter the ED50 of NE at either of the two concentrations used.

2. Angiotensin II

Angiotensin II in concentrations of 10^{-7} M to 10^{-6} M produced little or no vasoconstriction. Subthreshold concentrations of angiotensin II did not exert a significant effect on either phase 2 of the ESR (Figures 13a and 13b) or on responses to NE (Figures 14a and 14b) except at the 3×10^{-9} M concentration of NE where 10^{-8} M angiotensin II exerted a significant ($p < 0.05$) inhibitory effect.

D. Effect of Drugs Acting on Adrenergic Receptors

1. Alpha-adrenergic receptor antagonists

a. Prazosin This α_1-adrenergic receptor antagonist, as expected, significantly inhibited phase 2 of the response to ES and the contractile responses to NE in turkey aortae (Figures 15-17). Phase 1 was not altered as much as phase 2. The ED50 (Table 2) for NE in the presence of prazosin was significantly ($p < 0.01$) different from control.

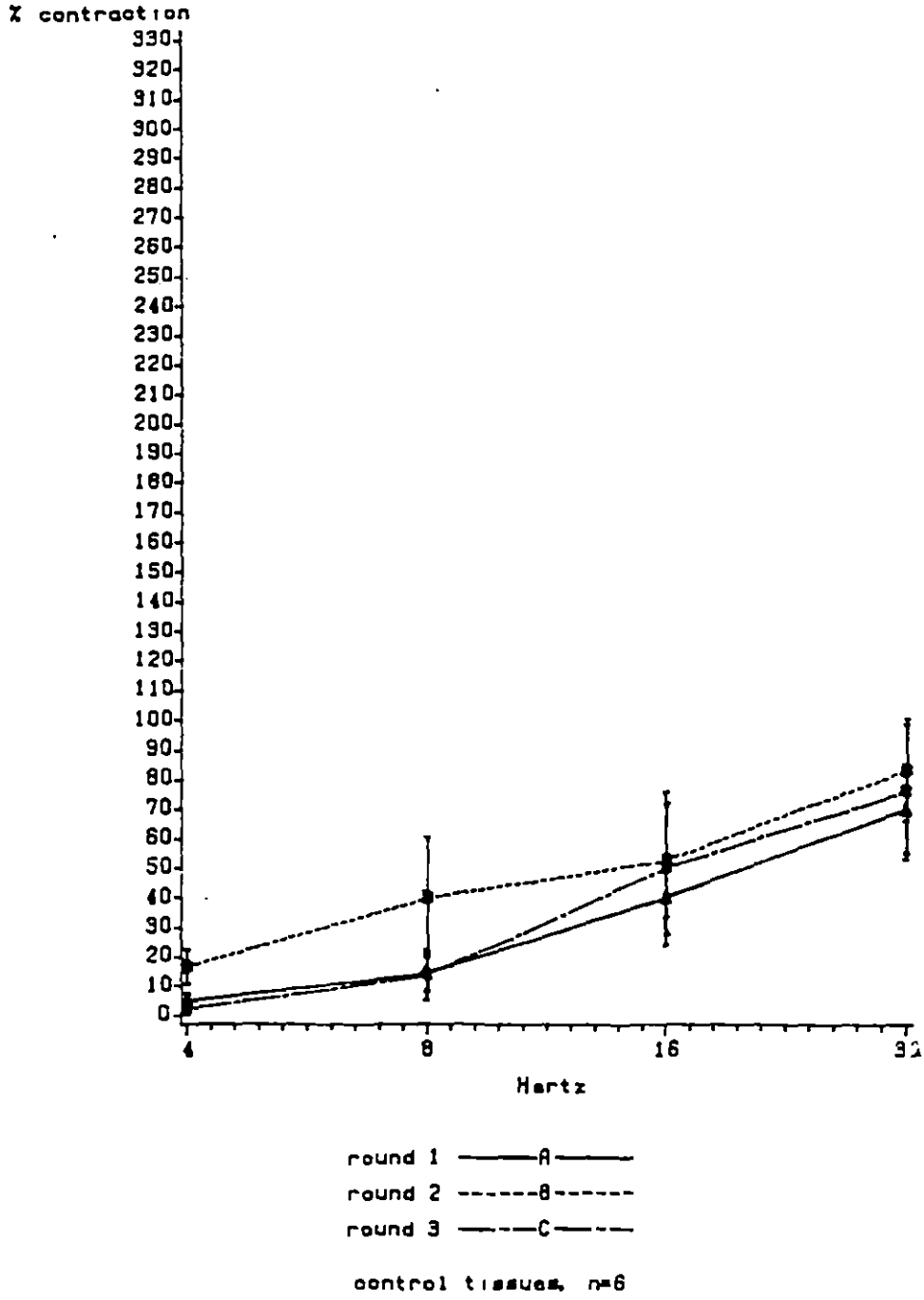


Figure 11a. Control of Figure 11b

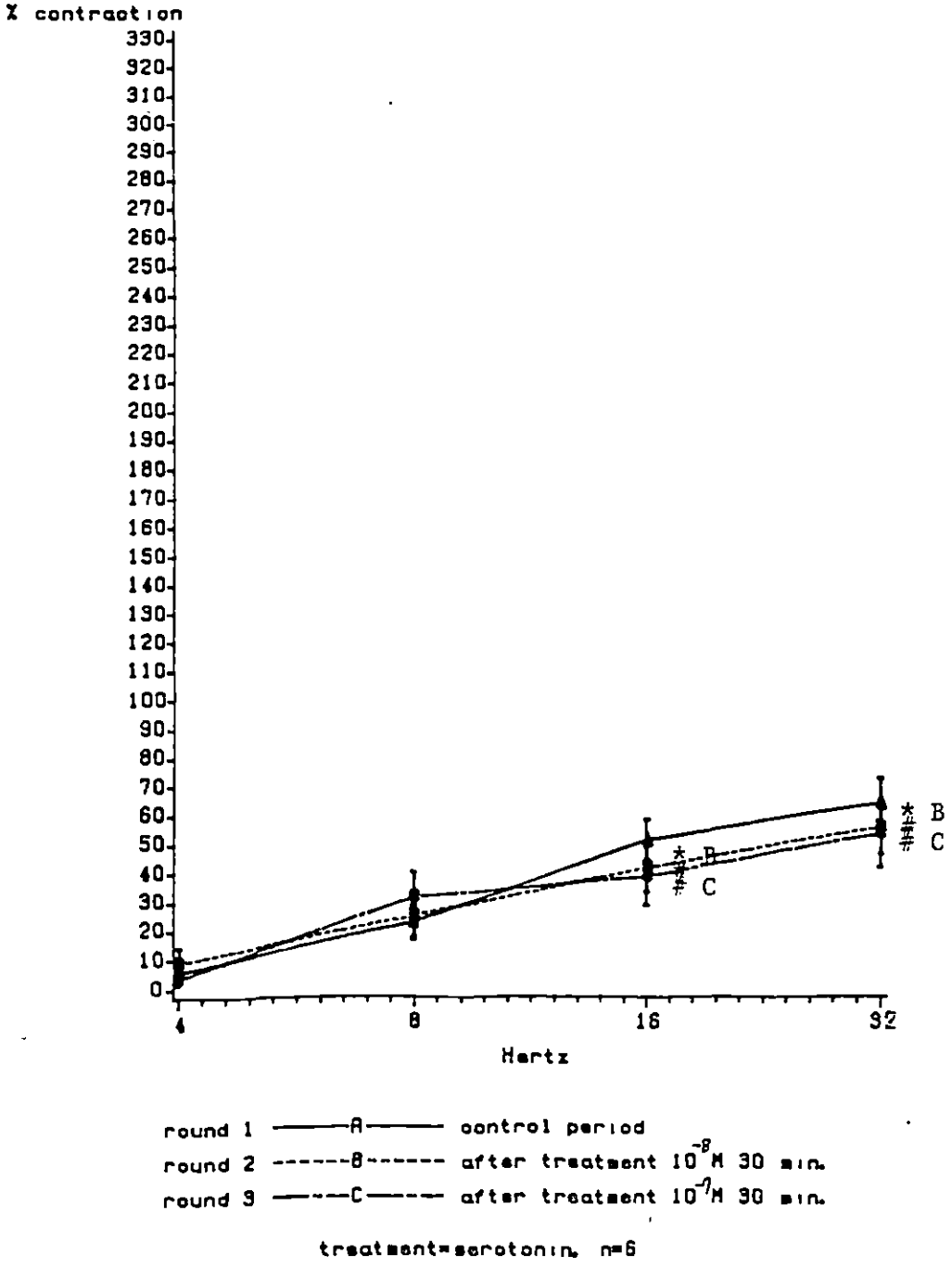


Figure 11b. Effect of Serotonin on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM)=100% contraction. Control responses are shown in Figure 11a

Table 2. Effects of drugs on the shift of the ED50s in the NE dose response curves in turkey aortae[a]

Treatment (number of experiments)	Round (concentration)	Shift of ED50[b]	Mean of the net dose ratio at ED50
Iproniazid (7)	2 (100μg/ml)	0.11 ± 0.24	1.29
Serotonin (6)	2 (1×10^{-8}M)	0.33 ± 0.27	2.14
	3 (1×10^{-7}M)	0.001 ± 0.33	1.00
Angiotensin II (6)	2 (1×10^{-8}M)	0.12 ± 0.24	1.32
	3 (1×10^{-7}M)	0.043 ± 0.4	1.10
Prazosin (5)	2 (1×10^{-7}M)	1.2 ± 0.23**[c]	15.8
Yohimbine (3)	2 (1×10^{-6}M)	0.42 ± 0.03**[c]	2.63

[a]See Appendix C.

[b]Mean ± S.E.M. in logarithmic units.

[c]Statistical significance ($P<0.01$).

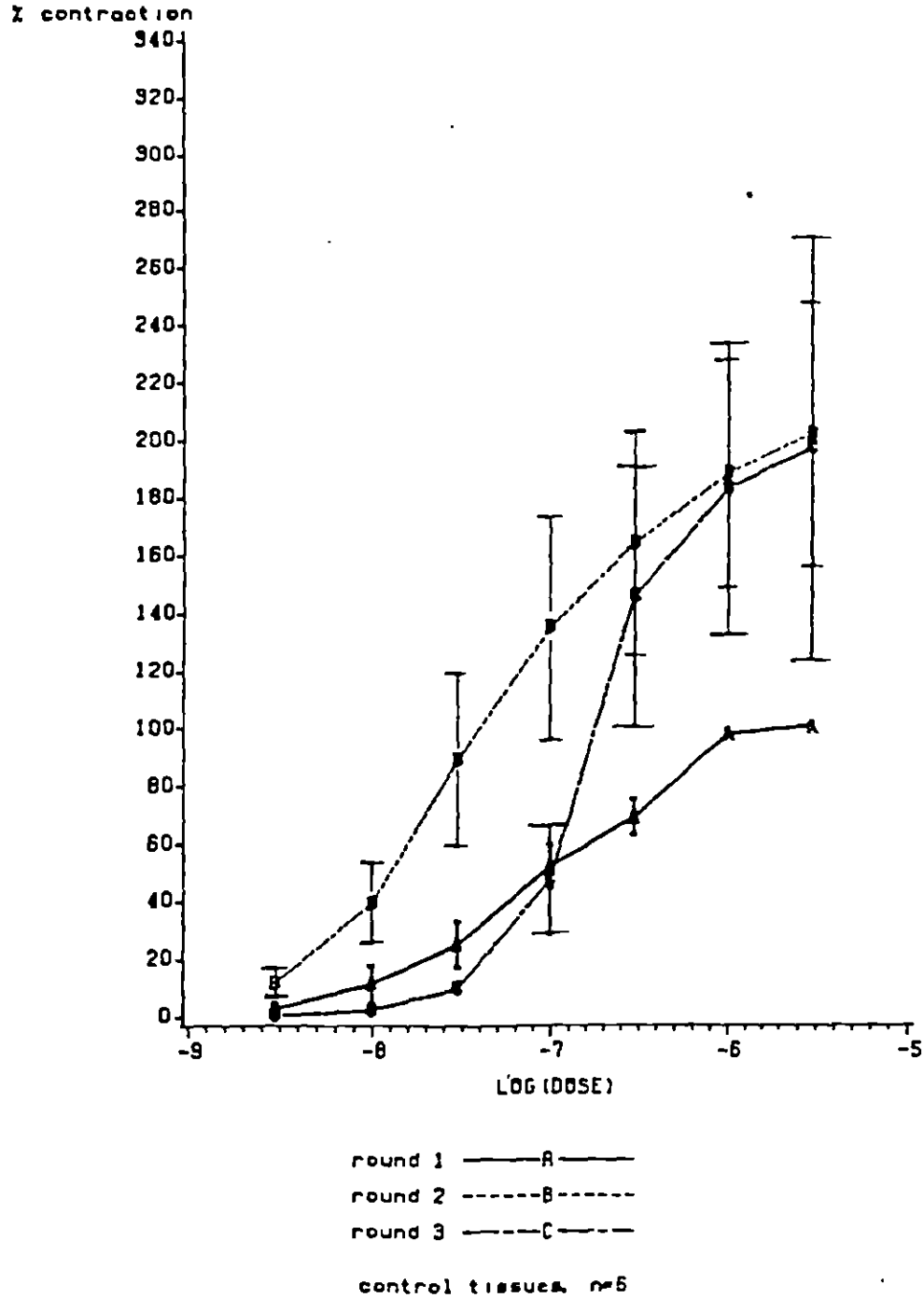


Figure 12a. Control of Figure 12b

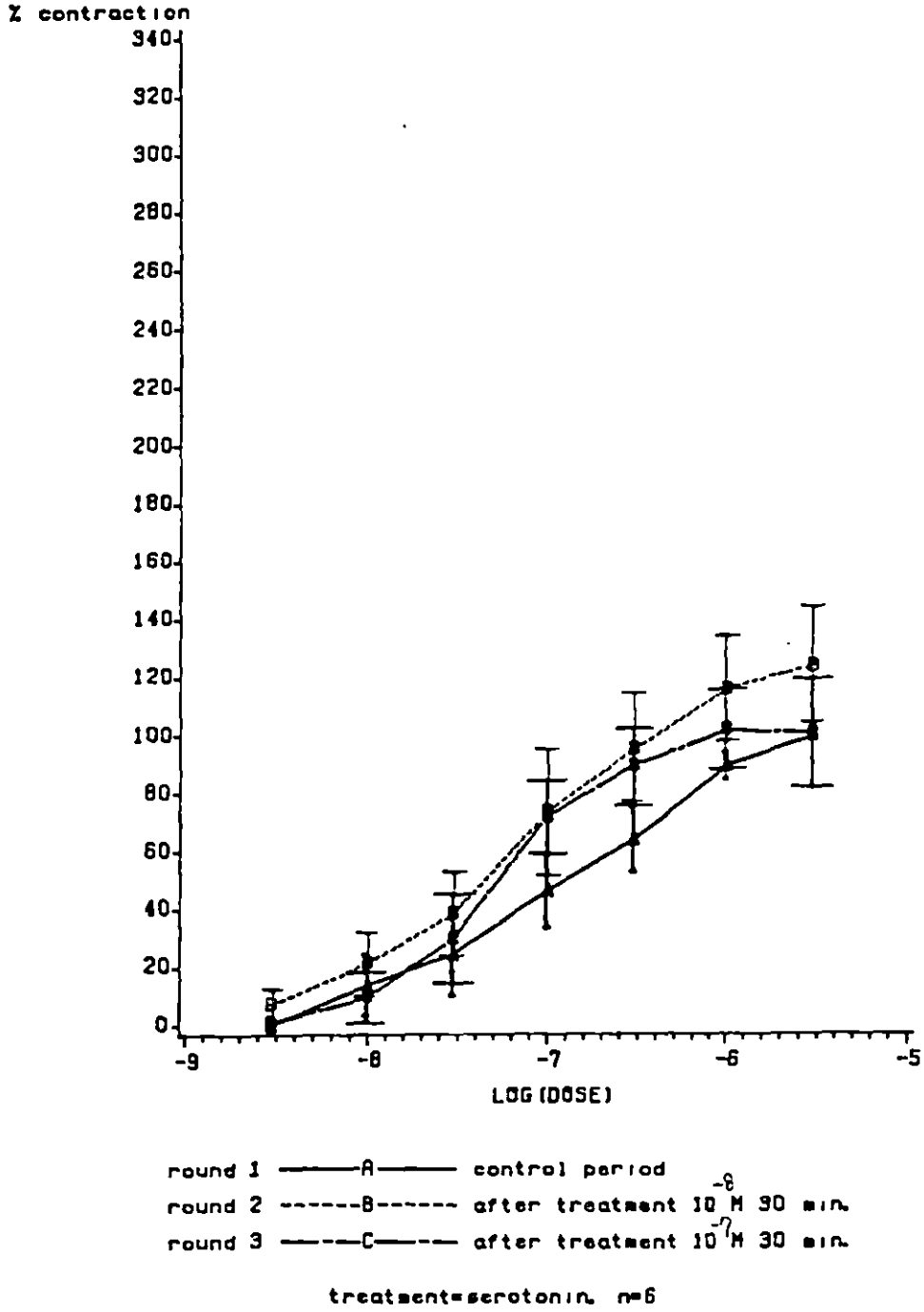


Figure 12b. Effect of serotonin on responses to norepinephrine (NE) in turkey aorta. The percent contraction was the maximal contraction to norepinephrine obtained in round 1. Control tissues is shown in Figure 12a

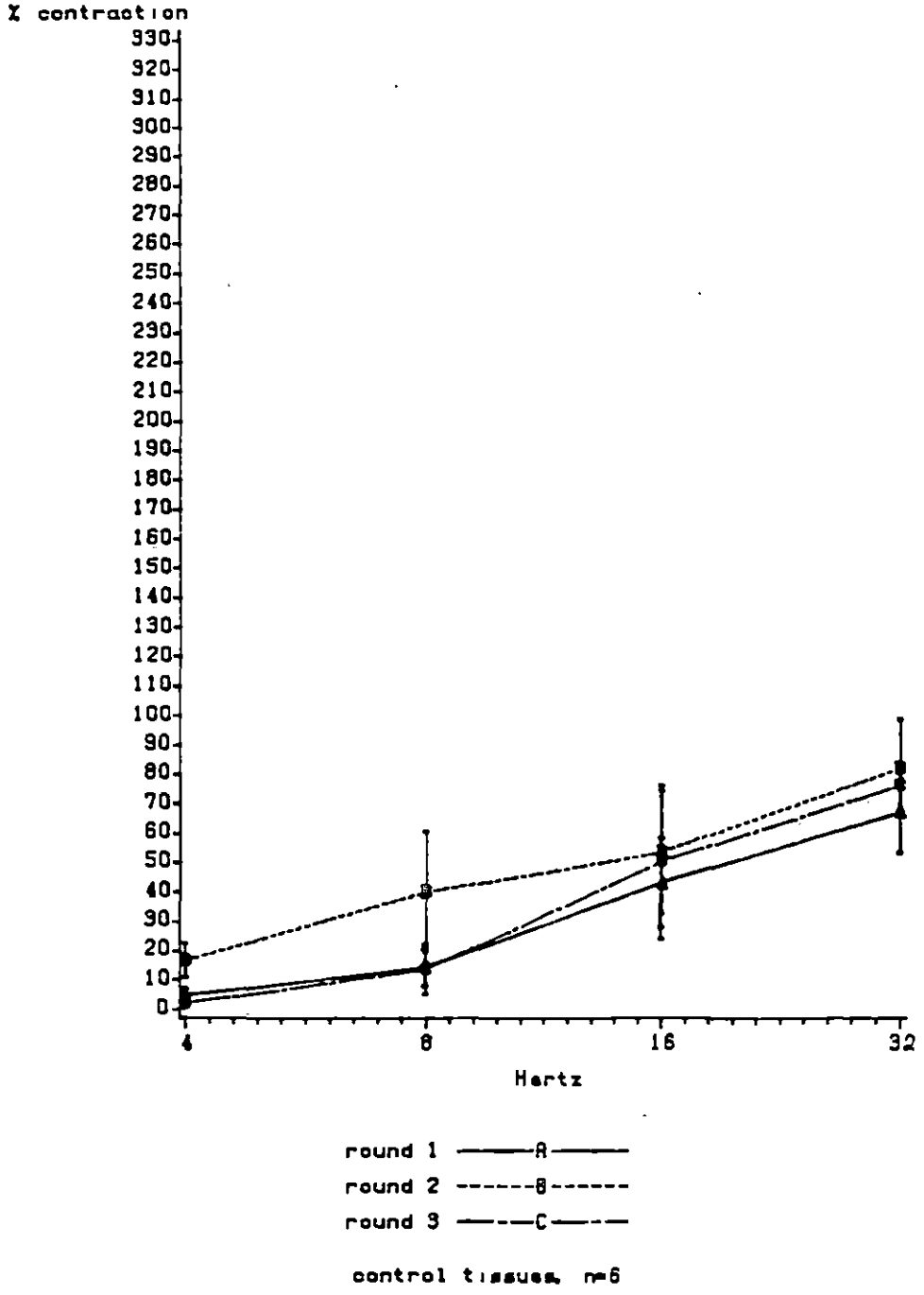


Figure 13a. Control of Figure 13b

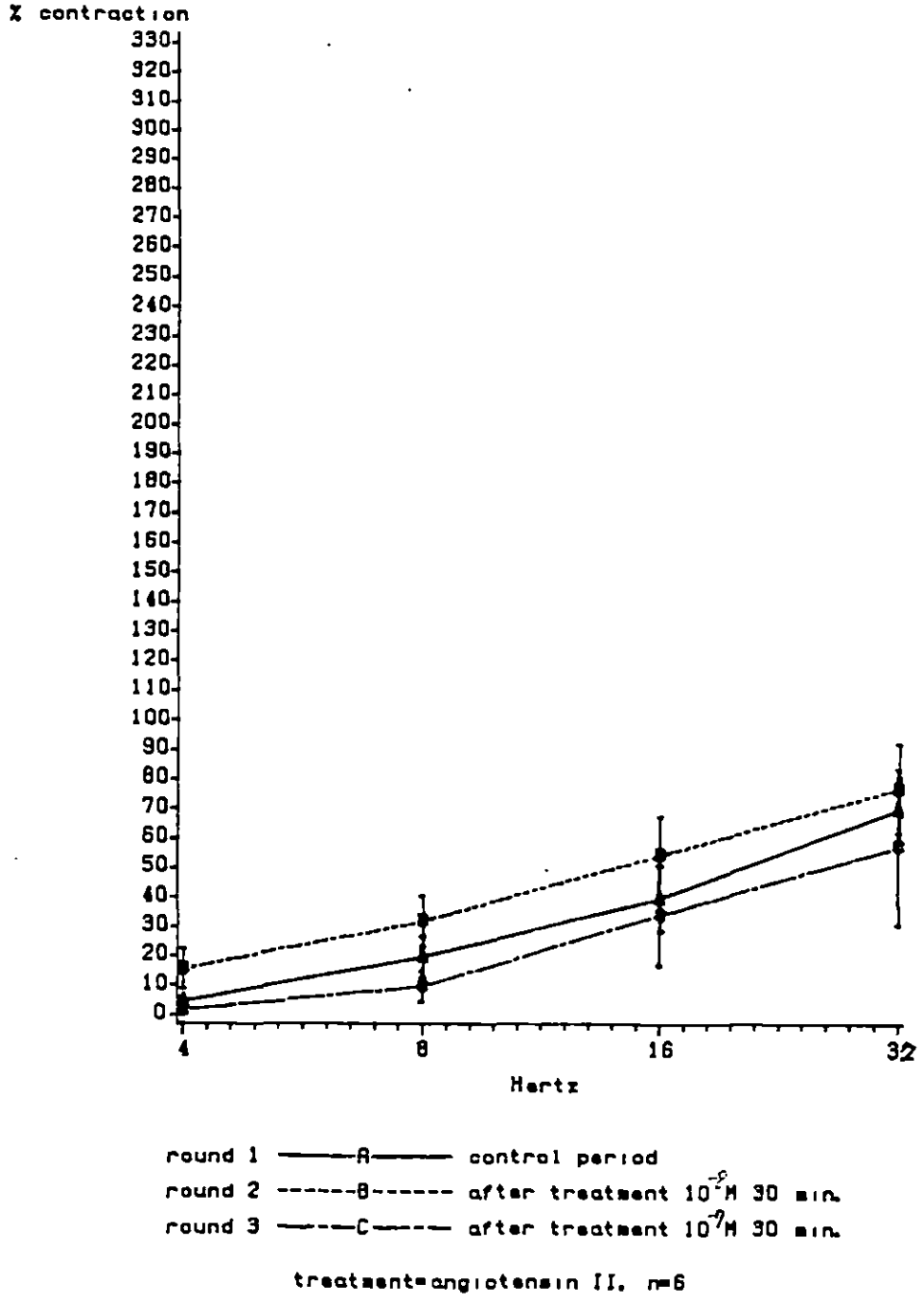


Figure 13b. Effect of angiotensin II on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM)=100% contraction. Control responses are shown in Figure 13a

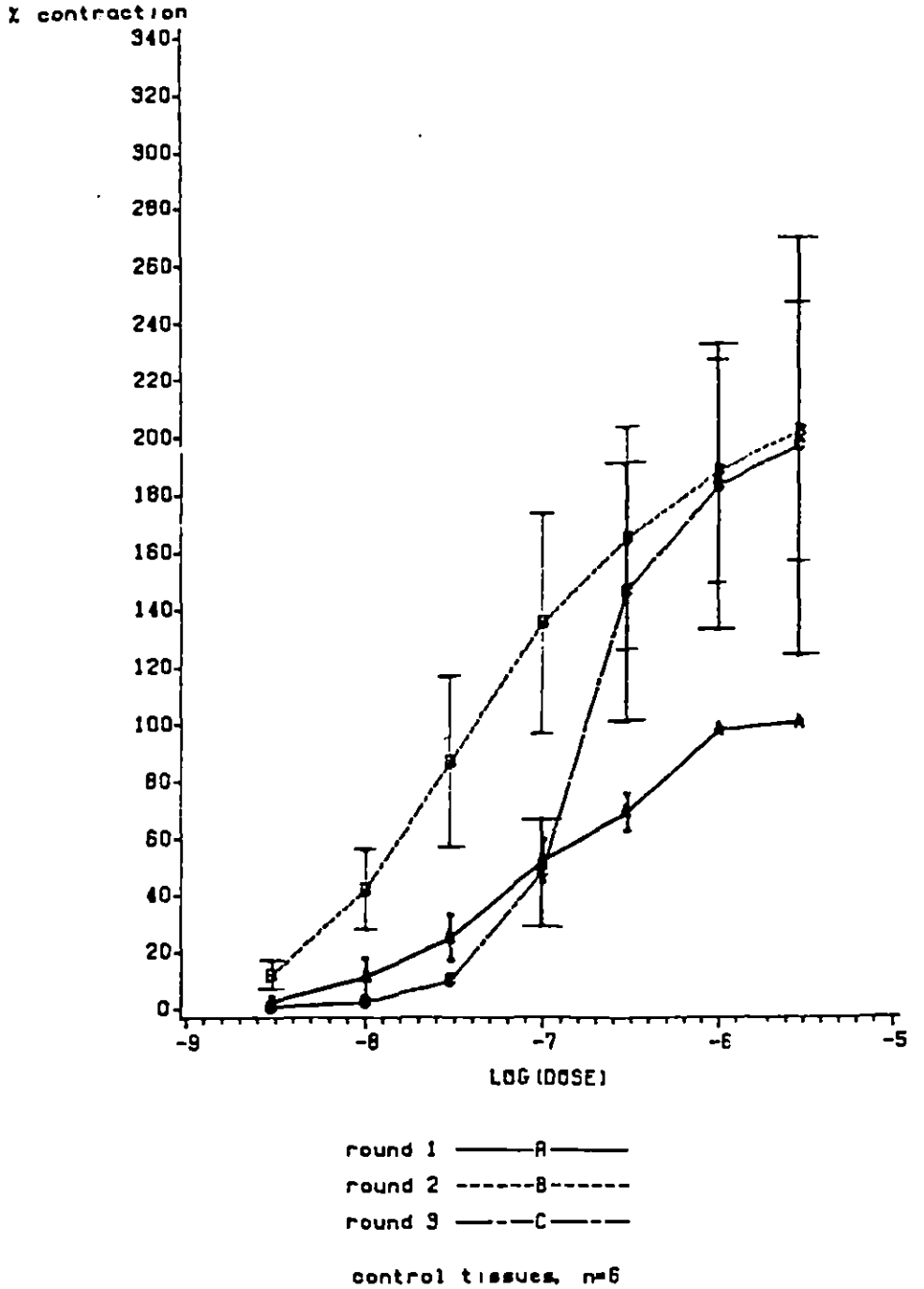


Figure 14a. Control of Figure 14b

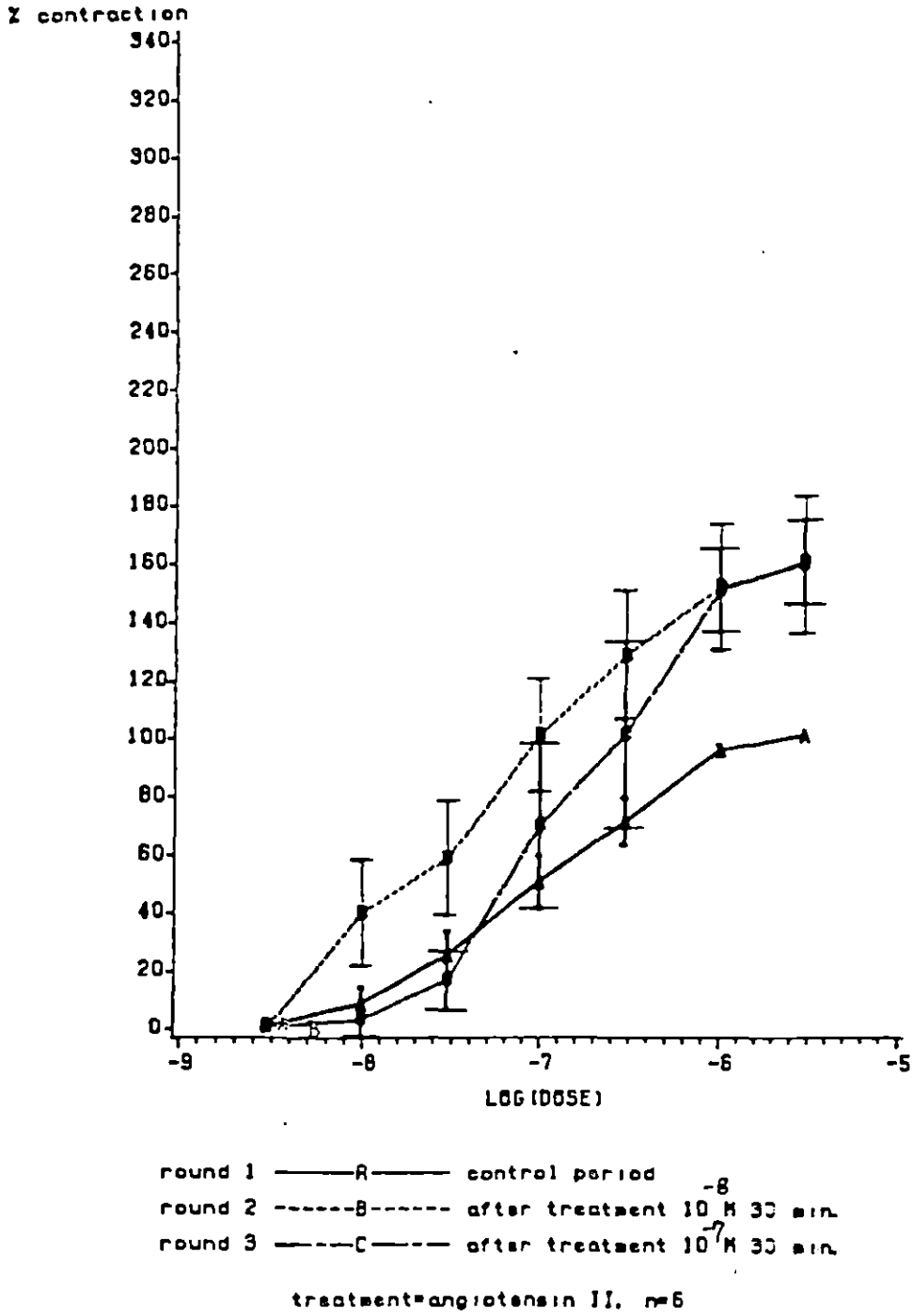


Figure 14b. Effect of angiotensin II on responses to norepinephrine in turkey aorta. The percent contraction was based on the maximal contraction to norepinephrine obtained in round 1. Control responses are shown in Figure 14a

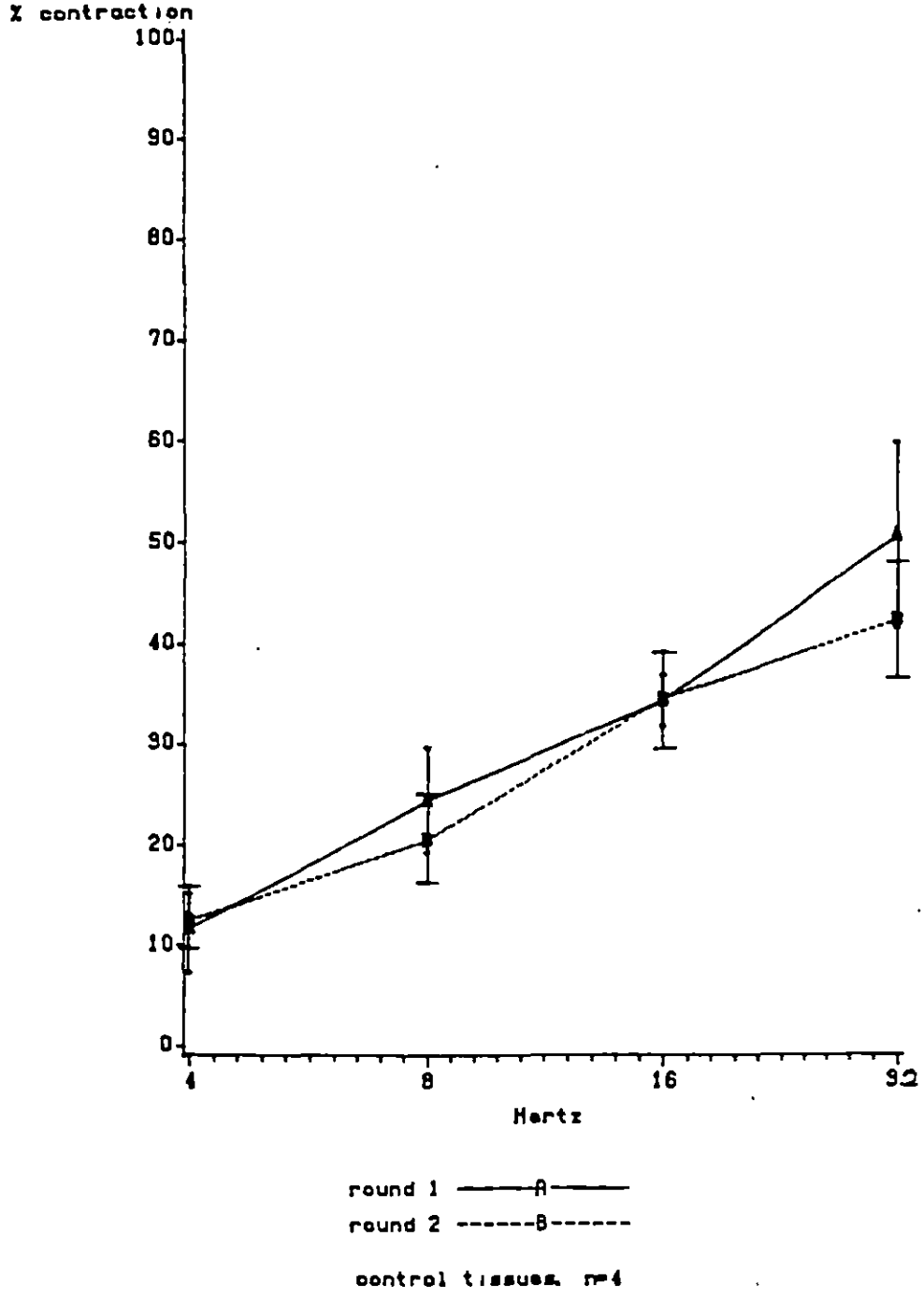


Figure 15a. Control of Figure 15b

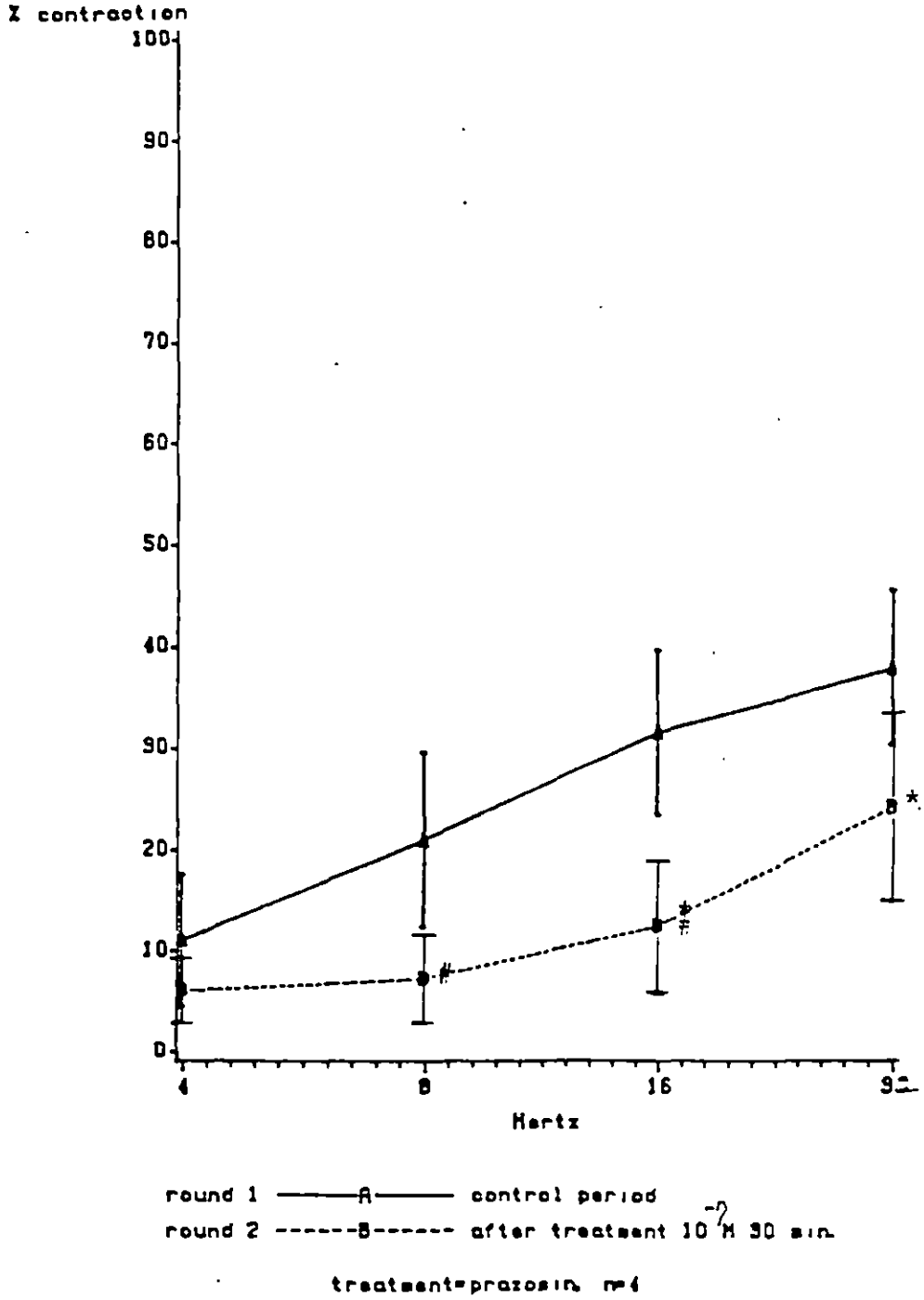


Figure 15b. Effect of prazosin on phase 1 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 15a

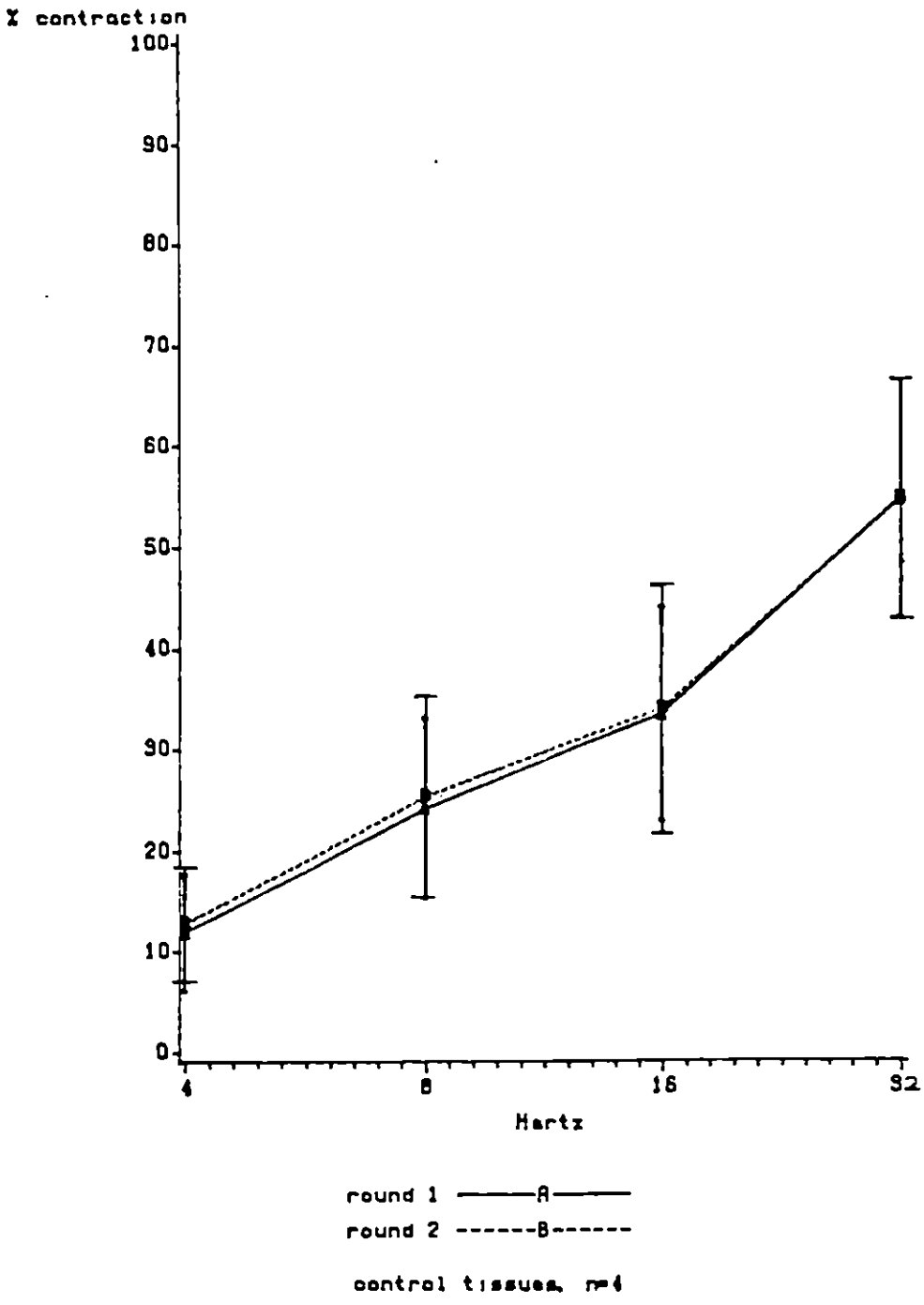


Figure 16a. Control of Figure 16b

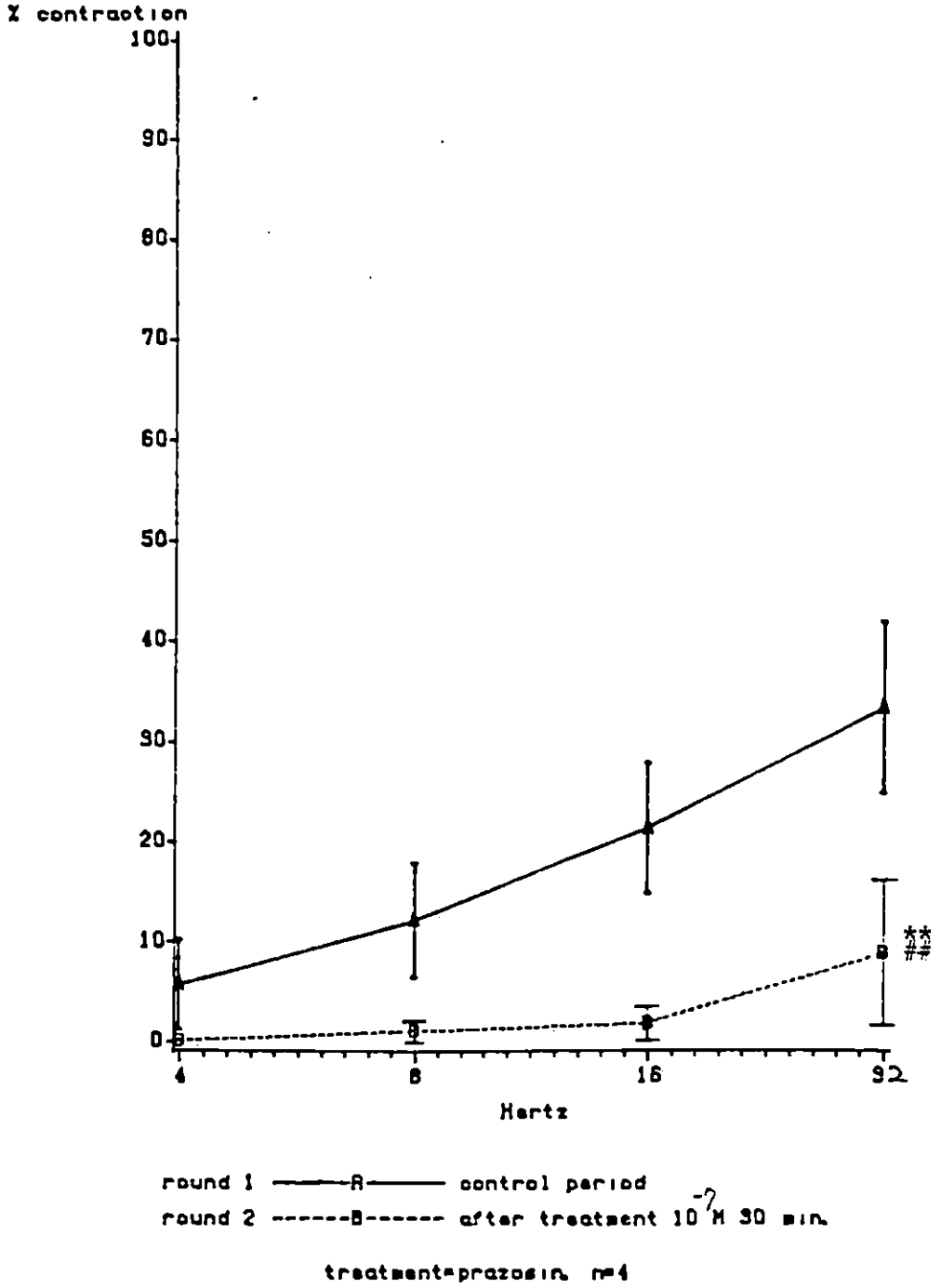


Figure 16b. Effect of prazosin on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 16a

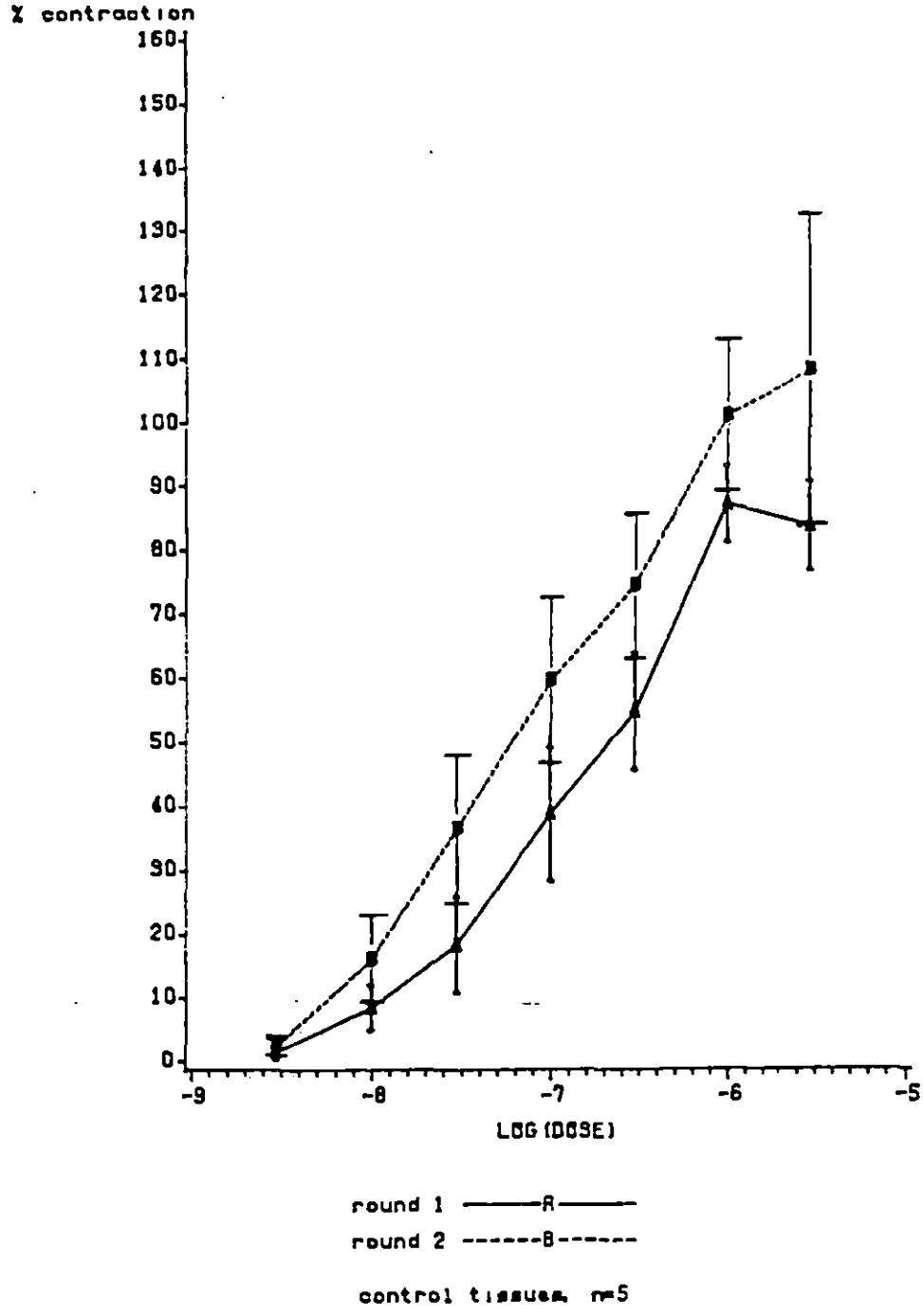


Figure 17a. Control of Figure 17b

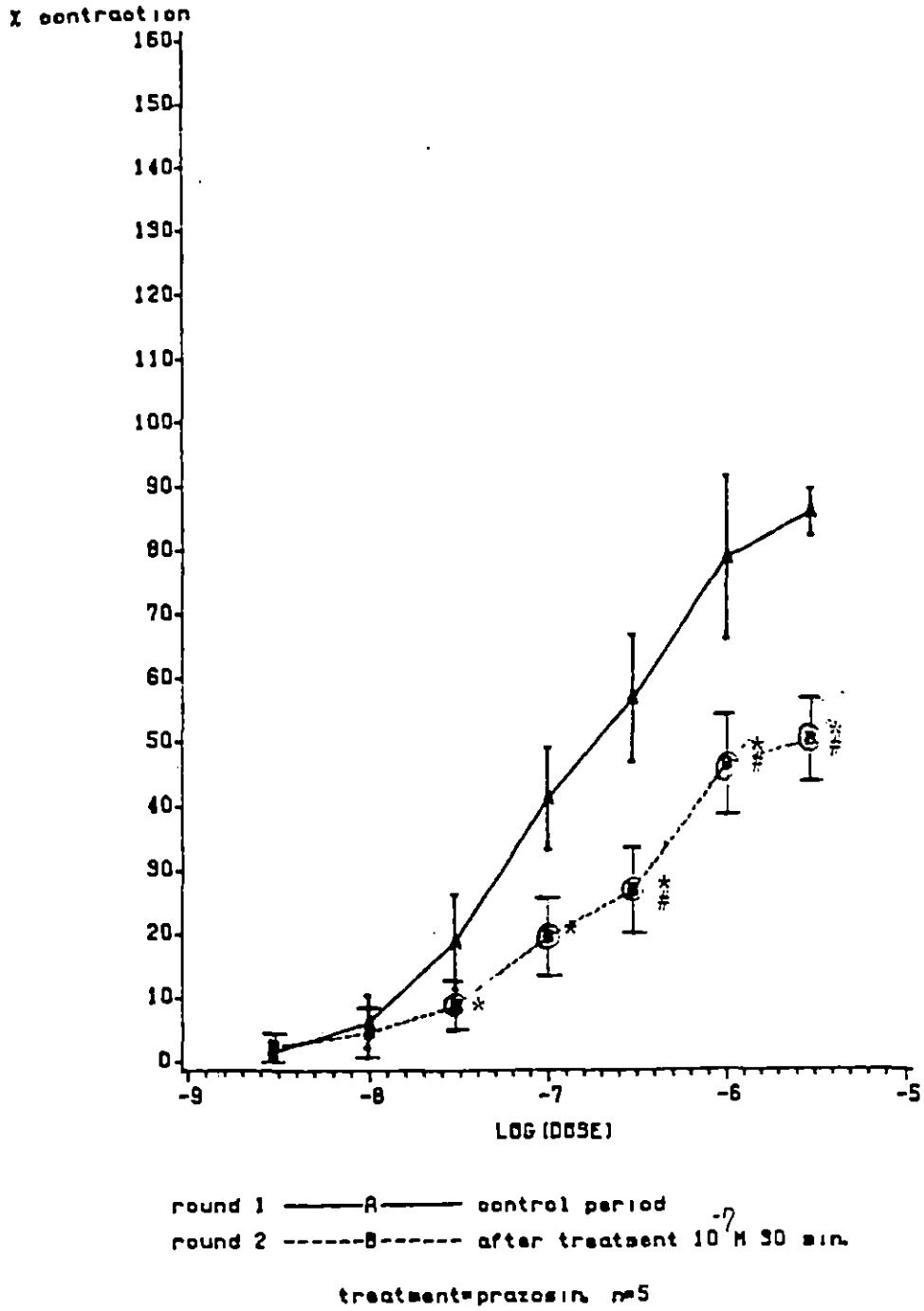


Figure 17b. Effect of prazosin on responses to norepinephrine (NE) in turkey aorta. The percent contraction was based on the maximal contraction to norepinephrine obtained in round 1. Control tissues is shown in Figure 17a

A Schild plot was used to further characterize α_1-adrenergic receptors in turkey aorta. The pA_2 value and slope was calculated as 7.92 and 0.50, respectively by mathematical equations. The graphic representation is presented in Figure 18.

b. Yohimbine This predominantly α_2-adrenergic receptor antagonist, unexpectedly exerted a significant inhibitory effect on both phase 2 of the response to ES and the response to NE (Figures 19-21). The ED50 of NE was also significantly ($p < 0.01$) different from control. A Schild plot for yohimbine is presented in Figure 22. The pA_2 value and slope were computed as 6.90 and 0.45, respectively.

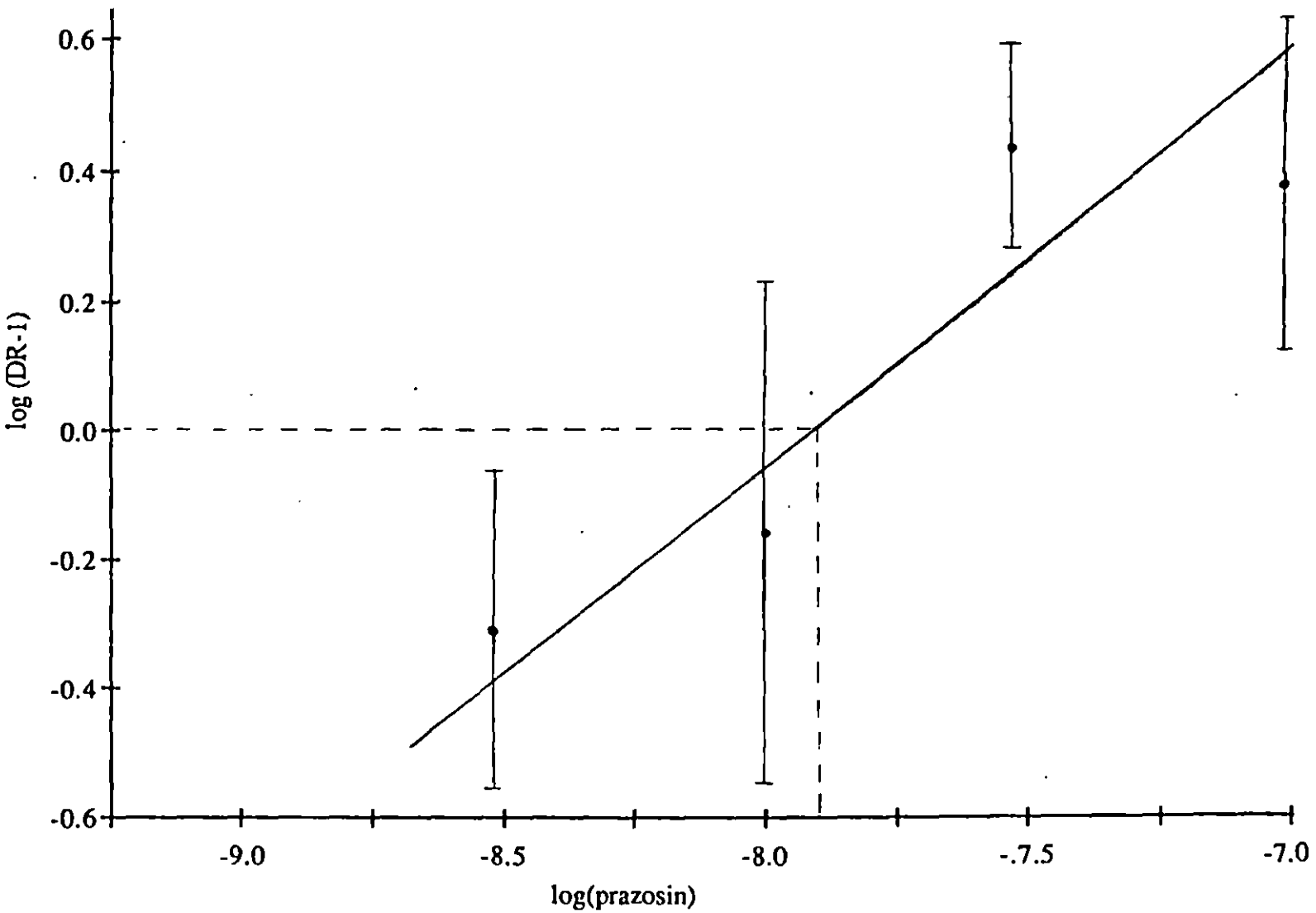
2. Alpha-adrenergic agonists

Comparisons of an α_1 agonist (methoxamine) to several α_2 agonists (B-HT 920, clonidine, and xylazine) were made by observing their responses produced in different aorta preparations. The maximal contractile response to methoxamine was much greater than that to B-HT 920, xylazine, or clonidine. The contractile response to xylazine was the least of the α_2-agonists studied, see Figure 2 part (H) and (I). To be more specific, if the maximal contraction of methoxamine = 100% (n=4), then the maximal response to B-HT 920 and clonidine = 20-40% (n=4) and that to xylazine = 3-7% (n=4).

3. Beta-adrenergic agonists

Isoproterenol was used to ascertain, in part, if β-adrenergic receptors existed in turkey aorta. In an equilibrated preparation, isoproterenol usually did not exert a significant relaxing effect on the turkey aorta smooth muscle. In preparations in which the tone of the isolated ring was increased by adding KCl (15 mM), or NE (1×10^{-7} M) to the bath, or by mechanically stretching the ring, the addition of isoproterenol to the bath produced very

Figure 18. Schild plot: pA_2 determination for prazosin using methoxamine as the agonist. Each point stands for the mean of four observations. The vertical bars are the standard error of the mean. DR = Dose Ratio. $pA_2 = 7.92$, slope = 0.50


0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
log (DR-1)
-9.0
-8.5
-8.0
-.7.5
-7.0
log(prazosin)

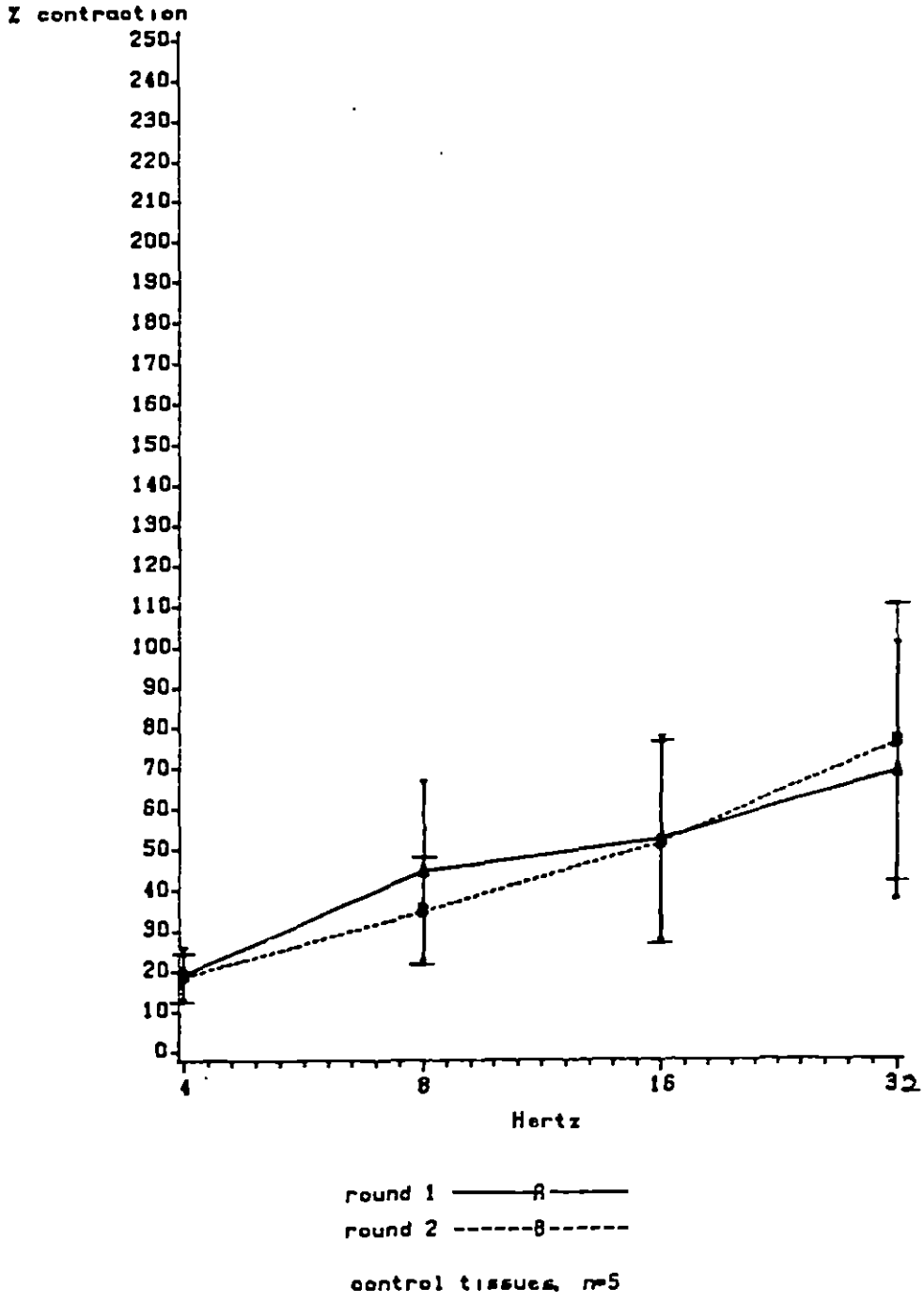


Figure 19a. Control of Figure 19b

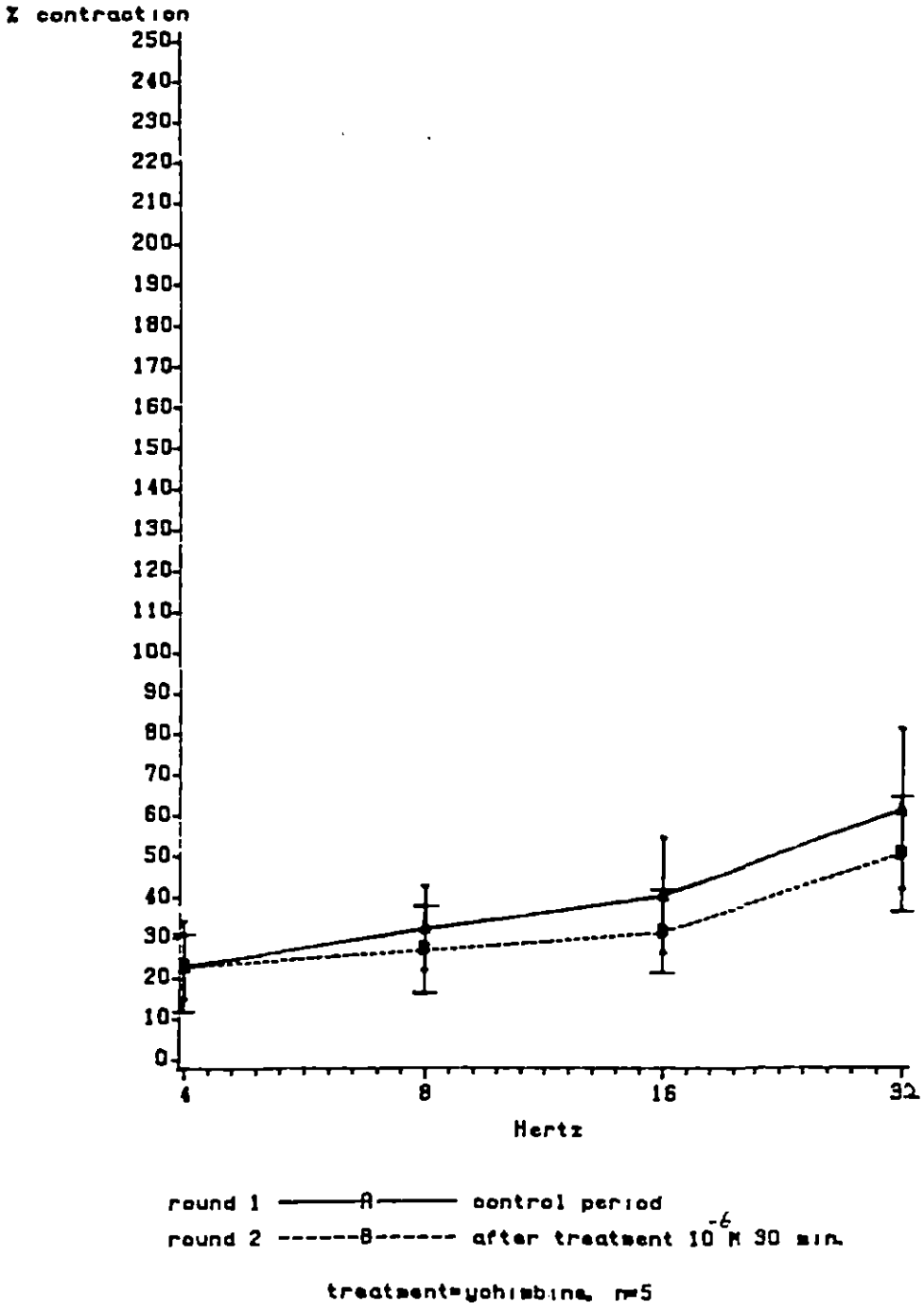


Figure 19b. Effect of yohimbine on phase 1 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 19a

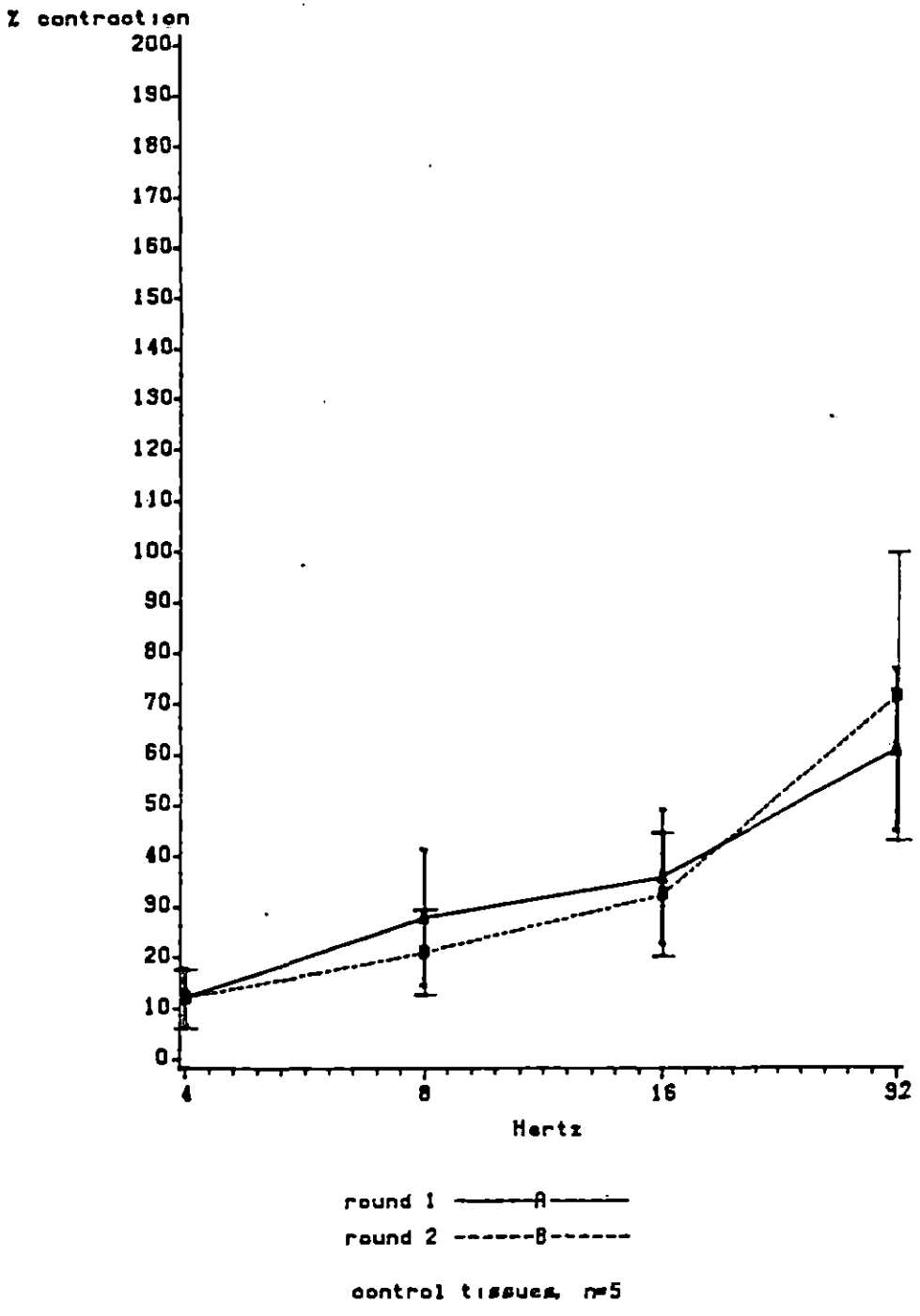


Figure 20a. Control of Figure 20b

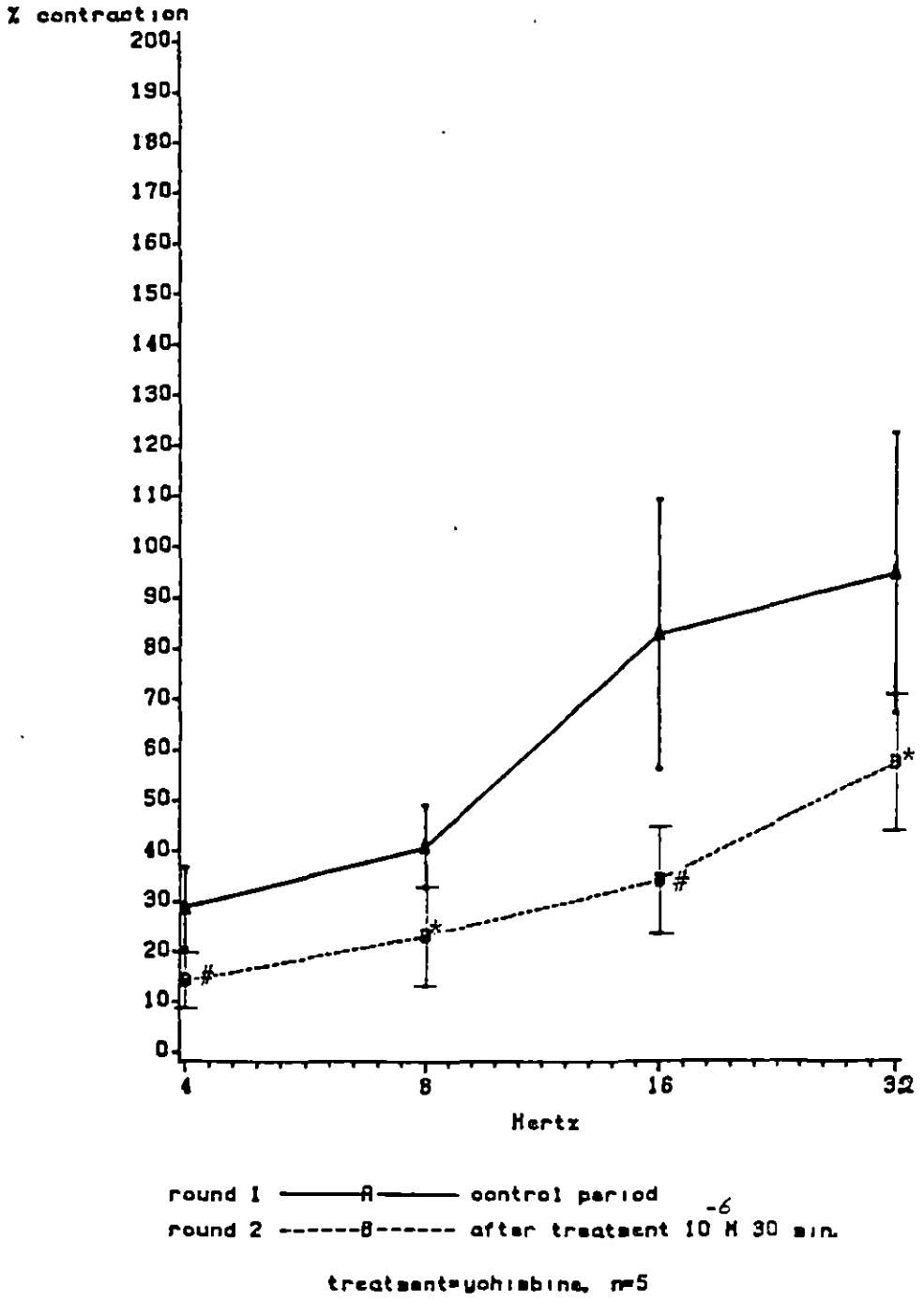


Figure 20b. Effect of yohimbine on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 20a

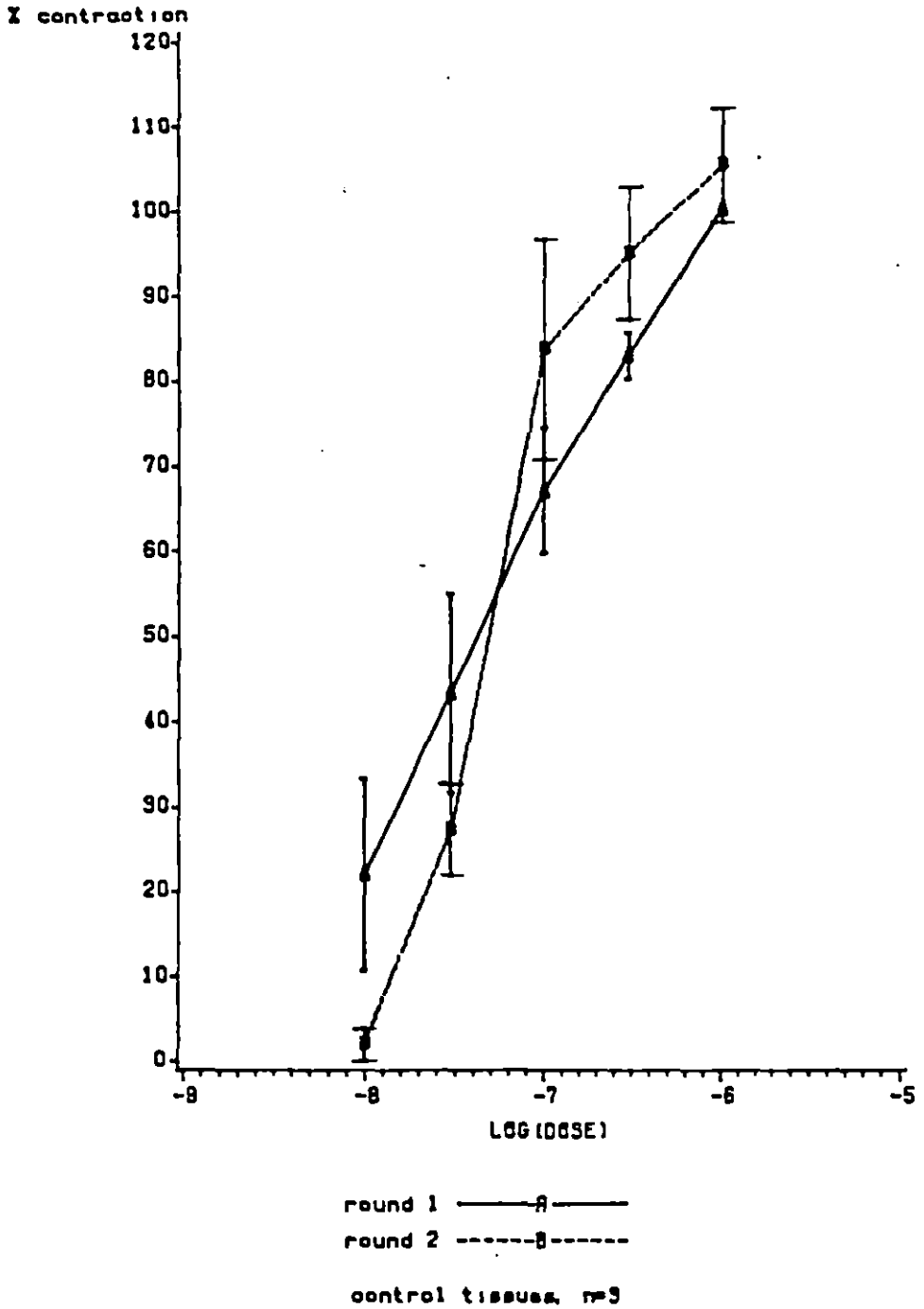


Figure 21a. Control of Figure 21b

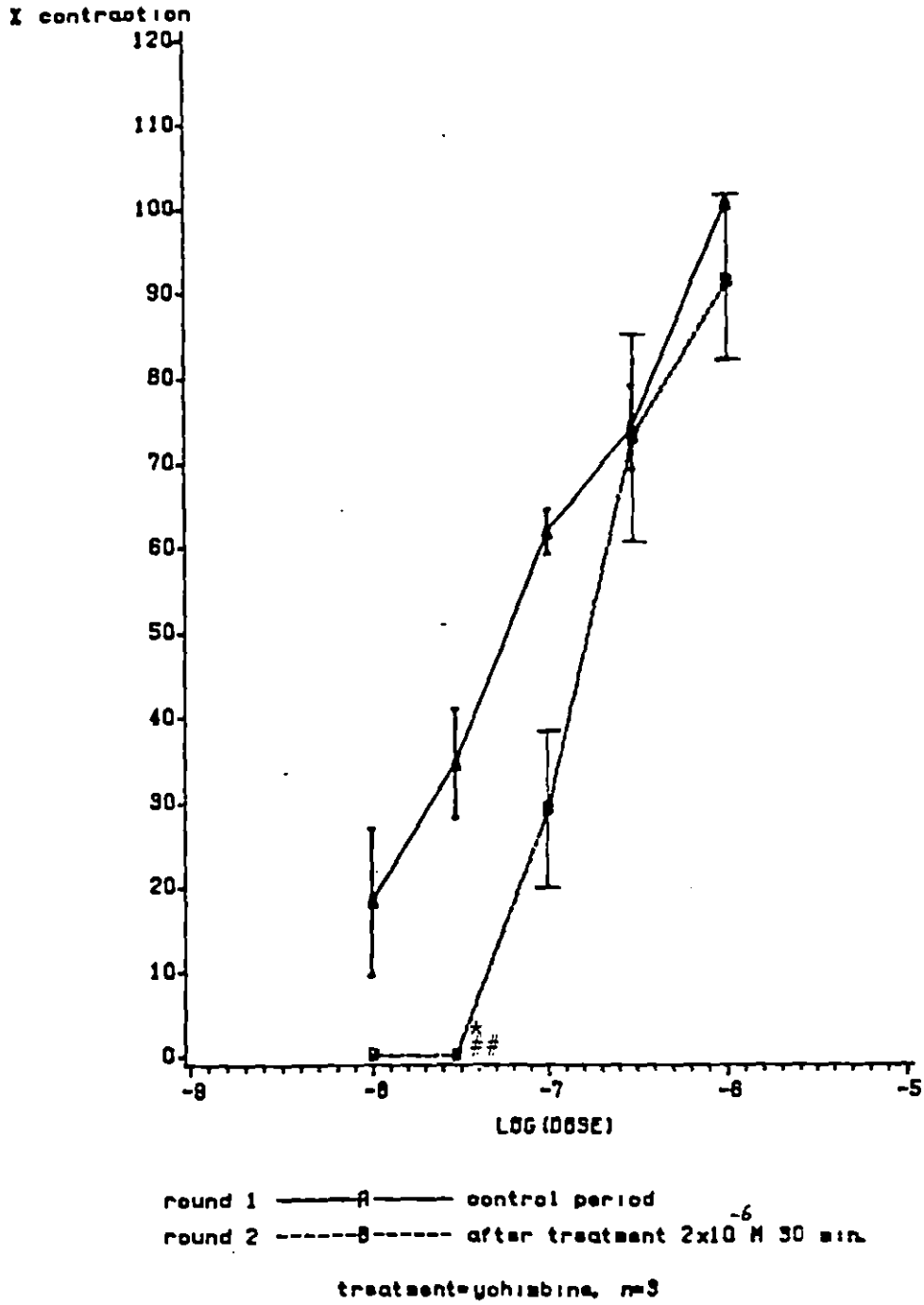
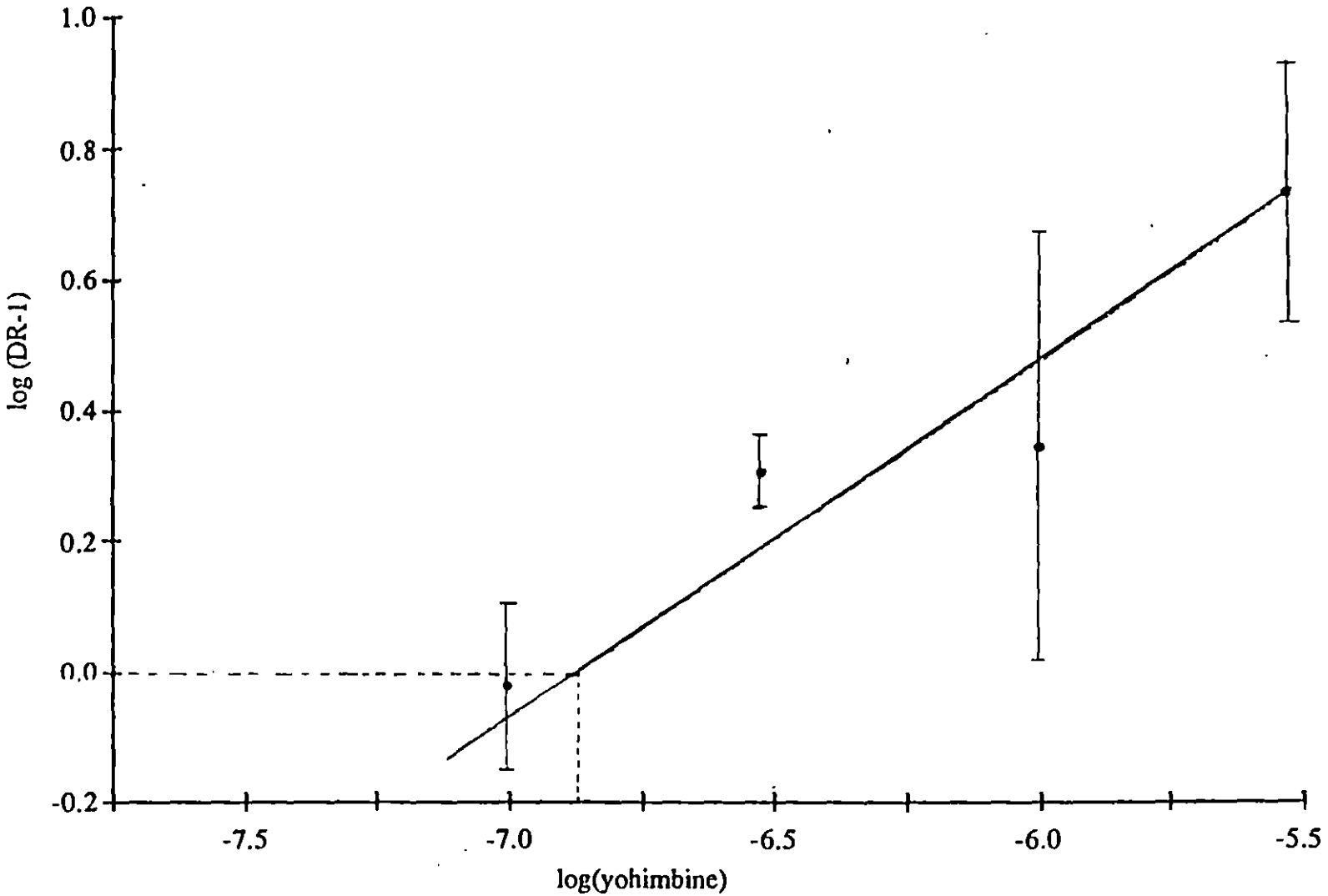


Figure 21b. Effect of yohimbine on responses to norepinephrine (NE) in turkey aorta. The percent contraction was based on the maximal contraction to norepinephrine obtained in round 1. Control tissues are shown in Figure 21a

Figure 22. Schild plot: pA_2 determination for yohimbine using methoxamine as the agonist. Each point stands for the mean of four observations. The vertical bars are the standard error of the mean. DR = Dose Ratio. $pA_2 = 6.9$, slope = 0.45


1.0
0.8
0.6
0.4
0.2
0.0
-0.2
log (DR-1)
-7.5
-7.0
-6.5
-6.0
-5.5
log(yohimbine)

small or no relaxation of such preparations. Figure 2 (G) is an example of one of the few cases in which isoproterenol relaxed the preparation.

E. Inhibitors of Uptake $_1$ and Uptake $_2$

1. Cocaine

This inhibitor of the adrenergic neuronal membrane pump (uptake 1), as expected, appeared to potentiate the contractile response of phase 2 (statistical significance was masked by large variance) (Figures 23a and 23b) and prolonged the duration (Figures 24a and 24b) of the ESR (given in duration units). The duration unit is the time for the tissue to return to baseline from the start of the contractile response to electrical stimulation (Figure 2B). Relaxation has been used as a tool to evaluate the importance of various termination mechanisms of the adrenergic transmitter (Kalsner and Nickerson, 1968). No significant effect was observed on phase 1 of ESR (data not shown).

2. Normetanephrine

This inhibitor of uptake$_2$ in adrenergically innervated tissues did not exert a significant effect on either the phase 2 response resulting from electrical stimulation or on the duration of the ESR. See Figures 25 and 26. No significant effect was observed on the phase 1 responses to electrical stimulation and responses to NE (data not shown).

F. Inhibitors of MAO and COMT

1. Iproniazid

This inhibitor of monoamine oxidase enhanced the contractility of the tissue during phase 2 of electrical stimulation (Figures 27a and 27b) and prolonged the duration of the ESR (Figures 28a and 28b). In contrast, iproniazid had no effect on responses to NE (Figures 29a and 29b), nor to phase 1 of the ESR, data not shown.

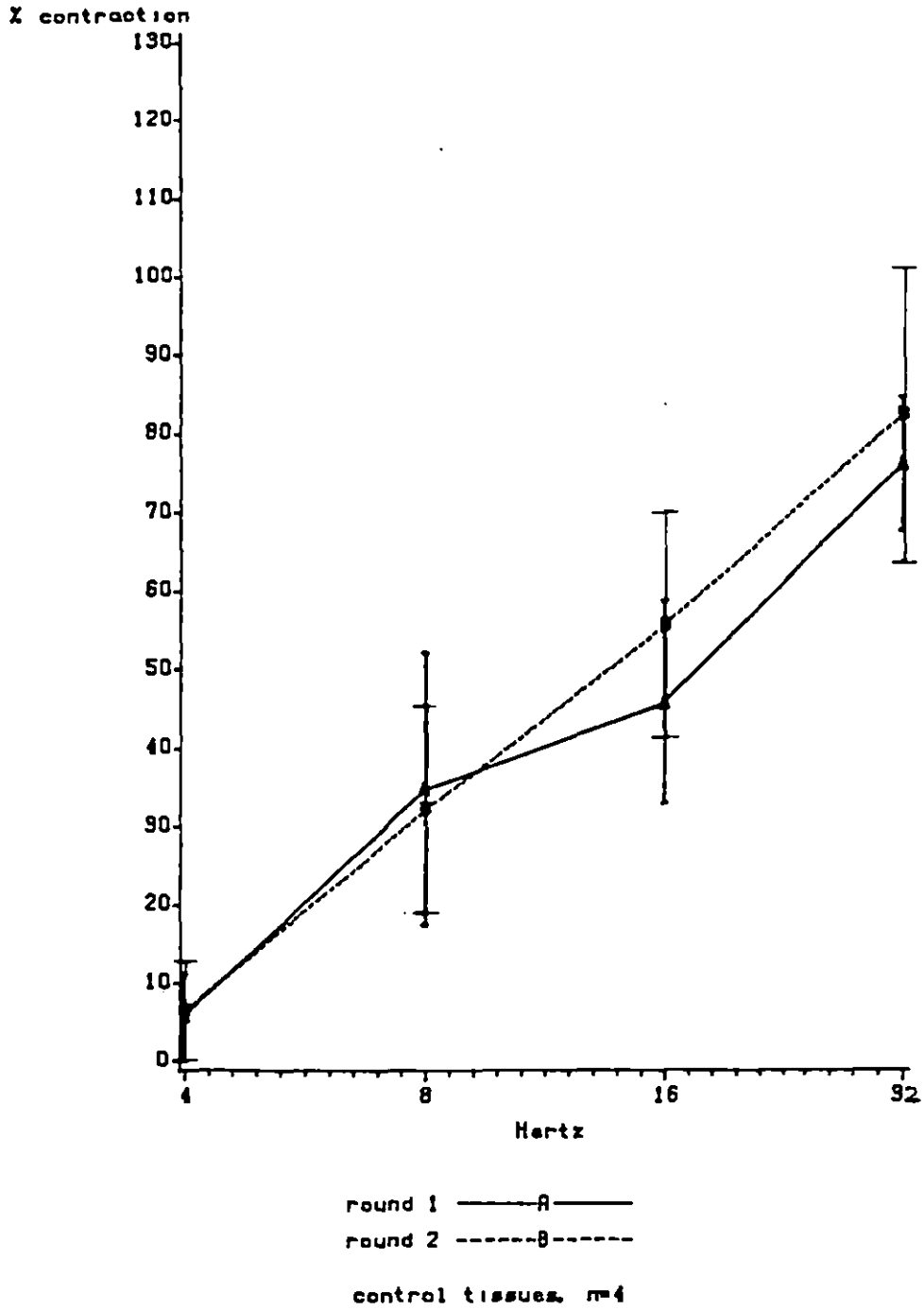


Figure 23a. Control of Figure 23b

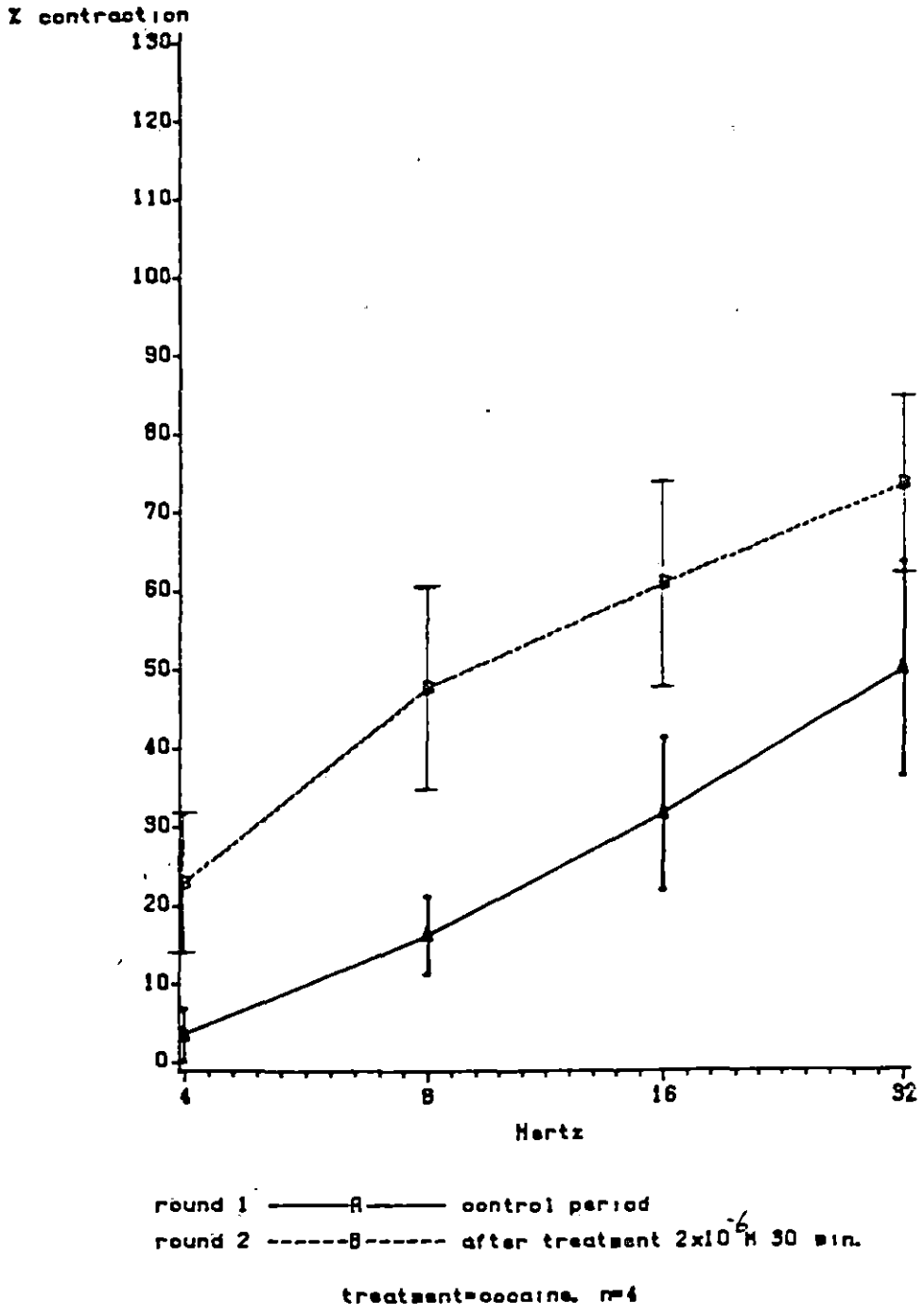


Figure 23b. Effect of cocaine on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 23a

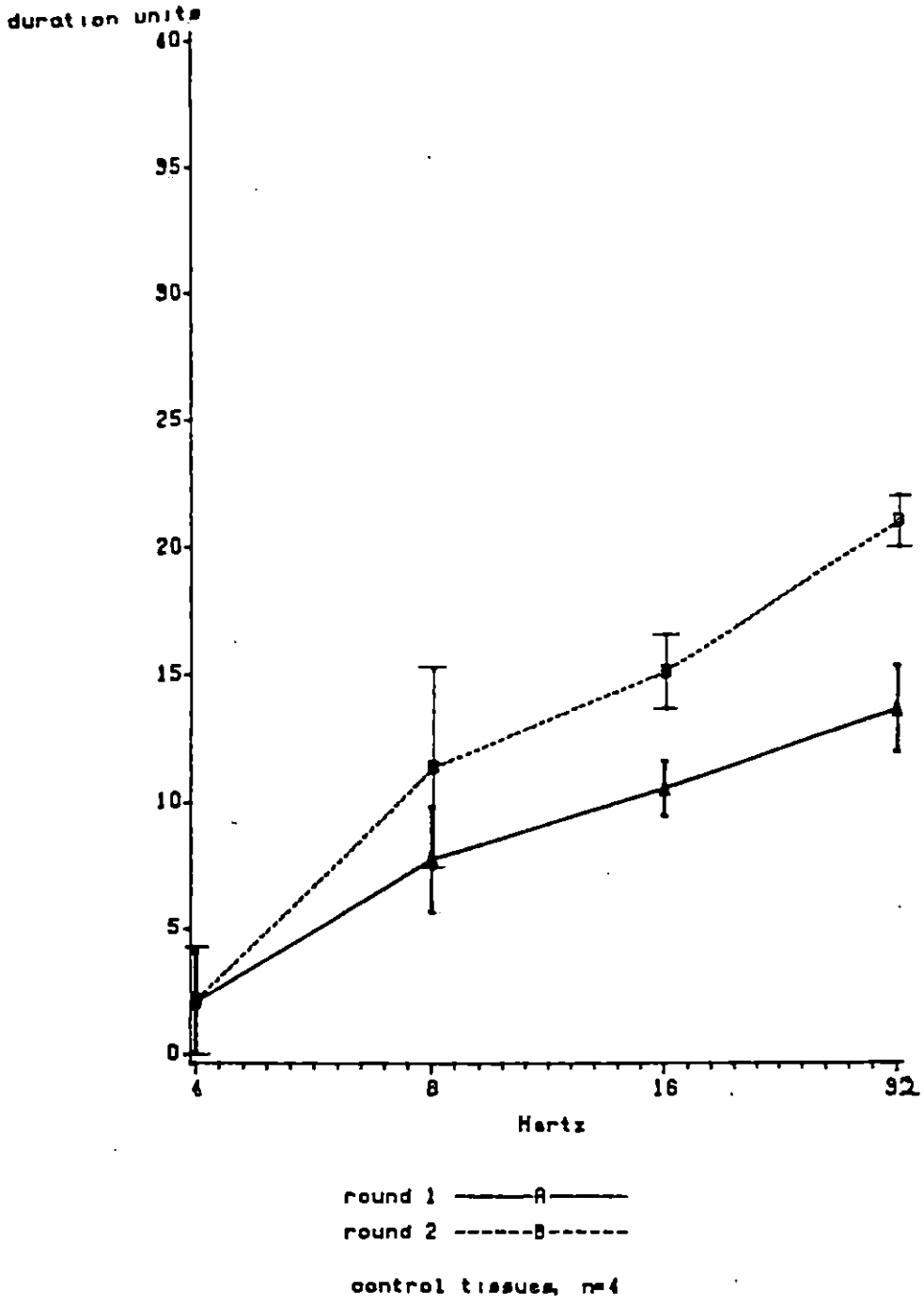


Figure 24a. Control of Figure 24b

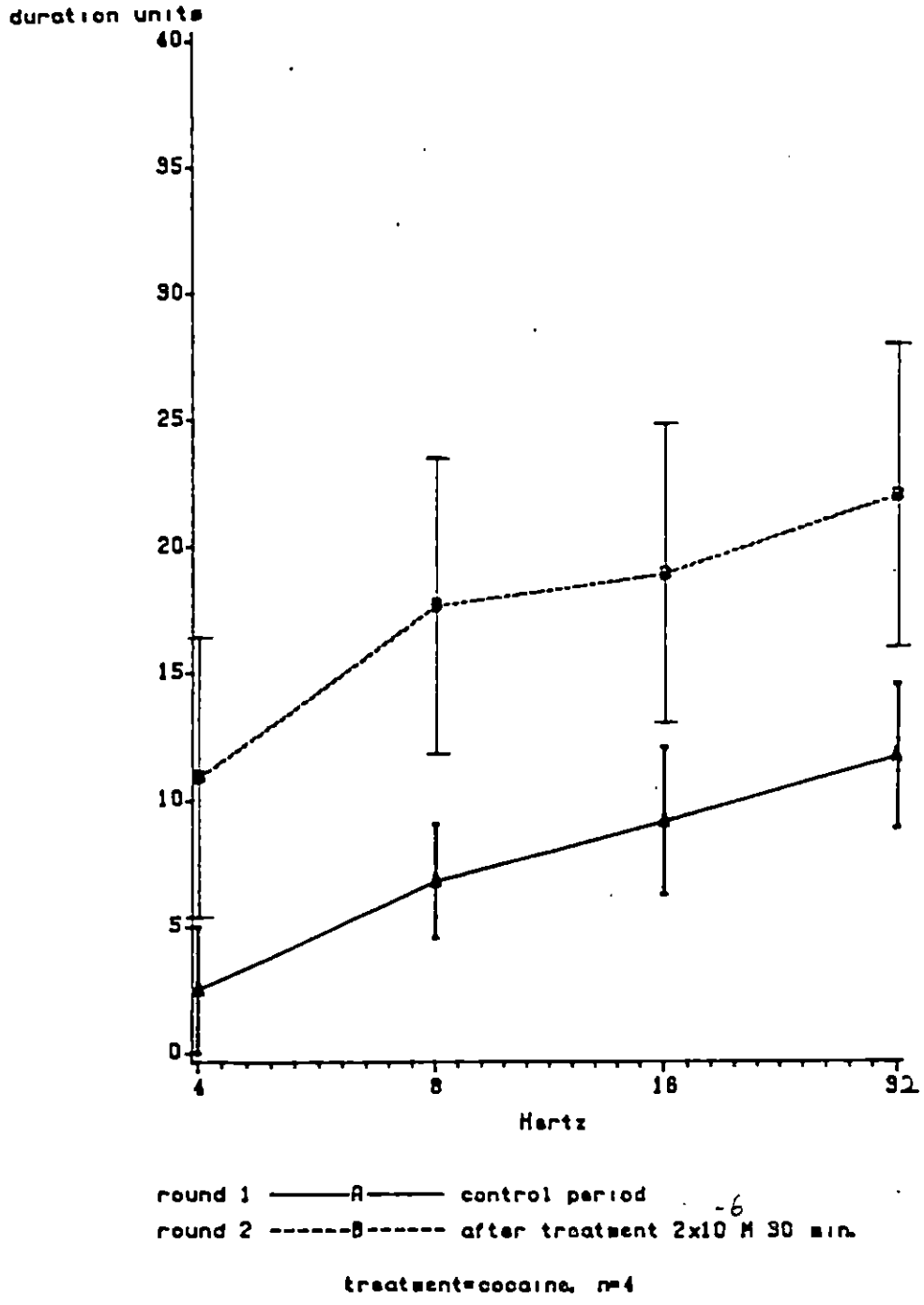


Figure 24b. Effect of cocaine on duration units (see text) of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 24a

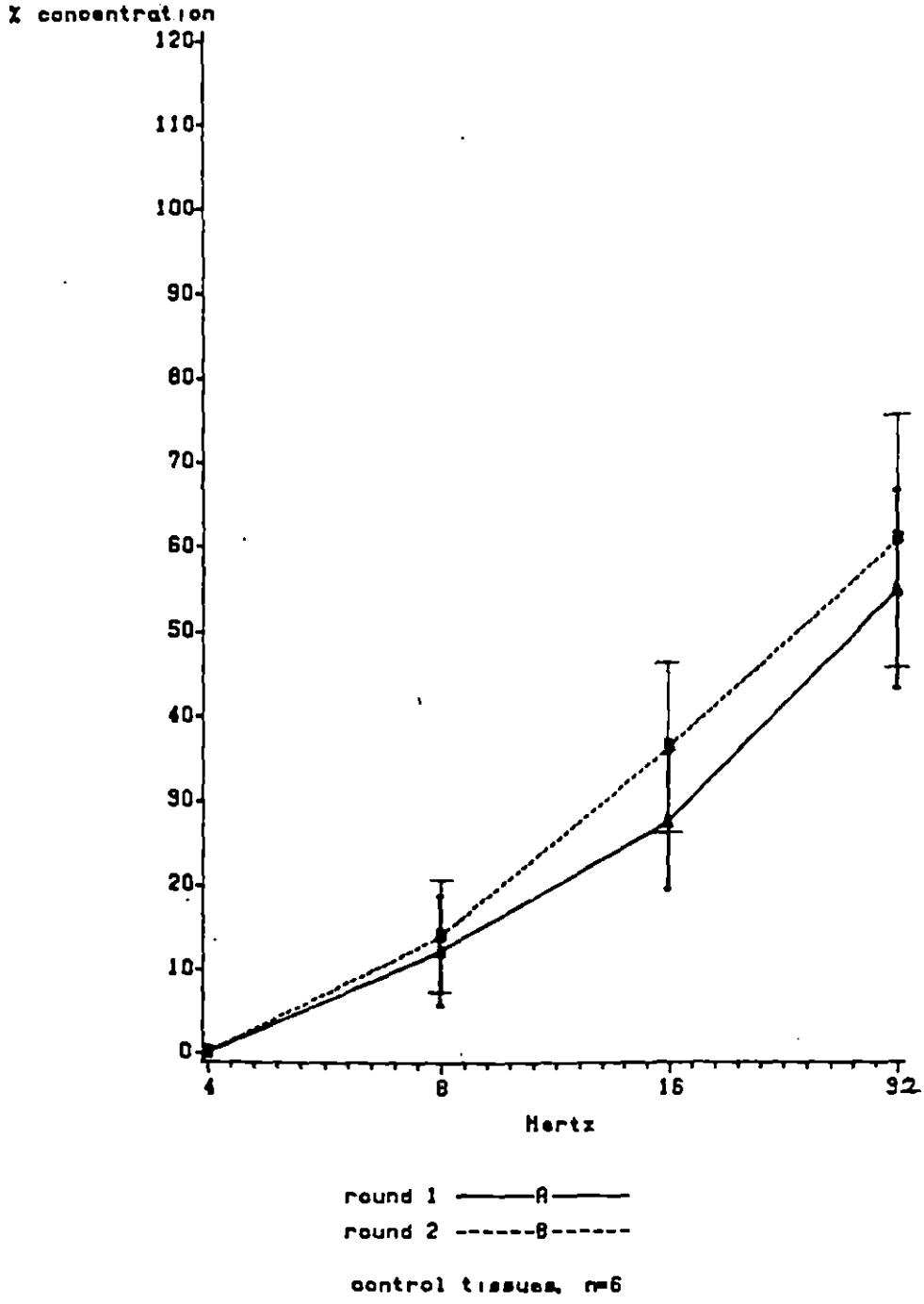


Figure 25a. Control of Figure 25b

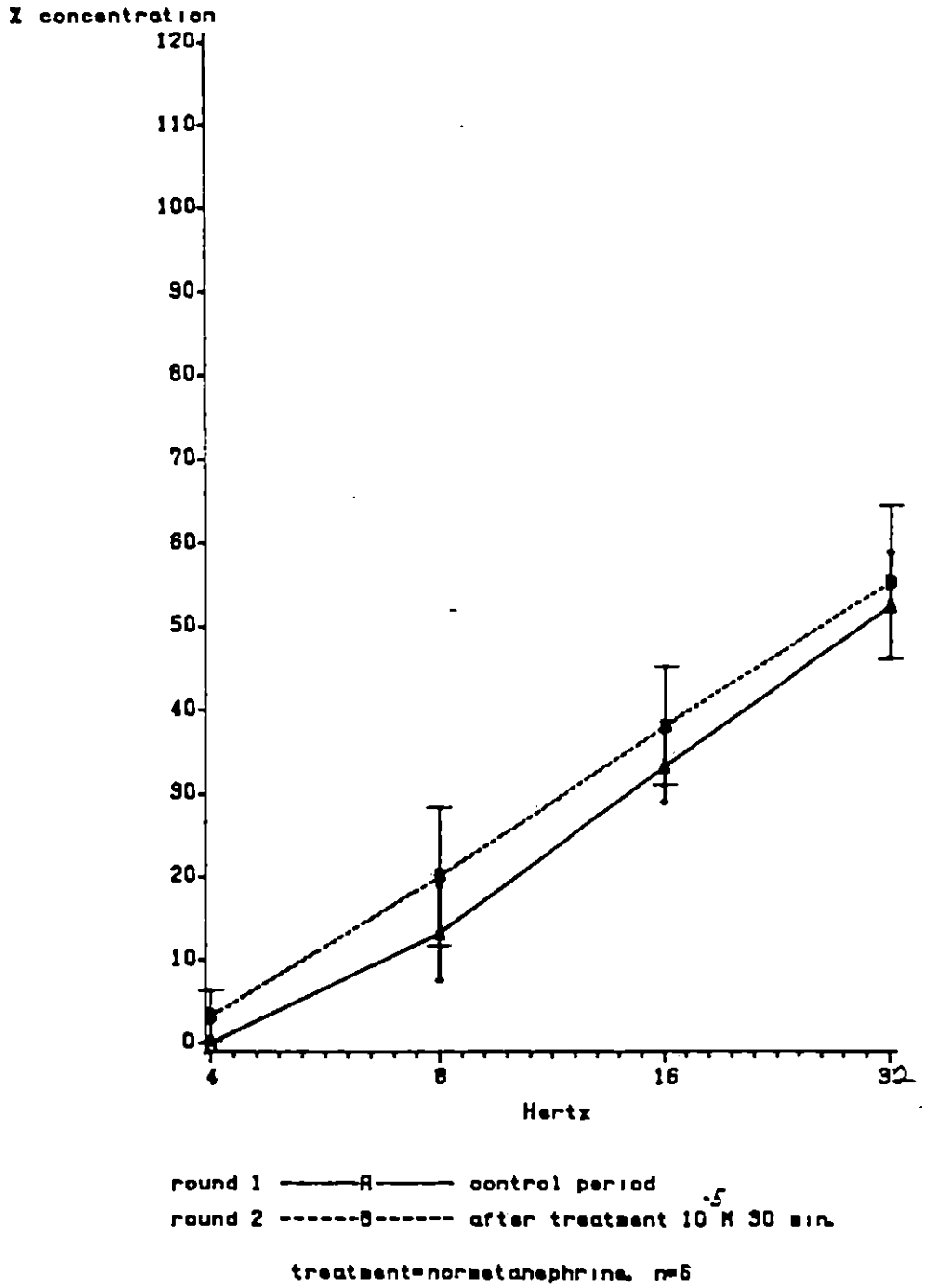


Figure 25b. Effect of normetanephrine on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 25a

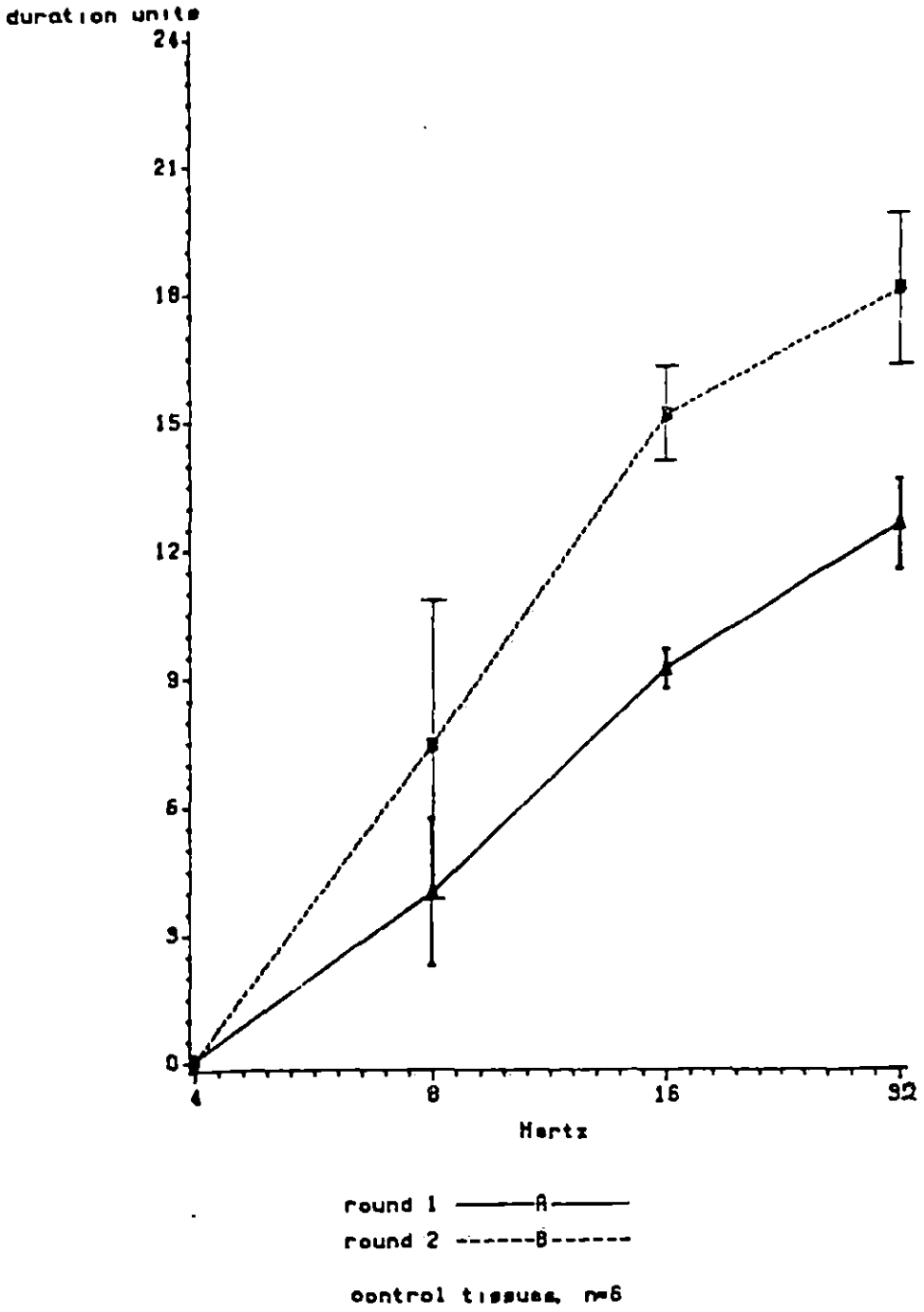


Figure 26a. Control of Figure 26b

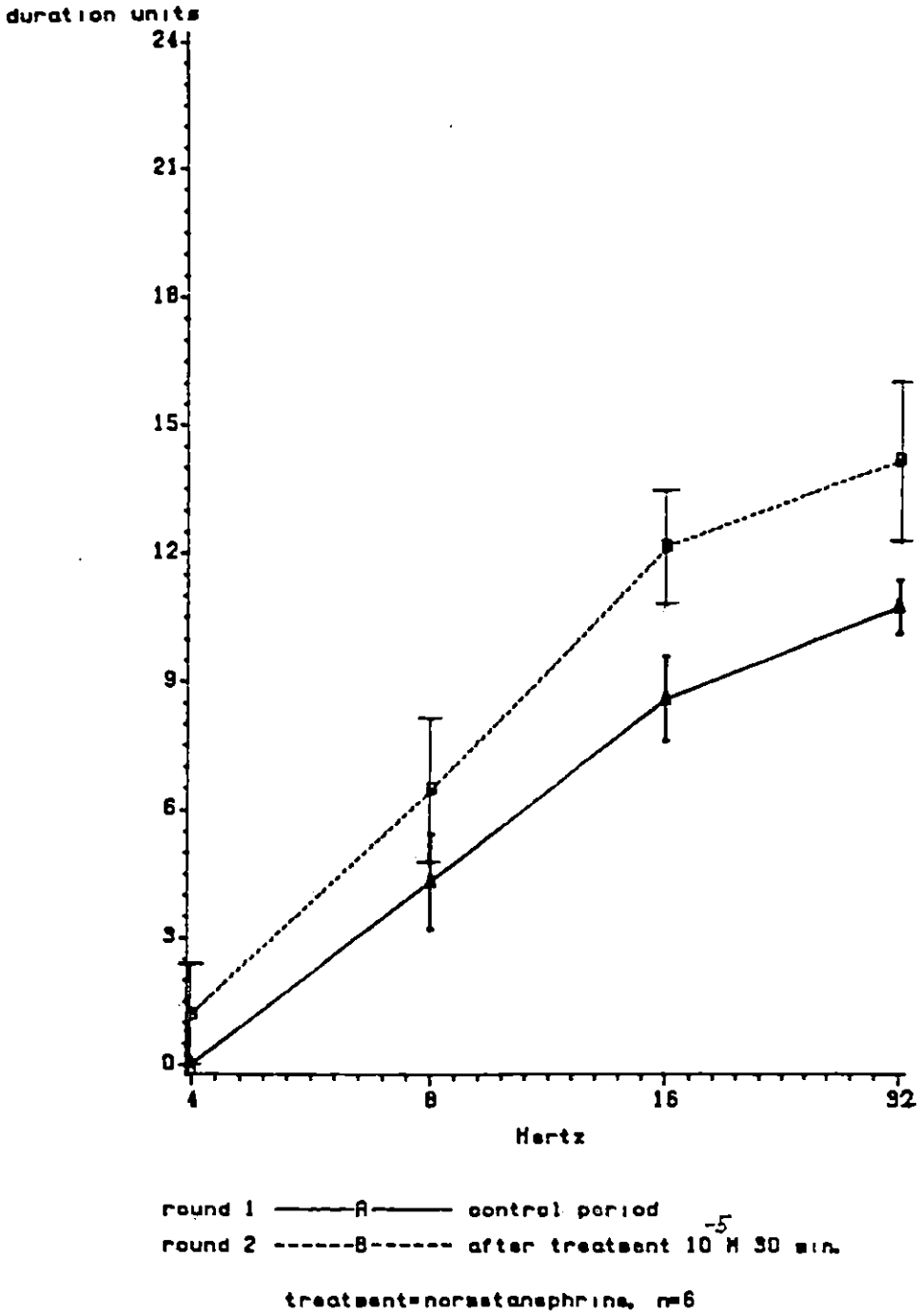


Figure 26b. Effect of normetanephrine on duration units (see text) of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 26a

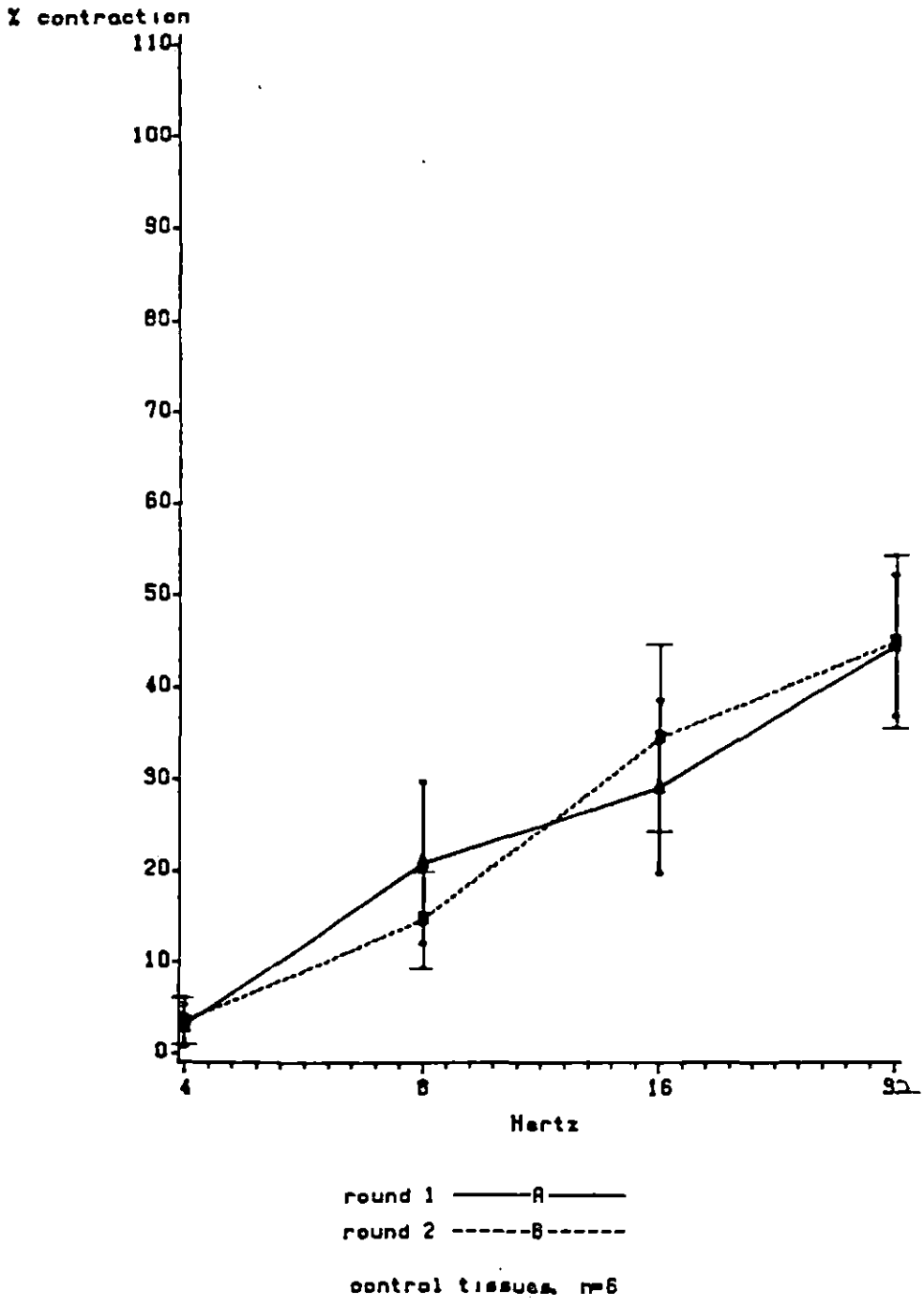


Figure 27a. Control of Figure 27b

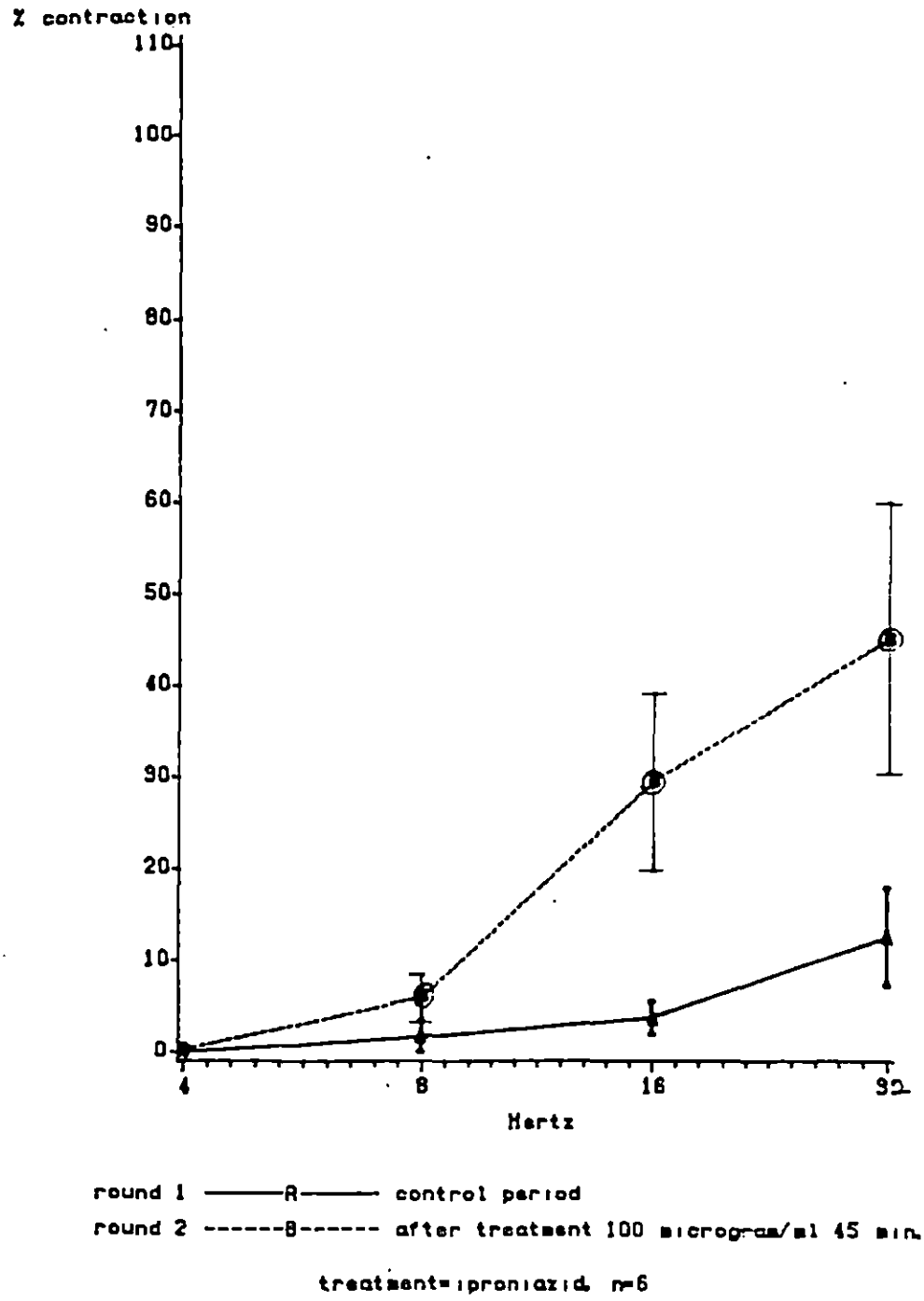


Figure 27b. Effect of iproniazid on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 27a

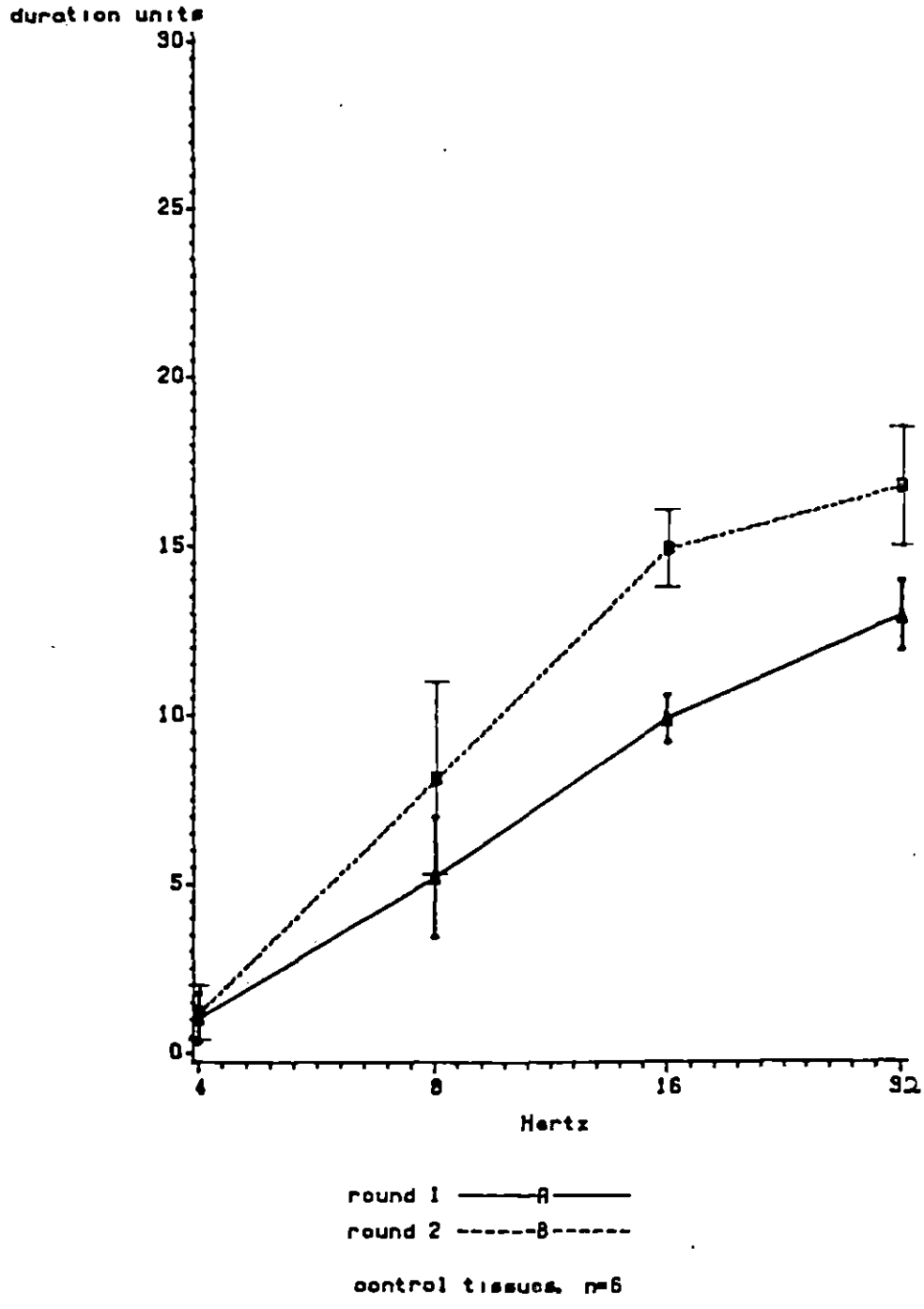


Figure 28a. Control of Figure 28b

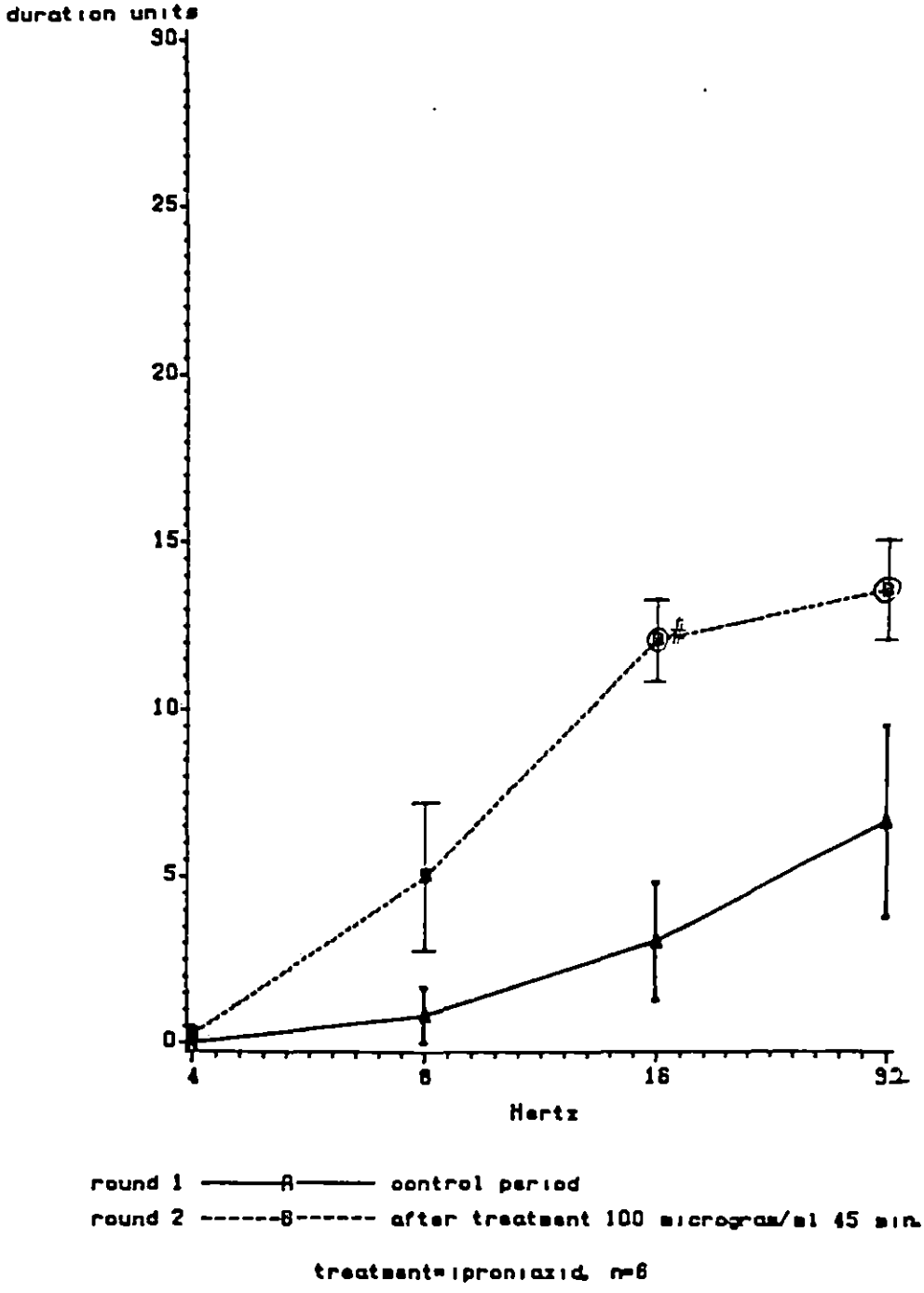


Figure 28b. Effect of iproniazid on duration units (see text) of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 28a

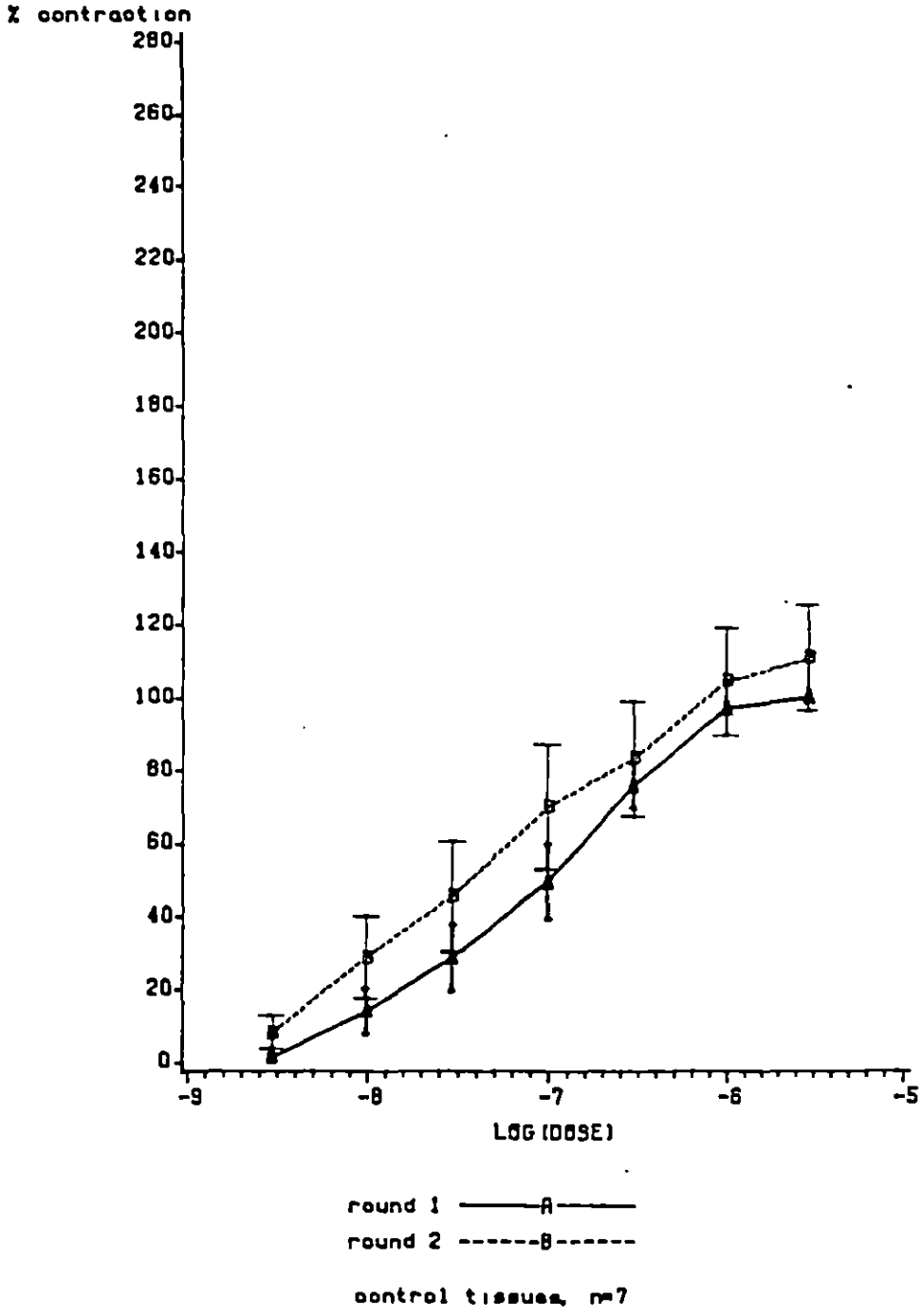


Figure 29a. Control of Figure 29b

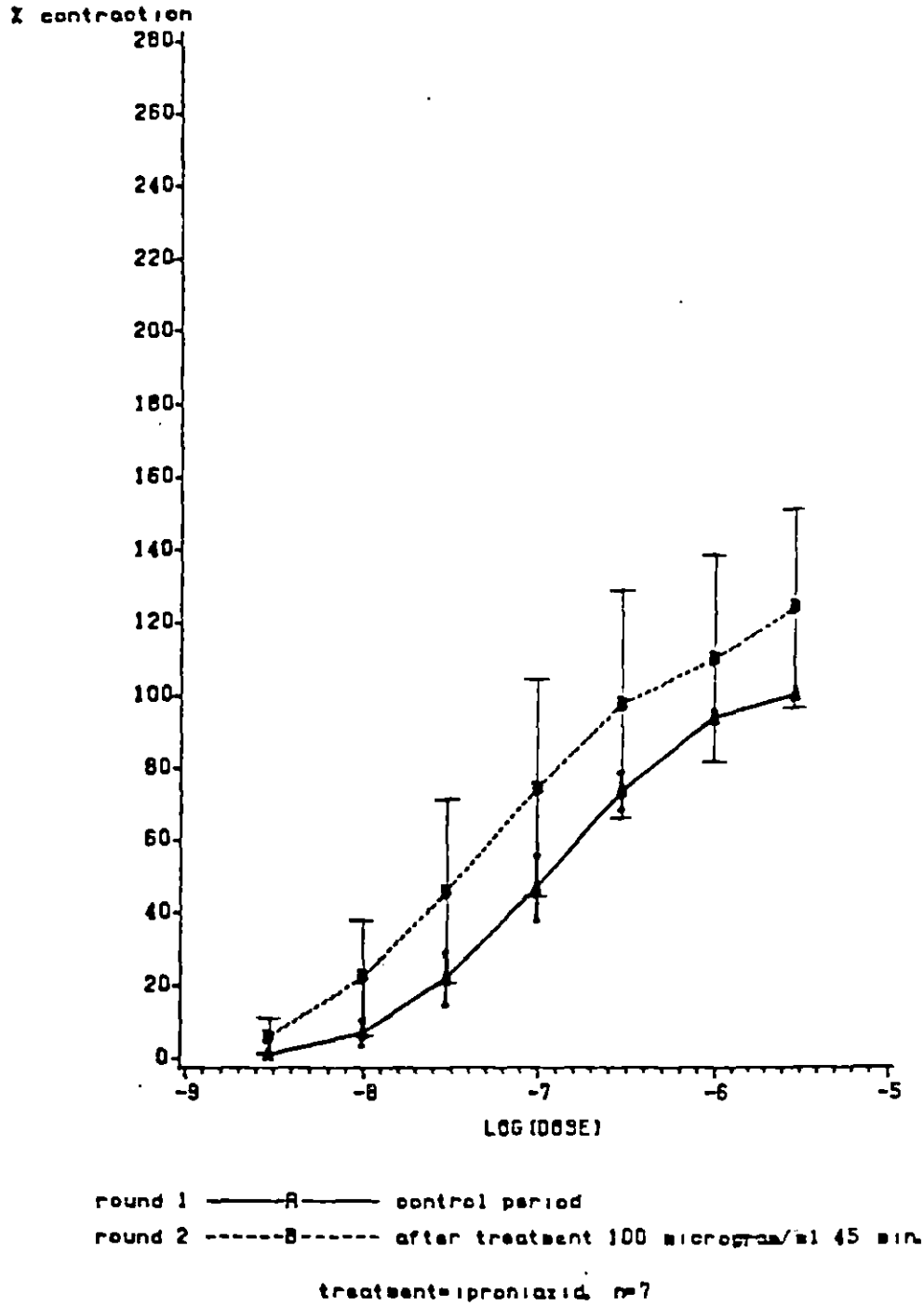


Figure 29b. Effect of iproniazid on responses to norepinephrine (NE) in turkey aorta. The percent contraction was the maximal contraction to norepinephrine obtained in round 1. Control tissues is shown in Figure 29a

2. Tropolone

This inhibitor of catechol-o-methyltransferase, did not exhibit any significant effect on the phase 1 and phase 2 response or on the duration of the phase 2 response (Figures 30 and 31).

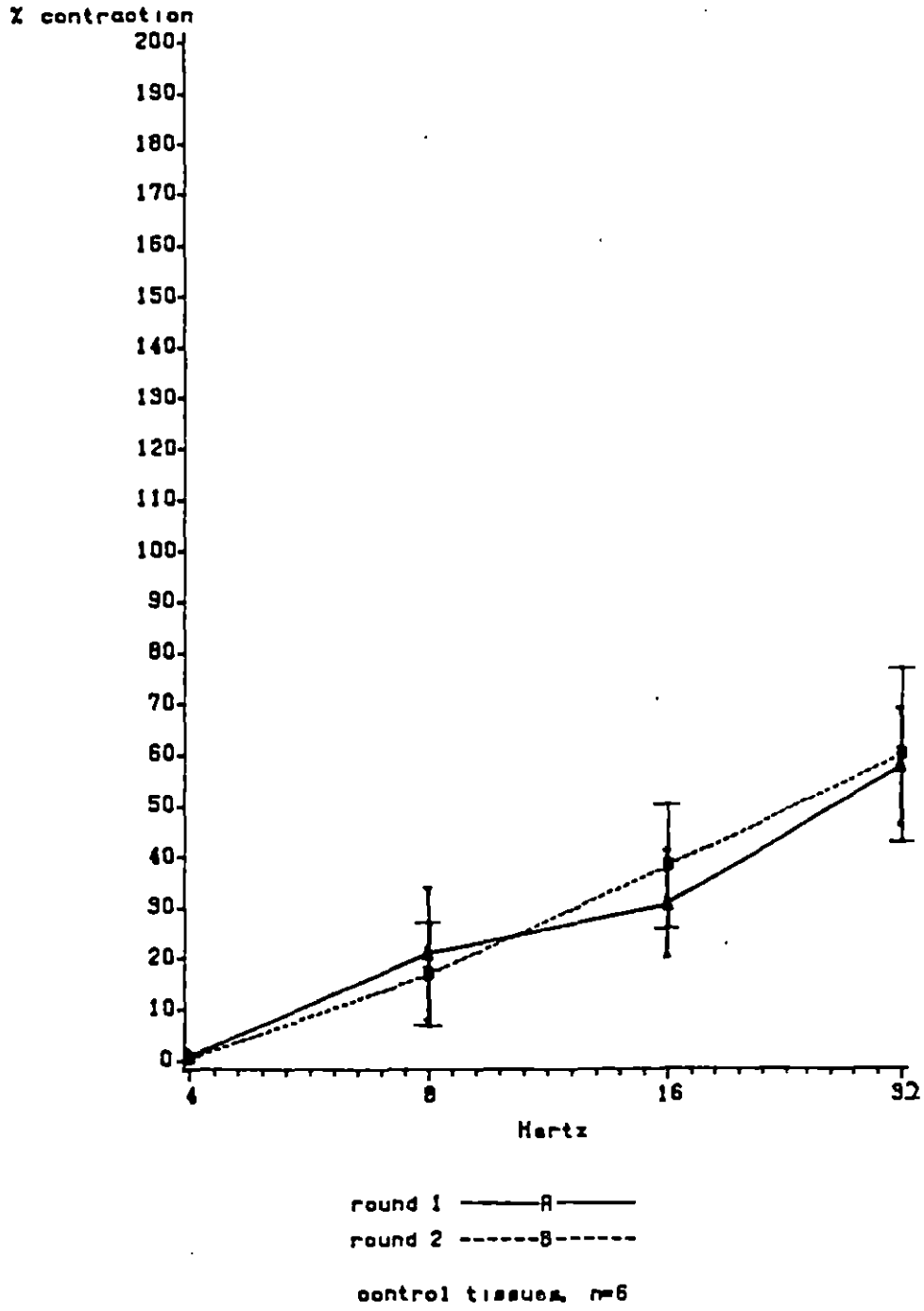


Figure 30a. Control of Figure 30b

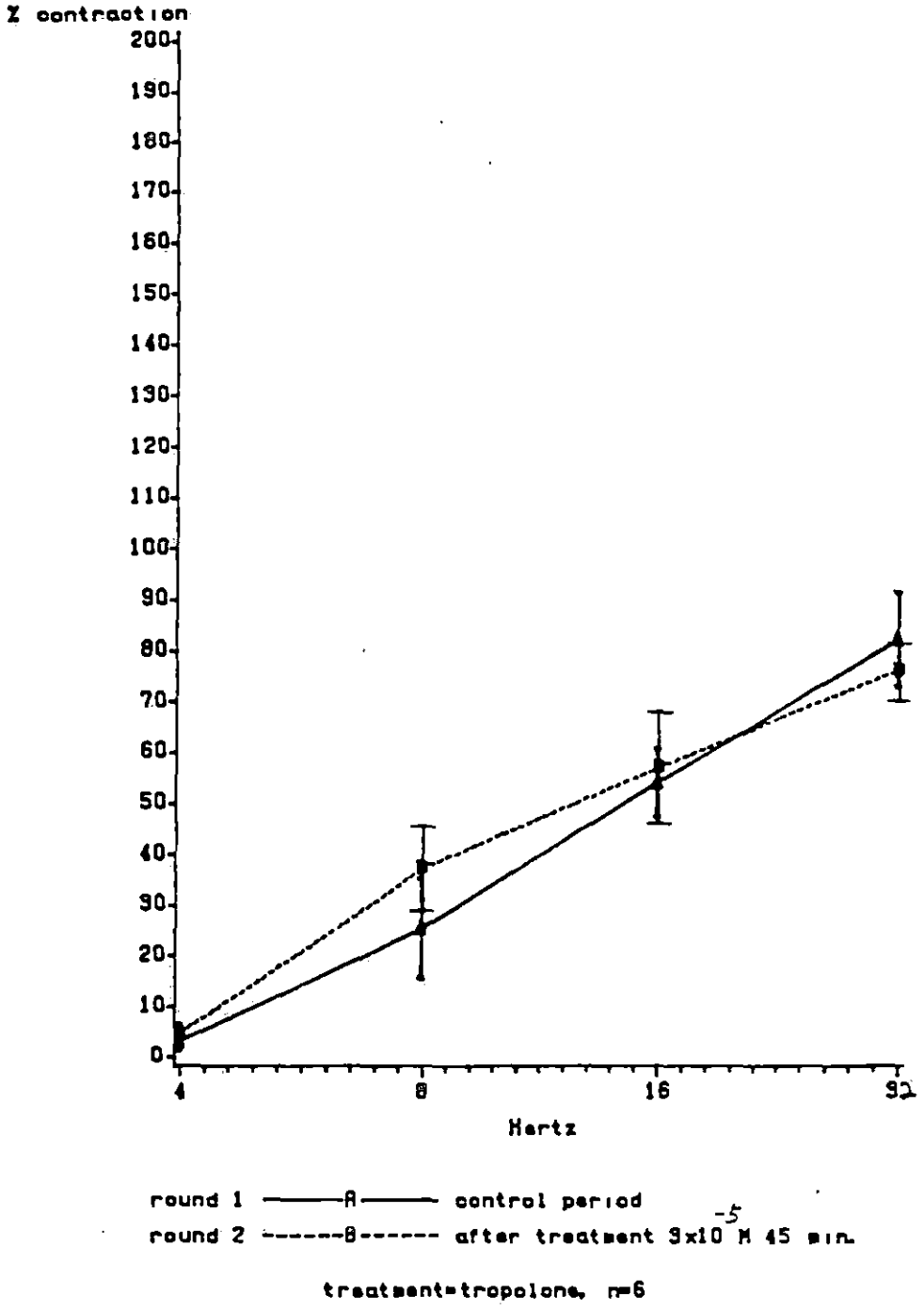


Figure 30b. Effect of tropolone on phase 2 of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 30a

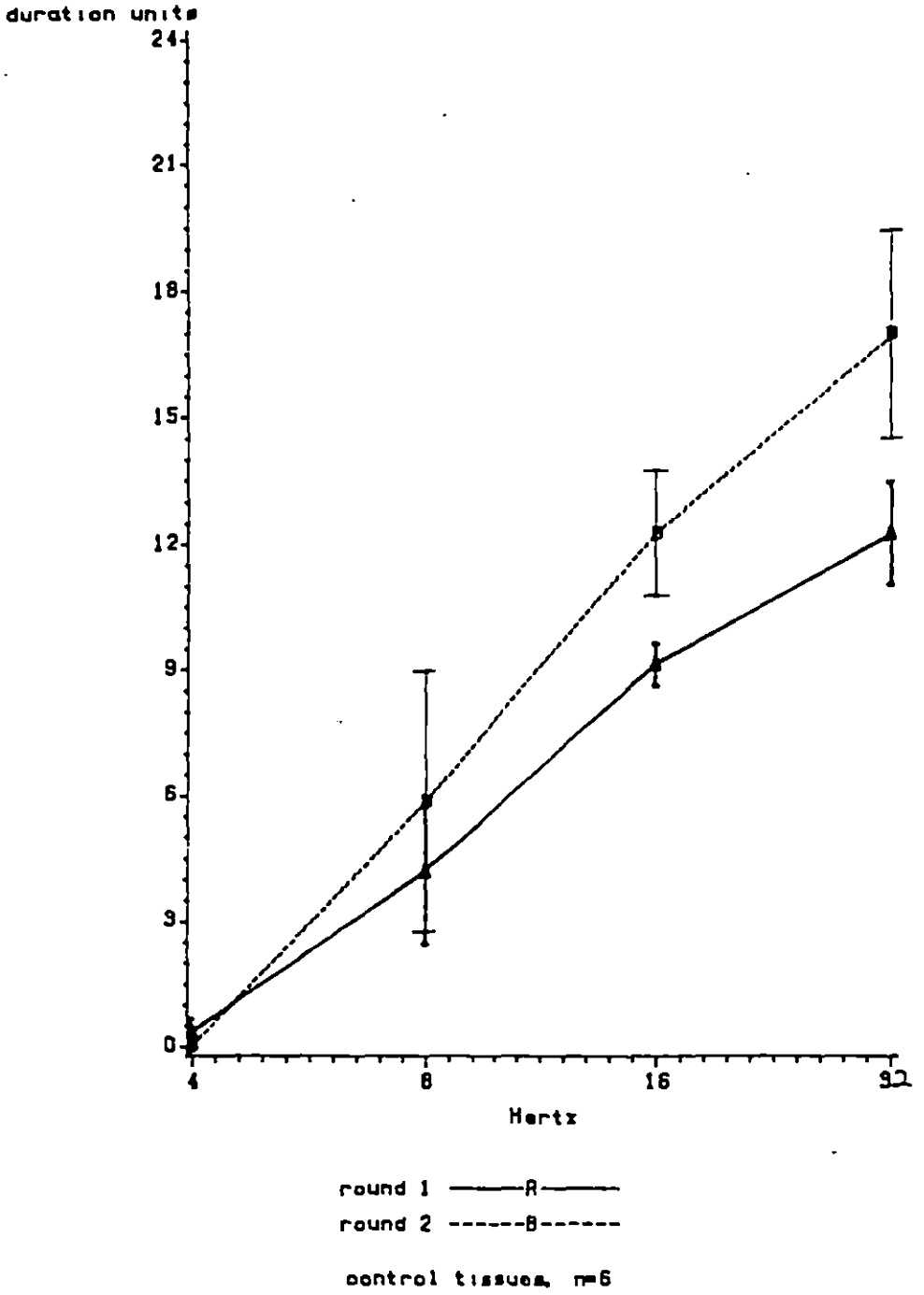


Figure 31a. Control of Figure 31b

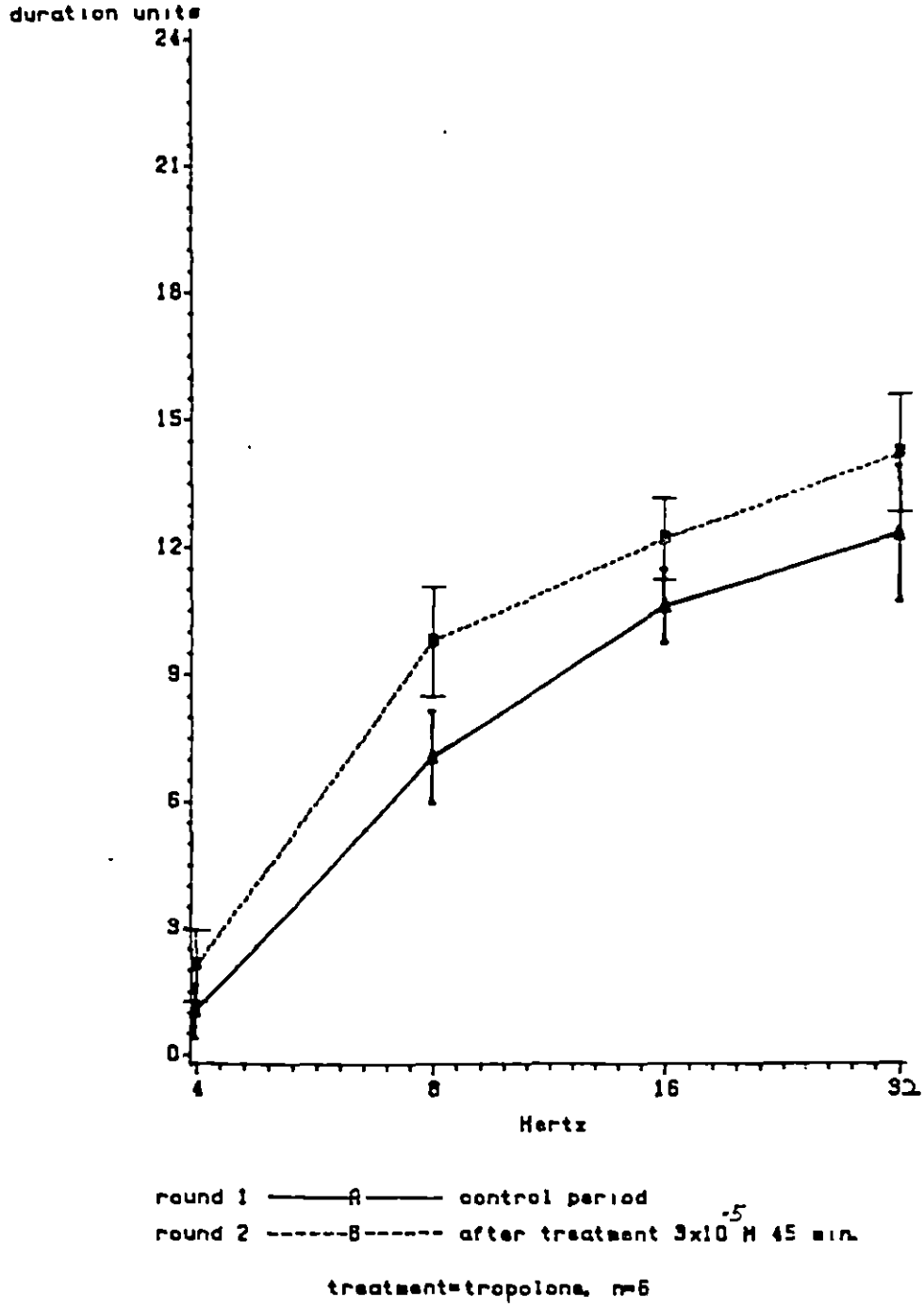


Figure 31b. Effect of tropolone on duration units of the electrical stimulation response (ESR) in turkey aorta. Response to KCl (120 mM) = 100% contraction. Control responses are shown in Figure 31a

VI. DISCUSSION

The two phased response to electrical field stimulation (Figure 2a) in the turkey aorta is similar to that described for the rabbit aorta (Furchgott, 1952; Yates and Gillis, 1963; Paterson, 1965). The evidence presented here seems to suggest that the secondary phase of the response to ES probably is the result of excitation of post-ganglionic adrenergic axons and the liberation of catecholamines as suggested by Paterson (1965). Evidence supporting this conclusion is based on the following evidence:

1) Bretylium inhibited the secondary phase of the ESR (Figures 4a and 4b). Bretylium has been shown to block transmission in adrenergic nerve terminals by decreasing transmitter release (Boura and Green, 1959; Su and Bevan, 1970).
2) Guanethidine also blocked the second phase of the ESR (Figures 6a and 6b). Guanethidine is thought to inhibit adrenergic function by impairing the release of NE from postganglionic adrenergic nerve terminals (Weiner and Taylor, 1985).
3) Tetrodotoxin (TTX) completely inhibited the phase 2 (Figures 8a and 8b) response to ES but not the phase 1 response (Figures 7a and 7b). Thus, TTX would impair the release of NE by stimulation of adrenergic nerves but would not hinder muscle response to ES (Kao, 1966).
4) Prazosin, a specific α_1-adrenergic antagonist abolished the second phase of ESR and shifted the NE dose response curve to the right (Figures 16-17).
5) Bretylium, guanethidine, and TTX appear to be exerting their typical pharmacological action in the isolated turkey aorta preparation in that they had no effect on the response to NE when it was added to the bath (data not shown). Thus these inhibitors do not depress the response to the presumed adrenergic transmitter in this preparation, namely, NE.

6) Drugs which have effects on adrenergic transmitter disposition such as: a) cocaine, an uptake$_1$ inhibitor, and b) iproniazid, a monoamine oxidase inhibitor, enhance the response of phase 2 and prolonged the duration of phase 2 (Figures 23, 24, 27, and 28). Both of these action are consistent with their known pharmacological response and that phase 2 involves an adrenergic transmitter (Maxwell et al., 1966; Zeller et al., 1952).

The first phase of ESR could be the result of any of the following:

1) Directly stimulating the muscle cell.
2) Stimulating a tetrodotoxin-resistant neuron (Senaratne and Kappagoda, 1984) and release of an unknown transmitter(s). While it is possible that two transmitters such as ATP and NE may be released by field stimulation of the turkey aorta just as ATP and NE are co-released by field stimulation of the rat or rabbit vas deferens (Sneddon et al., 1984), the evidence is against such a possibility. The primary fact which distinguishes the vas deferens from the turkey aorta is that TTX blocks both phase 1 and phase 2 in the vas deferens but only phase 2 in the turkey aorta and that phase 2 develops during stimulation in the vas deferens, not when stimulation ceases as in the turkey aorta preparation. Of course this argument becomes less convincing should there be nerves in the turkey aorta which are resistant to the TTX as has been described in the literature, (Senaratne and Kappagoda, 1984);
3) A combination of stimulating smooth muscle and adrenergic nerves simultaneously. This possibility is suggested since guanethidine (Figures 5a and 5b, TTX (Figures 7a and 7b), and prazosin (Figures 15a and 15b) could partially block the first phase.

According to Duckles and Silverman's (1980) experiments in rabbit ear arteries, it was possible to establish appropriate stimulation parameters to selectively activate nerves without stimulating smooth muscle directly. If phase 1 of the ESR is from stimulating smooth muscle, then by appropriately adjusting the stimulation parameter, it should have been possible to eliminate the phase 1 response while retaining the phase 2 response. Nevertheless, after I tried many turkey aortas (N>10), using all possible stimulation parameters such as voltage, duration, current, pulse number (stimulation time), or frequency from the point to evoke a threshold response to the point to cause the maximal contraction, I came to the conclusion that this first phase of ESR could never be completely eliminated at any stimulation parameter which could evoke a measurable response. There are two possible explanations for this experimental result: 1) something is wrong with the equipment. This possibility is unlikely since the equipment was tested by the departmental electronics technician (Chris Minor), and found to be in good working order or 2) the so-called "appropriate parameters" to selectively activate nerves without stimulating smooth muscle directly does not exist in all tissues, for example, in turkey aorta. This possibility seems likely since in pilot experiments using the coeliac (Figures 9a and 9b), testicular, and superior mesenteric arteries from the turkey, it was possible to eliminate the first phase of the response to ES. In addition, pilot experiments were performed on the rabbit aorta and uterus, rat and guinea pig vas deferens. The responses of these tissues to ES in our system were all similar to those reported in the literature. For example, TTX (10^{-6}M) blocked the whole ESR of rat or guinea pig vas deferens, whereas only the secondary phase of ESR in rabbit aorta was blocked by adrenergic neuronal blockers. In other pilot experiments using the female abdominal aorta (n=2), the responses to ES were only one-phased and this phase was blocked by prazosin.

It seems that in some tissues, such as turkey or rabbit aorta, the membranes of adrenergic axons (the voltage sensitive sodium channels) may not be as sensitive to electrical current flow as that of smooth muscle (the voltage-gated Ca^{+2} channels), so that the depolarization of smooth muscle and the subsequent opening of the voltage-gated Ca^{+2} channels may occur before or at the same time as the depolarization of the adrenergic axon and the opening of sodium channels. Thus muscle contraction may takes place before or at the same time the released transmitters reach their receptors. This may be the reason why phase 1 of ESR was not as sensitive to neuronal blockers as phase 2. After cessation of electrical current, both smooth muscle membranes and nerve membranes repolarized, but transmitters may continue to be released by exocytosis, or the released transmitters continue to find their postsynaptic receptors on the aortic smooth muscle membranes. The latter speculation would help in explaining the phase 2 response.

In tissues well innervated by adrenergic neurons, such as rabbit ear artery, and rabbit pulmonary artery, these nerve membranes (voltage sensitive sodium channels) may be more sensitive to ES than smooth muscle membranes (voltage-gated calcium channels). Depolarization of nerve membrane takes place before that of smooth muscle membrane until the stimulation parameters reach a threshold for actually stimulating the smooth muscle. This model could explain the observations that in rabbit ear artery the responses to lower voltage can be blocked by TTX or phentolamine while those to higher voltage can not. In the case of rat or guinea pig vas deferens, the smooth muscle membrane (the voltage-gated calcium channels) in these tissues is rather insensitive to electrical current flow, so that the responses in these tissues are all nerve related and TTX can completely block responses to field stimulation even at high stimulation parameter (>30 Hz)

Duckles and Silverman's (1980) model for explaining the relationship between stimulating parameters and the responses to electrical stimulus might have oversimplified the

real situation by failing to take into account the possible existence of various tissues from different animals that would not conform to this model. Certainly the turkey aorta preparation does not belong to this model.

To further establish the methodology and study adrenergic mechanisms in turkey aorta, two modulators of adrenergic transmission (in mammals) were used, namely, serotonin and angiotensin II.

Serotonin appeared to have a significant inhibitory effect on responses to endogenous (released by ES) and exogenous NE (figures 11 and 12). Serotonin did not amplify any of the responses as reported by Van Nueten (1983). It is unlikely that serotonin inhibited the release of the adrenergic transmitter since the NE dose response curve was also inhibited to about the same extent as was the frequency-response curve to ES.

It is unlikely that serotonin exerted a vasodilation (relaxed the smooth muscle) and that this effect inhibited contractions to norepinephrine or to ES. This is because relaxation responses to serotonin were never observed in an equilibrated preparation or in a preparation under tone. As the concentration of serotonin was increased ($2x10^{-7}$ to 10^{-6}M) in the bath, contractions were produced.

Another possibility which may explain the inhibitory effect of serotonin on responses to NE is that serotonin may have the ability to act on α-adrenergic receptors. Innes (1962) demonstrated that serotonin could interact with α-adrenergic receptors in a variety of tissues. Purdy et al., 1981, found that prazosin, an α_1-antagonist, blocked contractile responses to serotonin in the rabbit ear artery. In several pilot experiments, I found that prazosin (10^{-6}M) did not block contractions to serotonin (unreported data). Therefore, it seems unlikely that serotonin is acting as a partial agonist of α-adrenergic receptors and thereby reduces α-adrenergic activation.

Angiotensin II, a reported modulator of adrenergic neural transmission, inhibited responses to ES and to norepinephrine (Figures 13 and 14). I expected that angiotensin II might enhance responses to ES as has been reported for adrenergically innervated tissue from mammals (Westfall et al., 1985). The apparent reasons for this difference are elusive. It must be pointed out that angiotensin II apparently does not act in avian species as it does in mammals since little or no vasoconstriction was produced to angiotensin II in the turkey aorta ring preparation. In the mammalian cardiovascular system, angiotensin II is a very potent vasoconstrictor (Ganong, 1985). The possibility that angiotensin II's inhibitory effect might be related to the release and production of prostaglandins can not be ruled out but seems doubtful since vasodilation in turkey aorta by angiotensin II was never observed (using mechanical tension) in this research. Angiotensin II has been reported to stimulate the synthesis of prostaglandins in pig renal veins (Webb, 1982).

The pA_2 value for prazosin in turkey aorta is 7.29 with the slope=0.50. Both of pA_2 and slope are apparently different from those (Schulz and Westfall, 1982) in guinea pig thoracic aorta (pA_2=8.29, slope=0.86±0.23), rabbit renal vein (pA_2=8.27, slope= 0.95±0.55), rabbit renal artery (pA_2=8.34, slope=1.11±0.33), rabbit saphenous vein (pA_2=8.40, slope=1.18±0.41) and rabbit saphenous artery (pA_2=9.16, slope=0.71±0.43). The slope (0.50) of the Schild plot from turkey aorta is significantly different from 1. In the Schild analysis, competitive antagonisn is defined with a slope to 1. Thus the prazosin pA_2 value and slope indicates that prazosin is not acting as a competitive α_1-antagonist or that proper conditions were not met during the experiment. It should be pointed out that the Schild analysis does not always meet the theoretical expectations. For instance, Patil (1968) found the isolated trachea from the guinea pig to give a pA_2 value of 7.23 and a slope of 0.46 when butoxamine was used to study β-adrenergic receptors. Patil could not offer a satisfactory explanation for the low slope.

Whether the α_1-adrenergic receptor in turkey aorta is the same as that in other experimental animals is not certain at this point. The obvious variability between experiments and even between preparations cut from the same aorta tends to make one cautious. The assumption that methoxamine acts in turkey aorta as it does in mammalian tissues may not be correct. Should the turkey adrenergic nerve terminal actively take up methoxamine or the smooth muscle take up the drug, or if methoxamine were metabolized by the preparation, then the criteria developed by Furchgott (1972) would not be met and a slope different from unity would be expected. Another possibility that needs to be entertained in seeking explanations of the low slope is that there may be more than one type of α-adrenergic receptor in the aorta preparation and that each subtype of receptor may have a different response to methoxamine. Prazosin in a high concentration ($>10^{-6}$M) has been shown to cause the release of NE through the disruption of adrenergic vesicles (Anderson et al., 1979). Although the highest concentration used for pA_2 determination was 10^{-7}M, we are still not sure whether this concentration of prazosin can cause the release of transmitter in turkey aorta. Should prazosin promote the release of transmitter, then this would obviously alter the pA_2 determination.

Yohimbine (Figure 22) had a pA_2 value of 6.9 and a slope of 0.45. Yohimbine probably acts mostly as an α_1- rather than an α_2-adrenergic blocking agent in turkey aorta. In contrast, using the same agonist (methoxamine) and antagonist (yohimbine), a pA_2 value of 5.76 and slope of 0.96 was reported for rabbit pulmonary artery (Holck et al., 1983). Yohimbine significantly inhibited ($p<0.05$) responses to electrical stimulation (Figures 20a and 20b) at all frequencies and no enhancement was observed. If yohimbine was acting on presynaptic α_2-adrenergic receptors and provided that α_2-adrenergic receptors did not contribute to postsynaptic stimulation, then we would anticipate enhancement of the response to ES. Since we did not measure the release of NE during ES (alone) and in the presence of

yohimbine, no definitive statement can be made concerning the presence and importance of presynaptic α_2-adrenergic receptors. The possibility that the antagonism by yohimbine on the postsynaptic adrenergic receptors overcomes the increasing release of transmitters still exists. However, the weak contractile response produced to clonidine, xylazine, and BHT-920, all α_2-agonist, suggests that little activation of α_2-receptors is involved in postsynaptic actions of the adrenergic transmitter.

The possible "sites of loss" of an adrenergic transmitter include uptake$_1$, uptake$_2$, and metabolism by MAO and/or COMT (Furchgott, 1972). Various drugs were used which specifically block one of these four sites of loss. Using enhancement of the response to ES as an indicator, if enhancement of the response occurred to ES when a specific inhibitor was used, then the mechanism inhibited would likely play an important role in terminating the action of the neuronally-released transmitter.

Cocaine, an uptake$_1$ inhibitor, significantly enhanced phase 2 responses to ES as well as the duration of ESR, following ES (Figures 23-31). This suggests that the neuronal membrane pump (uptake$_1$) is important in regulating the amount of adrenergic transmitter in the synaptic cleft following the release of the transmitter.

Extraneuronal uptake (uptake$_2$) is probably less important physiologically in regulating NE in the synaptic cleft since normetanephrine, an inhibitor of uptake$_2$, did not enhance responses to ES. Extraneuronal uptake seems to be relatively less physiologically important than neuronal uptake as suggested by Iversen (1975) and Trendelenburg (1980). Since the principal neuronal enzyme involved in degrading catecholamines is MAO (Levin, 1974; Paiva and Guimaraes, 1978; Kopin, 1972), it is understandable how iproniazid (a MAO inhibitor) (Zeller et al., 1952) greatly potentiated the response to ES (Figures 27 and 28). Catechol-o-methyltransferase is probably of little importance in regulating neuronally-released norepinephrine since tropolone had essentially no effect on responses to ES

(Figures 30 and 31). Figures 29a and 29b show that iproniazid had no effect on the contractile response to NE.

The present experimental results are complicated by the large variation occurring between experimental days and even within an experiment. The possible reasons for this are many and a few possibilities will be discussed. While the turkeys were purchased from a common source, this does not ensure genetic similarity. The weight (and age) of the birds did vary somewhat, but since so little is known about the influence of age and weight on the response of the turkey cardiovascular system, further speculation would be futile. Except to point out that age has an effect of β-adrenergic responses in rat aorta (Fleisch et al., 1970). Another source of variance is that repeating the same stimulation did not always produce the same (or similar) degree of response for each tissue preparation. As shown in the figures, the responses of the second round (also 3rd round or 4th round) in the control tissues are not always similar to those of the first round. While the paired tissue method (Kenakin, 1984) or preparing tissues from a common artery usually decreases variation, this may not be adequate in the turkey, since the change of sensitivity during the course of the experiment is not always similar for each turkey aorta preparation. Adrenergic innervation may vary from one end of the abdominal aorta to the other as has been demonstrated for the rabbit ear artery (Griffith et al., 1982). Receptor distribution may also vary with ring segments prepared from the turkey abdominal aorta. Heterogeneous receptor distribution in vascular smooth muscle has been shown to occur for β-adrenergic receptors (Fleisch et al., 1970) and serotonin receptors (Gintautas et al., 1980). While care was taken to try and prepare the turkey aorta rings from day-to-day as uniformly as possible, I recognize that stretch of the smooth muscle or damage to the endothelial cell surface in some preparations is a possibility and that if this occurred, variations in responses to ES and agonists would be expected.

VII. SUMMARY

The lack of information available concerning the turkey cardiovascular system, the naturally occurring high blood pressure in the turkey, and the associated economic loss from aortic rupture led to this study. Using male turkey abdominal aortae to study responses to neuronal blockers, modulators of adrenergic transmission, antagonists of alpha adrenergic receptors, and inhibitors of adrenergic transmitter disposition, the following was concluded:

1) The methodology for isometric recording and field stimulation of turkey aortic rings was developed. However, the large variation in responsiveness of the aortic rings during the course of the experiment presents a problem which needs to be solved.

2) In turkey aorta, the responses to modulators of adrenergic transmission such as serotonin and angiotensin II are inhibitory and do not enhance adrenergic transmission as reported in the literature.

3) Alpha adrenergic receptors in turkey aorta may be different from those in other vertebrates, since the pA_2 values for prazosin and yohimbine are different. However, the results of these experiments did not meet the theoretical criteria established by Schild (1947) and need further study.

4) Inhibitors of catecholamine uptake and metabolism produced responses in the turkey aortic preparation which were similar to those produced in preparations of mammalian vascular smooth muscle. This suggests that comparable mechanisms of catecholamine disposition are operative in turkey and mammalian vasculature.

VIII. BIBLIOGRAPHY

Abrahamsen, J. and O. A. Nedergaard. 1985. Accumulation of ^{3}H-adrenaline by rabbit aorta. Blood Vessels 22:32-46.

Ahlquist, R. P. 1948. A study of the adrenotropic receptors. Am. J. Physiol. 154:586-600.

Akester, A. R. 1971. The blood vascular system. Chapter 32, pp. 783-837. In D. J. Bell and B. M. Freeman, eds. Physiology and biochemistry of the domestic fowl. Academic Press Inc., London.

Altura, B. M. 1975. Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J. Pharmacol. Exp. Ther. 193:403-412.

Altura, B. M. and B. T. Altura. 1970. Heterogeneity of drug receptors in different segments of rabbit thoracic aorta. Eur. J. Pharmacol. 12:44-52.

Anderson, M. J., D. Cambridge, M. J. Davey, R. Massingham, P. M. Vanhoutte, and T. Verbeuren. 1979. The effect of a high dose of prazosin, and transmural stimulation, on the disposition of transmitter noradrenaline in the rabbit pulmonary artery and the dog saphenous vein. Br. J. Pharmacol. 66:151-152.

Andrew, W. 1959. Textbook of comparative histology. Oxford University Press, Inc., New York.

Axelrod, J. 1957. O-methylation of catechol amines *in vitro* and *in vivo*. Science 126:400-401.

Axelrod, J. 1959. The metabolism of catechol amines *in vivo* and *in vitro*. Pharmacol. Rev. 11 (part 2):402-408.

Axelrod, J. 1973. The fate of norepinephrine in the sympathetic neuron. Harvey Lect. 67:175-197.

Axelrod, J. and M. J. Laroche. 1959. Inhibitor of O-methylation of epinephrine and norepinephrine *in vitro* and *in vivo*. Science 130:800.

Axelrod, J. and R. Tomchick. 1958. Enzymatic O-methylation of NE and other catechols. J. Biol. Chem. 233:702-705.

Axelrod, J., H. Weil-Malherbe, and R. Tomchick. 1959a. The physiological disposition of H^{3}-epinephrine and its metabolite metanephrine. J. Pharmacol. Exp. Ther. 127:251-256.

Axelrod, J., R. W. Albers, and C. D. Clemente. 1959b. Distribution of catechol-o-methyl transferase in the nervous system and other tissues. J. Neurochem. 5:68-72.

Belis, J. A., J. E. Colby, and D. P. Westfall. 1982. Effects of α-adrenoceptor agents on norepinephrine release from vas deferens of several species including man. Eur. J. Pharmacol. 78(4):487-490.

Belleau, B. and J. Burba. 1961. Tropolone: A unique class of potent noncompetitive inhibitors of S-adenosylmethionine-catechol methyltransferase. Biochim. Biophys. Acta (Amst.) 54:195-196.

Bevan, J. A. and C. Su. 1964. The sympathetic mechanism in the isolated pulmonary artery of the rabbit. Br. J. Pharmacol. Chemother. 22:176-182.

Blaschko, H. 1974. The natural history of amine oxidases. Rev. Physiol. Biochem. Pharmacol. 70:81-148.

Bloom, W. and D. W. Fawcett. 1962. A textbook of histology. 8th ed. W. B. Saunders Company, Philadelphia, London.

Blumberg, A. L., S. E. Denny, G. R. Marshall, and P. Needleman. 1977a. Blood vessel-hormone interaction: Angiotensin, bradykinin, and prostaglandins. Am. J. Physiol. 232:H305-H310.

Blumberg, A. L., K. Nishikawa, S. E. Denny, G. R. Marshall, and P. Needleman. 1977b. Angiotensin (AI, AII, AIII) receptor characterization. Correlation of prostaglandin release with peptide degradation. Circulation Res. 41:154-158.

Bohr, D. F. and K. H. Berecek. 1976. Relevance of vascular structure and smooth muscle sensitivity changes in hypertension. Aust. N. Z. J. Med. 6,Suppl. 2:26-34.

Bolton, T. B. 1968. Studies on the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. J. Physiol., Lond. 196:273-281.

Boura, A. L. A. and A. F. Green. 1959. The actions of bretylium: Adrenergic neurone blocking and other effects. Br. J. Pharmacol. 14:536-548.

Burn, J. H. 1933. A pharmacological approach to the cause of asthma. Proc. Roy. Soc. Med. 27:31-41.

Burn, J. H. and M. J. Rand. 1958. Noradrenaline in artery walls and its dispersal by reserpine. Br. Med. J. 1:903-908.

Carnaghan, R. B. A. 1955. Atheroma of the aorta associated with dissecting aneurysms in turkeys. Vet. Rec. 67:568.

Cebeddu, L., E. Barnes, and N. Weiner. 1975. Release of norepinephrine and dopamine β-hydroxylase by nerve stimulation. I. V. An evaluation of a role for cyclic adenosine monophosphate. J. Pharmacol. Exp. Ther. 193:105-107.

Cohen, M. L. and B. A. Berkowitz. 1974. Age-related change in vascular responsiveness to cyclic nucleotides and contractile agonists. J. Pharmacol. Exp. Ther. 191:147-155.

Cohen, M. L. and K. S. Wiley. 1977. Specific enhancement of norepinephrine-induced contraction in rat veins after beta adrenergic antagonists. J. Pharmacol. Exp. Ther. 201:406-416

Cowen, T., D. E. M. MacCormick, W. D. Toff, G. Burnstock, and J. S. P. Lumley. 1982. The effect of surgical procedures on blood vessel innervation. Blood Vessels 19:65-78.

Crout, J. R., C. R. Creveling, and S. Udenfriend. 1961. Norepinephrine metabolism in rat brain and heart. J. Pharmacol. Exp. Ther. 132:269-277.

De Clerck, F. and P. M. Vanhoutte. 1982. 5-Hydroxytryptamine in peripheral reactions. Raven Press, New York.

De la Lande, I. S. 1975. Adrenergic mechanisms in rabbit ear artery. Blood Vessels 12:137-160.

Duckles, S. P. and R. W. Silverman. 1980. Transmural nerve stimulation of blood vessels *in vitro*: A critical examination. Blood Vessels 17:53-57.

Dyer, D. C. and S. D. Badr. 1985. Characterization of drug-induced responses of isolated turkey aorta. The Pharmacologist 27:199.

Eisenfeld, A. J., J. Axelrod, and L. Krakoff. 1967. Inhibition of extraneuronal accumulation and metabolism of norepinephrine by adrenergic blocking agents. J. Pharmacol. Exp. Ther. 156:107-113

Euler, U. S. V. 1972. Synthesis, uptake and storage of catecholamine in adrenergic nerves, the effect of drugs. Chapter 6, pp. 186-230. In H. Blaschko and E. Muscholl, eds. Handbook of experimental pharmacology. Vol. 33. Springer-Verlag, Berlin.

Fleisch, J. H, H. M. Maling, and B. B. Brodie. 1970. Beta receptor activity in aorta: Variations with age and species. Circulation Res. 26:151-162.

Folkow, B., M. Hallback, Y. Lundgren, R. Sivertsson, and L. Weiss. 1973. Importance of adaptive changes in vascular design for establishment of primary hypertension, studied in man and in spontaneously hypertensive rats. Circulation Res. 32/33, Suppl. 1:2–16.

Foster, R. W. 1967. The potentiation of the responses to noradrenaline and isoprenaline of the guinea-pig isolated trachea chain preparation by desipramine, cocaine, phentolamine, phenoxybenzamine, guanethidine, metanephrine and cooling. Br. J. Pharmacol. 31:466-482.

Foster, R. W. 1968. A correlation between inhibition of the uptake of ^{3}H from (±)-^{3}H-noradrenaline and potentiation of the responses to (-)-noradrenaline in the guinea-pig isolated trachea. Br. J. Pharmacol. 33:357-367.

Foster, R. W. 1969. An uptake of radioactivity from (±)-^{3}H-isoprenaline and its inhibition by drugs which potentiate the responses to (-)-^{3}H-isoprenaline in the guinea-pig isolated trachea. Br. J. Pharmacol. 35:418-427.

Furchgott, R. F. 1952. Effect of dibenamine on response of arterial smooth muscle to epinephrine nor-epinephrine, electrical stimulation and anoxia. Fed. Proc. 11:217.

Furchgott, R. F. 1972. The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. Chapter 9, pp. 283-335. In H. Blaschko and E. Muscholl, eds. Handbook of experimental pharmacology. Vol. 33. Springer-Verlag, Berlin.

Furchgott, R. F. 1983. Role of endothelium in responses of vascular smooth muscle. Cir. Res. 53:557-573.

Ganong, W. F. 1985. Review of medical physiology. Lange Medical Publications, Los Altos, California.

Geddes, L. A. 1970. The direct and indirect measurement of blood pressure. Year Book Medical Publishers, Inc., Chicago, Illinois.

Gillis, C. N. and C. M. Yates. 1960. Effect of reserpine on the response of isolated aorta to electrical stimulation. Fed. Proc.19:105.

Gintautas, J., B. J. Krayrack, and G. B. Racz. 1980. Responsiveness of isolated cerebral arteries to various pharmacologic agents and to transmural electrical stimulation. Proc. West. Pharmacol. Soc. 23:63-67.

Göthert, M. 1984. Adrenergic transmitter release. Blood Vessels 21:117-125.

Green, H. and B. W. Erickson. 1960. Effect of trans-2-phenylcyclopropylamine upon norepinephrine concentration and monoamine oxidase activity of rat brain. J. Pharmacol. Exp. Ther. 129:237-242.

Griffith, S. G., R. Crowe, J. Lincoln, A. J. Haven, and G. Burnstock. 1982. Regional differences in the density of perivascular nerves and varicosities, noradrenaline content and responses to nerve stimulation in the rabbit ear artery. Blood Vessels 19:41-52.

Grollman, A., C., Ashworth, and W. Suki. 1963. Atherosclerosis in the chicken. Arch. Pathol. 75:56.

Guldberg, H. C. and C. A. Marsden. 1975. Catechol-O-methyltransferase: Pharmacological aspects and physiological role. Pharmacol. Rev. 27:135-206.

Hansen, T. R. and D. F. Bohr. 1975 Hypertension, transmural pressure, and vascular smooth muscle response in rats. Circulation Res. 36:590-598.

Head, R. J., L. A. Cassis, R. L. Robinson, D. P. Westfall, and R. E. Stitzel. 1985. Altered catecholamine contents in vascular and nonvascular tissues in genetically hypertensive rats. Blood Vessels 22:196-204.

Hodges, R. D. 1974. The histology of fowl. Academic Press Inc., New York.

Holck, M. I., C. H. M. Jones, and G. Haeusler. 1983. Differential interactions of clonidine and methoxamine with the postsynaptic α-adrenoceptor of rabbit main pulmonary artery. J Cardiovascular Pharmacol. 5:240-248.

Hughes, A. F. W. 1943. The histogenesis of the arteries of the chick embryo. J. Anat. 77:266-287.

Innes, I. R. 1962. An action of 5-hydroxytryptamine on adrenaline receptors. Br. J. Pharmacol. 19:427-441.

Iversen, L. L. 1965. The uptake of catechol amines at high perfusion concentrations in the rat isolated heart: A novel catechol amine uptake process. Br. J. Pharmacol. 25:18-33.

Iversen, L. L. 1967. The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press, London.

Iversen, L. L. 1974. Uptake mechanisms for neurotransmitter amines. Biochem. Pharmacol. 23:1927-1935.

Iversen, L. L. 1975. Uptake processes for biogenic amines. Pages 381-442 in L. L. Iversen, S. D. Iversen, and S. H. Snyder, eds. Handbook of psychopharmacology. Vol. 3. Plenum Press, New York.

Jones, A. W. and R. G. Hart. 1975. Altered in transport in aortic smooth muscle during deoxycorticosterone acetate hypertension in the rat. Circ. Res. 37:333-341.

Jones, D. R. and K. Johansen. 1972. The blood vascular system of birds. Chapter 4. In D. S. Farner, J. R. King, and K. C. Parkes, eds. Avian biology. Volume II. Academic Press, New York.

Kalsner, S. 1982. Evidence against the unitary hypothesis of agonist and antagonist action at presynaptic adrenoceptors. Br. J. Pharmacol. 77:375-380.

Kalsner, S. 1983a. Evidence that transmitter release in sympathetic nerves is not set by feedback via presynaptic receptors. Can. J. Physiol. Pharmacol. 61:1197-1201.

Kalsner, S. 1983b. The effect of yohimbine on presynaptic and postsynaptic events during sympathetic nerve activation in cattle iris: A critique of presynaptic receptor theory. Br. J. Pharmacol. 78:247-253.

Kalsner, S. and M. Nickerson. 1968. A method for the study of mechanisms of drug disposition in smooth muscle. Can. J. Physiol. Pharmacol. 46:719-730.

Kalsner, S. and M. Quillan. 1984. A hypothesis to explain the presynaptic effects of adrenoceptor antagonists. Br. J. Pharmacol. 82:515-522.

Kao, C. Y. 1966. Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmacol. Rev. 18:997-1049.

Katzung, B. G. 1984. Basic & clinic pharmacology. 2nd ed. Lange Medical Publications, California.

Kawasaki, H., W. H. Cline, and S. Su. 1982. Enhanced angiotensin mediated facilitation of adrenergic neurotransmission in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 221:112-116.

Kenakin, T. P. 1980. On the importance of agonist concentration-gradients within isolated tissues. Increased maximal responses of rat vasa deferentia to (-)-noradrenaline after blockade of neuronal uptake. J. Pharm. Pharmacol. 32:833-838.

Kenakin, T. P. 1984. The classification of drugs and drug receptors in isolated tissues. Pharmacol. Rev. 36:165-222.

Kopin, I. J. 1972. Metabolic degradation of catecholamines. The relative importance of different pathways under physiological conditions and after administration of drugs. Chapter 8, pp. 270-282. In H. Blascho and E. Muscholl, eds. Handbook of experimental pharmacology. Vol. 33. Springer-Verlag, Berlin.

Krista, L. M., P. E. Waibel, R. N. Schoffner, and J. H. Sautter. 1970. A study of aortic rupture and performance as influenced by selection for hypertension and hypotension in the turkey. Poultry Sci. 49:405.

Lands, A. M., A. Arnold, J. P. McAuliff, F. P. Luduena, and T. G. Brown. 1967a. Differentiation of receptor systems activated by sympathomimetic amines. Nature, 214:597-598.

Lands, A. M., F. P. Luduena, and H. J. Buzzo. 1967b. Differentiation of receptors responsive to isoproterenol. Life Sci. 6:2241-2249.

Langer, S. Z. 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23: 1793-1800.

Langer, S. Z. 1980. Presynaptic regulation of the release of catecholamines. Pharmacol. Rev. 32:337-363.

Lake, C. R., M. G. Ziegler, M. D. Coleman, and S. J. Kopin. 1977. Age-adjusted plasma norepinephrine levels are similar in normotensive and hypertensive subjects. New Engl. J. Med. 296:208-209.

Lee, R. M. K. W. 1985. Vascular changes at prehypertensive phase in the mesenteric arteries from spontaneously hypertensive rats. Blood Vessels 22:105-126.

Leighton, H. J. 1982. Quantitative assessment of the pre- and postsynaptic alpha adrenoceptor antagonist potency of amitriptyline. J. Pharmacol. Exp. Ther. 220:299-304.

Levin, J. A. 1974. The uptake and metabolism of ^{3}H-L and ^{3}H-DL-norepinephrine by intact aorta and by isolated adventitia and media. J. Pharmacol. Exp. Ther. 190:210-226.

Macmillan, W. H. 1959. A hypothesis concerning the effect of cocaine on the action of sympathomimetic amines. Br. J. Pharmacol. 14:385-391.

Marley, E. and J. D. Stephenson. 1972. Central action of catecholamine. Chapter 12. In H. Blaschko and E. Muscholl, eds. Handbook of experimental pharmacology. Springer-Verlag, Berlin.

Maxwell, R. A., W. B. Wastila, and S. B. Eckhardt. 1966. Some factors determining the response of rabbit aortic strips to dl-norepinephrine-7-H^{3}-hydrocholride and the influence of cocaine, guanethidine and methylphenidate on these factors. J. Pharmacol. 151:253-261.

McLeod, W. M. 1964. Avian anatomy. Burgess Publishing Company, Minneapolis, Minnesota.

Miller, R. C., M. Mony, V. Schini, P. Schoeffter, and J. C. Stoclet. 1984. Endothelial mediated inhibition of contraction and increase in cyclic GMP levels evoked by the α-adrehoceptor agonist B-HT 920 in rat isolated aorta. Br. J. Pharmacol. 83:903-908.

Moulds, R. F. 1983. Techniques for testing isolated blood vessels. Gen. Pharmacol. 14:47-53.

Muscholl, E. 1980. Peripheral muscarinic control of norepinephrine release in the cardiovascular system. Am. J. Physiol. 239:H713-H720.

Nelson, W. L., J. E. Wennerstrom, D. C. Dyer, and M. Engel. 1977. Absolute configuration of glycerol derivatives. 4. Synthesis and pharmacological activity of chiral 2-alkylamino 2-alkylaminomethyl benzodioxans, competitive α-adrenergic antagonists. J. Med. Chem. 20 880-885.

Nishino, K., T. Irikura, and I. Takayanagi. 1970. Mode of action of 5-hydroxytryptamine on isolated rat vas deferens. Nature, Lond. 228:564-565.

Ohhashi, T. and T. Azuma. 1980. Paradoxical relaxation of arterial strips induced by vaso-constrictive agents. Blood Vessels 17:16-26.

Osa, T. and T. Ogasawara. 1984. Changes in the adrenergic effects and membrane activity of circular muscle of rat uterus during late pregnancy and postpartum. Jpn. J. Physiology 34:113-126.

Ott, L., D. Weiner, H. Cheng, and J. Woodward. 1981. Estimating pA_2 values for different designs. J. Pharmacol. Methods 5:75-92.

Owen, M. P., D. A. Nay, R. D. Bevan, and J. A. Bevan. 1983. Importance of 'cut end' effects in *in vitro* artery segments. Blood Vessels 20:306-312.

Paiva, M. Q. and S. Guimaraes. 1978. A comparative study of the uptake and metabolism of noradrenaline and adrenaline by isolated saphenous vein of the dog. Naunyn-Schmiedeberg's Archs. Pharmacol. 303:221-228.

Pang, S. C-N and T. M. Scott. 1985. An examination of the arterial media in transplanted arteries of spontaneously hypertensive (SHR) and normotensive Wistar Kyoto rats (WKY). Artery 12:382-387.

Paterson, G. 1965. The response to transmural stimulation of isolated arterial strips and its modification by drugs. J. Pharm. Pharmacol. 17:341-349.

Patil, P. N. 1968. Steric aspects of adrenergic drugs. VIII. optical isomers of beta adrenergic receptor antagonist. J. Pharmacol. Exp. Ther. 160:308-314.

Paton, D. M. 1976. The mechanism of neuronal and extraneuronal transport of catecholamines. Raven Press, New York.

Paton, W. D. M. 1954. The response of the guinea-pig ileum to electrical stimulation by coaxial electrodes. J. Physiol. 127:40-41.

Purdy, R. E., D. E. Hurlbut, and L. A. Rains. 1981. Receptors for 5-hydroxytryptamine in rabbit isolated ear artery and aorta. Blood Vessels 18:16-27.

Pure, E. and P. Needleman. 1979. Effect of endothelial damage on prostaglandin synthesis by isolated perfused rabbit mesenteric vaculature. J. Cardiovasc. Pharmacol. 1:299-309.

Rennick, B. R. and H. Gandia. 1954. Pharmacology of smooth muscle value in renal portal circulation. Proc. Soc. Exp. Biol. Med. 85:234-236.

Ringer, R. K. and K. Rood. 1959. Hemodynamic changes associated with aging in the broad-breasted Bronze turkey. Poultry Sci. 38:395.

Robinson, B. F. 1983. Differences in blood vessels between species: Relation to differences in blood vessels of varing type within the same species. Gen. Pharmacol. 14:43-46.

Robinson, M. C. 1970. Laboratory anatomy of the domestic chicken. Wm. C. Brown Company Publishers, Dubuque, Iowa.

Schild, H. O. 1947. A new scale for the measurement of drug antagonism. Br. J. Pharmacol. 2:189-206.

Schayer, R. W., R. L. Smiley, and J. Kennedy. 1953. Metabolism of epinephrine containing isotopic carbon (III). J. Biol. Chem. 202:425-430.

Schayer, R. W., R. L. Smiley, K. J. Davis, and Y. Kobayashi. 1955. Role of monoamine oxidase in noradrenaline metabolism. Am. J. Physiol. 182:285-286.

Schulz, J. C. and D. P. Westfall. 1982. A pharmacological analysis of the α-adrenoceptor antagonism by prazosin in arteries and veins. Blood Vessels 19:79-87.

Senaratne, M. and T. Kappagoda. 1984. Tetrodotoxin-resistant relaxation to transmural nerve stimulation in isolated saphenous vein. Am. J. Physiol. 247 (Heart Circ. Physiol. 16):H952-H959.

Simmonds, M. A. and C. N. Gillis. 1968. Uptake of normetanephrine and norepinephrine by cocaine treated rat heart. J. Pharmacol. Exp. Ther. 159:283-289.

Sneddon, P., D. P. Westfall, J. Colby, and J. S. Fedan. 1984. A pharmacological investigation of the biphasic nature of the contractile response of rabbit and rat vas deferens to field stimulation. Life Sciences 35:1903-1912.

Somlyo, A. P. and C. Woo. 1967. β-adrenergic auto-inhibitor of the effect of noradrenaline on avian pulmonary artery. J. Pharm. Pharmacol. 19:59-61.

Speckmann, E. W. and R. K. Ringer. 1963. The cardiac output and carotid and tibial blood pressure of the turkey. Can. J. Biochem. Physiol. 41:2337.

Starke, K. 1972. Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves: Evidence for an α-receptor-mediated feed-back inhibition of noradrenaline release. Naunyn-Schmiedebergs Arch. Pharmacol. 275:11-23.

Starke, K. 1981. Presynaptic receptors. A. Rev. Pharmacol. Toxicol.. 21:7-30.

Stevens, M. J., R. E. Rittinghausen, R. L. Medcalf, and R. F. W. Moulds. 1982. Prejunctional β-adrenoceptors in human digital arteries. Eur. J. Pharmacol. 83:263-270.

Sturkie, P. D. 1976. Avian physiology. 3rd ed. Springer-Verlag, New York.

Su, C. and J. A. Bevan. 1970. The release of H^3-norepinephrine in arterial strips studied by the technique of superfusion and transmural stimulation. J. Pharmacol. Exp. Ther. 172:62-68.

Trendelenburg, U. 1959. The supersensitivity caused by cocaine. J. Pharmacol. Exp. Ther. 125:55-65.

Trendelenburg, U. 1980. A kinetic analysis of the extraneuronal uptake and metabolism of catecholamines. Rev. Physiol. Biochem. Pharmacol. 87:33-115.

Trendelenburg, U., R. A. Maxwell, and S. Pluchino. 1970. The importance of intraneuronal uptake in the cat's nictitating membrane as assessed with methoxamine, a sympathomimetic amine which is not taken up by adrenergic nerves. J. Pharmacol. Exp. Ther. 172:91-99.

Ursillo, R. C. and J. Jacobson. 1965. Potentiation of norepinephrine in the isolated vas deferens of the rat by some CNS stimulants and antidepressants. J. Pharmacol. Exp. Ther. 148:247-251.

Vanhoutte, P. M. 1982. 5-Hydroxytryptamine, vasospasm, and hypertension. Pages 163-174 in F. De Clerck and P. M. Vanhoutte, eds. 5-Hydroxytryptamine in peripheral reactions. Raven Press, New York.

Vanhoutte, P. M. 1983. 5-Hydroxytryptamine and the vascular system—Hypertension Thematic Symposium. Fed. Proc. 42:211-237.

Van Nueten, J. M. 1983. 5-Hydroxytryptamine and Precapillary vessels. Fed. Proc. 42:223-227.

Webb, R. C. 1982. Angiotensin II-induced relaxation of vascular smooth muscle. Blood Vessels 19:165-176.

Webb, R. C. and P.M. Vanhoutte. 1985. Responsiveness to serotonergic stimuli of the hypertensive blood vessel wall. In P. M. Vanhoutte ed. Serotonin and the cardiovascular system. Raven Press, New York (in press).

Weil-Malherbe, H., L. G. Whitby, and J. Axelrod. 1961. The uptake of circulating ^{3}H-norepinephrine by the pituitary gland and various areas of the brain. J. Neurochem. 8:55-64.

Weiner, N. and P. Taylor. 1985. Neurohumoral transmission: The autonomic and somatic motor nervous system. Chapter 4, pp. 66-99. In A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad, eds. The pharmacological basis of therapeutics. Macmillan Publishing Company, New York.

Westfall, T. C., C. S. Xue, M. J. Meldrum, and L. Badino. 1985. Effects of angiotensin on ^{3}H-norepinephrine release in the rat portal vein. Blood Vessels 22:13-24.

Westfall, T. C., M. J. Meldrum, L. Badino, and J. Earnhardt. 1984. Noradrenergic transmission in the isolated portal vein of the spontaneously hypertensive rat. Hypertension 6:267-274.

Whiteman, C. E. and A. A. Bickford. 1983. Avian disease manual. American Association of Avian Pathologists. Poultry Pathology Laboratory, University of Pennsylvania, Kennett Square, Pennsylvania.

Wooten, G. F., N. B. Thoa, I J. Kopin, and J. Axelrod. 1973. Enhanced release of dopamine β-hydroxylase and norepinephrine from sympathetic nerves by dibutyryl cyclic adenosine-3', 5'-monophosphate and theophylline. Molec. Pharmacol. 9:178-183.

Yates, C. M. and C. N. Gillis. 1963. The response of rabbit vascular tissue to electrical and drug stimulation. J. Pharmacol. 140:52-59.

Zeller, E. A., J. Barsky, E. R. Berman, and J. R. Fouts. 1952. Action of isonicotinic acid hydrazide and related compounds on enzymes of brain and other tissues. J. Lab. Clin. Med. 40:965-966.

Ziegler, M. G., C. R. Lake, and S. J. Kopin. 1976. Plasma noradrenaline increases with age. Nature 261:333-335.

IX. ACKNOWLEDGMENTS

I appreciate very much my advisor Dr. Dyer, for his help and advice throughout this project. I would like to greatly thank the other members of my advisory committee, Drs. Ahrens and Ford for their being nice and helpful to this program.

I am very grateful to Dr. Koehler for his very useful statistical computer program and his supply of computer money, and I thank Mr. Thompson for his help with the computer plots. I owe a debt of thanks to many members in the Department of Statistics for their being pleasant and patient to my continual visits.

I am especially thankful to other people in my department: Mr. Bud Maakestad who made the tissue holders and electrodes and other equipment, Mr. Chris Minor who helped solve the electronic problems in my research, Kieran Sehe who helped with some laboratory work, and to fellow students Mr. Zhang, Dr. Ryu, Dr. Kunkle who helped in various ways in the completion of my thesis.

I also thank Dr. Hsu and Mr. Danny Brass for their being helpful to my work. I appreciate Mrs. Bishop for her being nice and patient to my continual questions.

Finally to my Mom, Dad, my sister, and my brothers. They offered me a chance to go abroad to study, and this is a very important step for my future career. In the same manner, I would like to express my great gratitude to the admission and financial support offered from my department, by Dr. Ahrens, and other faculty.

If I have a girl friend or so-called wife, I would appreciate very much her patient waiting. It seems that this chance has not shown up. I would like to thank Mrs. Dyer and the Dyer family, instead, for sharing Dr. Dyer.

X. APPENDIX A

Composition of modified Krebs-Henseleit solution:

	gram/liter
NaCl	6.74
KCl	0.35
$CaCl_2 \cdot 2H_2O$	0.20
$MgSO_4 \cdot 7H_2O$	0.14
KH_2PO_4	0.16
Na_2(EDTA)	0.01
$NaHCO_3$	1.86
Dextrose	1.42

Na_2(EDTA), disodium ethylenediamine tetraacetate, is used to chelate heavy metals which may promote the oxidation of catecholamines.

XI. APPENDIX B

Effects of neuronal blocking agents on the responses of other tissues to electrical stimulation (ES)[1]

animal	tissue	type of response[2]	TTX[3] (10^{-6}M)	Bret.[3] (1.6 $\mu g/_{ml}$)	Gua.[3] (10^{-5}M)	Pz.[3] (10^{-6}M)	effects
rat	aorta	2 phases	(3)[4]			(3)	blocked phase 2
	vas deferens	1 phase	(6)	(6)	(2)	(3)	TTX blocked, the other drugs partially blocked
guinea pig	vas deferens	1 phase	(3)	(2)	(2)		same as rat
rabbit	uterus	1 phase	(4)				mostly blocked
	pulmonary artery	2 phases	(2)		(2)		blocked phase 2 and most of phase 1
	aorta	2 phases	(3)			(3)	blocked phase 2

[1]The parameters used: 1 ms pulse, 8-10 v., 4-32 Hz for 20 seconds.

[2]The definition for phase 1 is the tension generated only during electrical current flow, and phase 2 the contractile response which occurs after the cessation of electrical current flow.

[3]TTX=tetrodotoxin, Bret.=Bretylium, Gua.=Guanethidine, Pz.=Prazosin.

[4]Number of experiments is given within the brackets.

XII. APPENDIX C

A. Calculation of ED50

Suppose (x_i,y_i) are those points in the linear portion of a NE dose response curve. The linear portion of the curve was picked up as described in the Methods section on statistical analysis. If y_i = percentage contraction and x_i = log(concentration) then the slope and the intercept of the regression line for the linear portion of the NE dose response curve can be calculated by Equations 2 and 3.

For the regression line y=mx+b, when y=50% then 0.5=mx+b and x= ED50 =0.5–b/m in logarithmic units. ED50 calculation is for NE dose response curve only.

B. Calculation of the Shift of ED50 in Table 2

Suppose ED1 = ED50 for round 1 in treated tissues,

ED2 = ED50 for round n (n=2,.3, or 4) in treated tissues,

ED3 = ED50 for round 1 in control tissues, and

ED4 = ED50 for round n (n=2,.3, or 4) in control tissues (See Figure 32).

For the Xth (x=1, 2, 3, ... X) experiment, the shift of the ED50, Sx, can be calculated as follows:

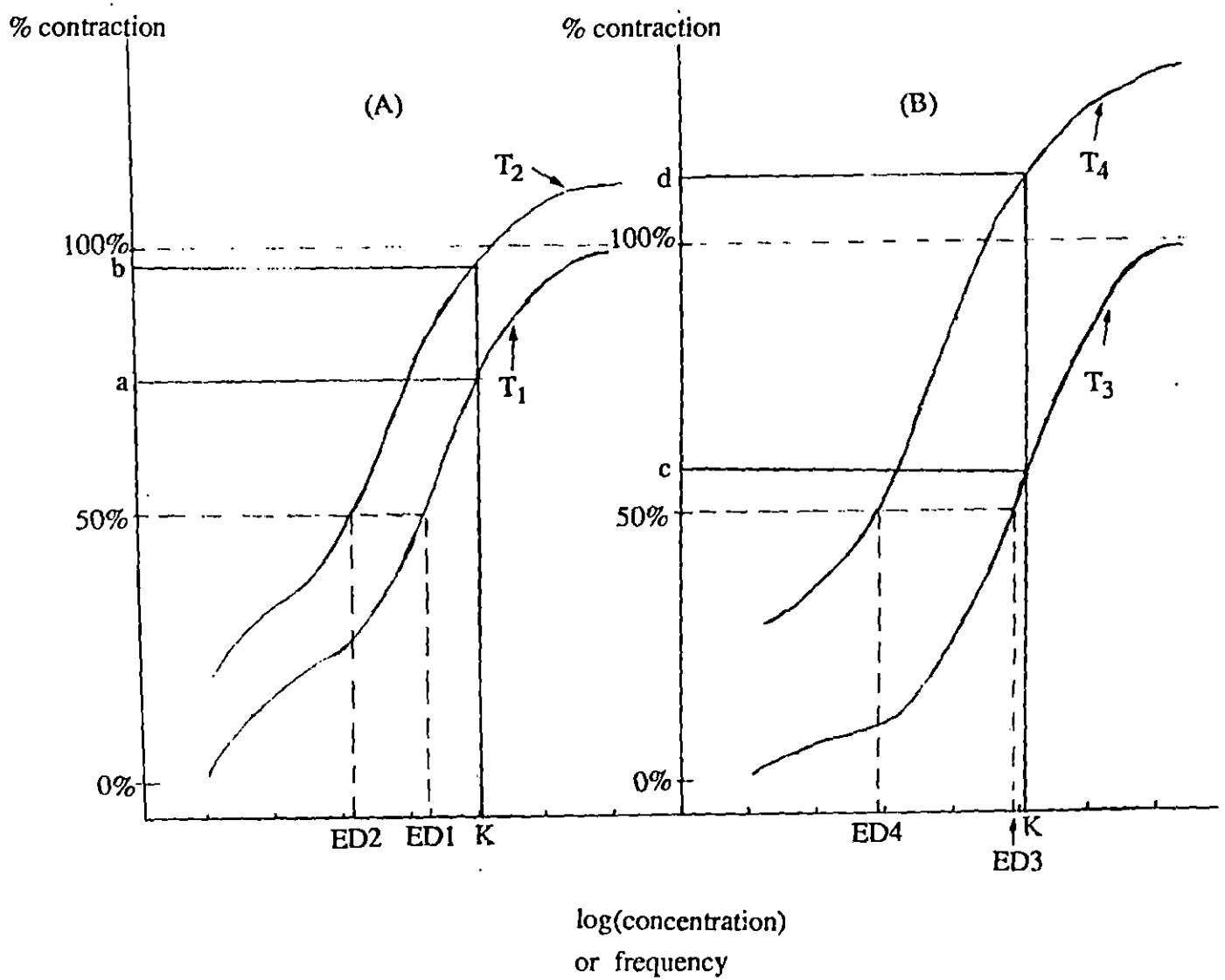
Sx = (ED2 - ED1) - (ED4 - ED3)

The mean of Sx (x - 1, 2, 3, ... x) from several different experiments was computed and presented in Table 2. Student t-values from these Sx's then were used to determine statistical significance.

Sx is the quantitative measurement of the shift in the ED50 in logarithmic units. Positive values of Sx mean the net effect of the treatment (drugs) (treated tissue compared to

Figure 32. Illustration of mathematical calculation. In this figure, the abscissa is in the units of log(concentration) of NE. One hundred percent contraction equals maximal contraction obtained during the first round for both control and treated tissues. If the abscissa is in frequency units, the one hundred percent contraction is the response to KCl 120mM

ED1	ED50 of round 1 in treated tissues.
ED2	ED50 of round n (n=2, 3, or 4) in treated tissues.
ED3	ED50 of round 1 in control tissues.
ED4	ED50 of round n (n=2, 3, or 4) in control tissues.
(A)	treated tissues.
(B)	control tissues.
a	% contraction for round 1 in treated tissues.
b	% contraction for round n (n=2, 3, or 4) in treated tissues.
c	% contraction for round 1 in control tissues.
d	% contraction for round n (n=2, 3, or 4) in control tissues.
K	a certain dose or frequency (e.g., 3×10^{-9} M, 4 hertz).
T_1	NE dose response curve for round 1 in the treated tissue.
T_2	NE dose response curve for round n (n=2, 3, or 4) in treated tissues.
T_3	NE dose response curve for round 1 in the control tissue.
T_4	NE dose response curve for round n (n=2, 3, or 4) in control tissues.


% contraction
(A)
T2
100%
b
a
T1
50%
0%
ED2
ED1
K
% contraction
(B)
T4
d
100%
T3
c
50%
0%
ED4
K
ED3
log(concentration)
or frequency

control tissues) is inhibitory (the dose response curve is shifted to the right). While negative values indicate enhancement of the response (dose response curve was shifted to the left). In other words, one must use 10^{Sx} times the concentration of NE to produce the same response (50% contraction) after administration of the treatment. These concentration ratios are shown in the right column of Table 2.

C. Type II Analysis

The calculation of distant difference (D) and ratio difference (R) was shown in the section of statistical analysis. D values are more appropriate especially when the percentage contraction is very little and/or the ratio (d/c) is very large. In this case, we assume that the paired tissues should increase or decrease the same amount of percentage contraction when time is changed. On the other hand, ratio difference is more appropriate when the ratio (d/c) is in the middle range and/or the percentage contraction is strong enough to be measured. In this case, we assume the paired tissue increases or decreases at the same rate of percentage contraction, when time is changed.