of noradrenaline has been proved to be dependent on the concentration of the perfused precursor[11]. The concentrations used in the present experiments ($0.5-1 \times 10^{-4} M$ tyrosine) were very close to tke levels that give a maximum rate of synthesis in the guinea-pig heart[11]. Despite the network of adrenergic nerve fibres in the atria being denser than in the ventricles[4], there was no corresponding difference in the formation rate of noradrenaline between those regions of the fetal heart.

A small part (less than 8%) of the radioactive catecholamine recovered in the heart after the perfusion was, in some hearts, identified as dopamine. In several species of laboratory animals, dopamine in the heart seems to be localized to 'small intensely fluorescent cells'[12]. Such cells have been found also in the human fetal heart from the 10th week of gestation in the atria and around the base of the heart[4]. These cells are not confined to the ganglia, and it is interesting that high doses of nicotine have been found to produce a propranolol-blockable excitation of human fetal atria at an early stage where field stimulation had no effect, suggesting a non-neuronal store of catecholamine[13].

The ability to form noradrenaline early in the development of the human fetal heart seems of biological significance by contributing to the integrity of the intrinsic catecholamine stores in the intact fetus. Furthermore, the capacity here demonstrated of the isolated fetal heart to form noradrenaline from tyrosine seems to be a factor of importance for the feasibility of experimental work on isolated hearts. Noradrenaline is known to be released locally by electrical stimulation[14] and by reduced oxygen tension[15], and part of the cardiac noradrenaline content is washed out by perfusion in amounts dependent on the perfusion rate[11]. Noradrenaline lost by degradation by MAO and COMT and/or washed out by the perfusion might be replaced in the in vitro heart preparation not only by the various uptake mechanisms[16] but also by the formation of new catecholamines from a dietary precursor. In fact, inhibitors of tyrosine hydroxylase, which turn off endogenous noradrenaline synthesis, decrease the levels of endogenous noradrenaline in adrenergically innervated tissue[17]. Both fetal and adult hearts in isolated preparations have been extensively used in physiological work. As the presence of noradrenaline contributes to the cardiac ability to withstand asphyxia[3], the intact capacity to form the neurotransmitter from available precursors might be essential for the heart to remain in a functional state during longtime studies of isolated hearts, especially under conditions that promote the release of endogenous noradrenaline.

Summary. The capacity of noradrenaline synthesis was investigated in 6 isolated human fetal hearts (13–23 gestational week). The mean rate of transforming perfused labelled tyrosine to noradrenaline in atrial, ventricular, and mediastinal tissue was 0.175, 0.168, and 0.108 μg/g tissue/h, respectively.

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Nonsympathetic Dilator Innervation of Cat Cerebral Arteries

In 1928 FORBES and WOLFF[1] reported that stimulation of the vagus nerves in the neck caused pial artery dilation in the cat. This observation was confirmed by COBB and FINESINGER[2] and CHOROBSKI and PENFIELD[3], who also provided anatomical and physiological evidence that cerebral blood vessels receive a dilator innervation. Subsequently, although interest has been focussed mainly on sympathetic vasoconstrictor innervation, based on the orientation of agranular vesicles in surviving nerve terminals in sympathetically denervated tissue[4–6] and positive histochemical staining for acetylcholinesterase[5, 7], several authors have suggested that cerebral blood vessels are richly innervated by cholinergic neurons.

Several in vivo studies support the idea that cholinergic nerves may be involved in cerebral vasodilation. This conclusion is based on observations that intravascular acetylcholine dilates pial arteries or increases blood flow[1, 8], that pial artery dilation follows the local application of carbachol[9, 10], and that autoregulatory cerebral vasodilation following a decrease in systemic arterial blood pressure was blocked by atropine[11]. Not all observations, however, support such a conclusion; neurogenic vasodilation of dog cerebral arteries following electrical stimulation of the trigeminal nerve and medulla was not atropine sensitive[12] and no significant change in cerebral blood flow resulted when the petrosal nerve was stimulated[13]. It is also well known from studies at other sites that an atropine-sensitive response to parasympathomimetic drugs is by no means an indication of cholinergic innervation.

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Because of these conflicting opinions derived from complex in vivo studies, the characteristics of neurogenic vasodilation in cat cerebral arteries were examined in vitro where many of the factors difficult to regulate in vivo can be precisely controlled.

Methods. 10 adult cats (2.2–3.1 kg) of either sex were anesthetized with pentobarbital (50 mg/kg i.p.) and exsanguinated. Ring segments (4 mm long) of intracranial arteries with an outer diameter of approximately 0.4 mm were removed for isometric recording in Kreb's bicarbonate solution equilibrated with 95% O_2 and 5% CO_2 at 37 °C as previously described[14]. Transmural nerve stimulation was carried out using trains of 100 biphasic square wave pulses, 0.3 msec duration, delivered at supramaximal voltage with varying frequencies. Adjacent arterial segments were processed for catecholamine fluorescence[15]. 3 cats were reserpinized (Serpasil, 3 mg/kg i.p. per day) for 2 days and sacrificed 24 h later. Bilateral superior cervical ganglionectomy was accomplished on 3 cats. Some arterial segments from control, reserpine-treated and sympathetically denervated animals were stored in Krebs' bicarbonate solution at 4 °C for 7 days to achieve cold storage denervation[16]. The disappearance of catecholamine fluorescence was taken as an indication of complete adrenergic denervation.

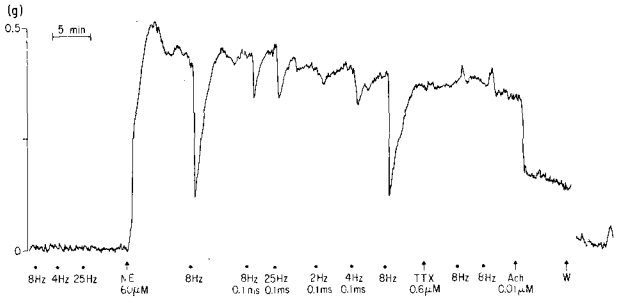

Fig. 1. Responses of cat cerebral (posterior communicating) artery ring to transmural nerve stimulation (TNS) and acetylcholine (Ach). In presence of active muscle tone produced by 1-norepinephrine (NE), TNS and Ach elicited a dilation; TNS-induced response was abolished by tetrodotoxin (TTX). TNS: 0.3 msec. 100 pulses.

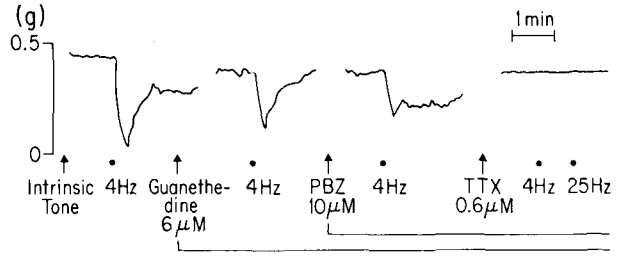

Fig. 2. Effect of adrenergic blocking agents on the dilator responses of cerebral (basilar) artery to transmural nerve stimulation (TNS). Neither guanethidine (6 μM) nor phenoxybenzamine (PBZ, 10 μM) blocked the response.

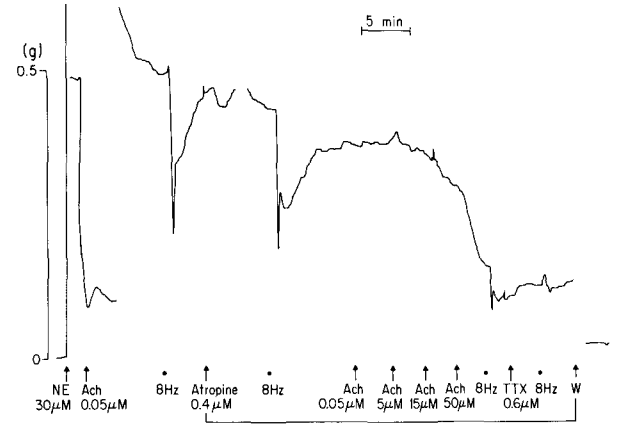

Fig. 3. Effect of atropine on the dilator responses of cerebral (basilar) artery ring. Atropine did not affect the response to transmural nerve stimulation (TNS) but blocked the response to acetylcholine (Ach) up to 5 μM.

The following drugs were employed: l-norepinephrine bitartrate (Sigma), dopamine hydrochloride (Calbiochem), tetrodotoxin (Sankyo-Tokyo), reserpine (Ciba), guanethidine sulfate (Ciba), bretylium tosylate (Burroughs Wellcome), phenoxybenzamine hydrochloride (Smith, Kline and French), phentolamine methane sulfonate (Ciba), propranolol hydrochloride (Ayerst), acetylcholine chloride (Calbiochem), atropine sulfate (Merck), physostigmine salicylate (Merck), serotonin creatinine sulfate (Calbiochem).

Results and discussion. All the cerebral arteries examined, including the basilar, anterior, middle and posterior cerebral, and posterior communicating arteries, dilated in response to transmural nerve stimulation (TNS) with a frequency of 2–32 Hz (Figure 1). This response can only be observed in vessels exhibiting active muscle tone. 13 out of 43 preparations developed spontaneous muscle tone 30 min after they were mounted in the tissue bath; the others gave no response to TNS between 2–32 Hz. However, when tone was induced by norepinephrine (30–60 μM), dopamine (30 μM), or serotonin (0.7 μM), these arteries dilated in response to TNS. The dilator responses to TNS were frequency dependent (Figure 1) reaching a maximum at 8 Hz. At greater frequencies (8–32 Hz), TNS elicited a quick dilation followed by a rapid recovery while at lower frequencies (2–4 Hz), a slower time course of dilation and recovery was observed. TNS-induced relaxations were abolished by tetrodotoxin (0.6 μM) and prevented by previous cold storage. The dilator response was seen when pulses as short as 0.1 msec were employed. These features indicate that the vasodilator responses were of neurogenic origin.

All whole-mount preparations of cat cerebral arteries exhibited catecholamine fluorescence which disappeared after reserpinization or bilateral superior cervical ganglionectomy. These two treatments, however, neither prevented the dilator response nor influenced the frequency-response relationship. This indicated that the vasodilator responses are not dependent upon adrenergic neurons nor are they included in the sympathetic outflow through the superior cervical ganglia. This conclusion was further supported by the finding that neurogenic vasodilation was not affected by guanethidine (6 μM), bretylium (7 μM), phenoxybenzamine (10 μM), phentolamine (10 μM) and propranolol (1 μM), (Figure 2).

Acetylcholine (0.01–10 μM) added to cerebral artery segments with active muscle tone caused dilation and 10 μM, in the absence of muscle tone, constriction. Clearly active muscle tone is crucial for the demonstration of the dilator action of acetylcholine and may explain why

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other authors only demonstrated vasoconstriction to acetylcholine in cat cerebral arteries in vitro[17]. The vasodilation caused by acetylcholine (0.01–10 μM) was abolished by atropine (0.4 μM), a muscarinic receptor blocking agent. However, neurogenic vasodilation to TNS was unaffected by this concentration of atropine (Figure 3). The maximum dilation to TNS (25 Hz) was matched by acetylcholine at 0.01–0.05 μM.

Preliminary electronmicroscopic observations (LEE, SU and BEVAN, unpublished data) do not support the idea that the neuromuscular gap in cat cerebral arteries is too narrow to permit the entry of atropine to block the action of the transmitter, as has been proposed for the rat vas deferens[18]. There may, however, be atropine-resistant cholinergic receptors, such as have been described in bladder smooth muscle[19]. Neurogenic vasodilation (8 Hz), however, was not potentiated by physostigmine (7 μM) suggesting that neither the muscarinic nor the nicotinic effect of acetylcholine was involved.

It is of interest that in the presence of muscle tone, cat cerebral arteries invariably relaxed to nerve stimulation. Rabbit cerebral arteries, on the other hand, showed predominantly a contraction upon stimulation of the intramural nerves. Only occasionally was a small relaxation clearly apparent after adrenergic blockade (LEE, SU and BEVAN 1975)[20]. In the dog basilar artery neither contraction nor relaxation has been demonstrated[21], even though relaxation to potassium ions does occur[22]. These observations suggest that significant species differences occur not only in types of innervation but in their relative importance in one particular species.

Summary. The results presented provide strong support for the presence of vasodilator innervation in the cat cerebral arteries. The dilator innervation is neither adrenergic nor cholinergic and does not originate in the superior cervical ganglia. The nature of the vasodilator transmitter is unidentified. Such innervation, however, may be involved in the regulation of cerebral blood flow, especially in view of the capability of some cat cerebral vessels to develop intrinsic muscle tone.

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Altered Arterial Connective Tissue in Racing Greyhound Dogs

The greyhound breed of dog has been shown to have hemodynamic characteristics different from those of mongrel dogs[1–3]. The greyhound is under evaluation in our laboratories as a possible animal model for hypertension, since it has hemodynamic characteristics resembling those described[4] for human essential hypertension, i.e., high cardiac index (CI), low total peripheral resistance (TPR) in young greyhounds, changing to low CI, high TPR in older greyhounds[3].

In recent years vascular wall components have received attention as to their role in vascular disease from two aspects: 1. the role of wall changes in contributing to the pathological state, and 2. the response of wall components to mechanical and chemical stress of the disease state[5–9], such as increased pressure in hypertension. These two aspects are very difficult to separate.

We have been particularly concerned with collagen and elastin changes in vascular wall in pathological states. We have shown previously that renal hypertensive dog vessels tend to have a lower collagen to elastin ratio, a finding that would imply increased distensibility of the vessel[10]. We have also shown that human coronary arteries which are calcified exhibit a lower collagen to elastin ratio, indicating a response of the vessel to pathological change[11].

Because of the increased blood pressure and increased cardiac index of these greyhound dogs we thought that they would be a useful animal model for documenting changes in wall components in relation to hemodynamic mechanical stress. This report, accordingly, presents the findings as to vascular connective tissue in greyhound dogs as compared to that in normal mongrel dogs, previously reported[12].

Methods. 9 healthy greyhound dogs, obtained from racing kennels, were anesthetized with pentobarbital, 30 mg/kg, and studied for hemodynamic characteristics[1–3]. At the end of the hemodynamic studies specimens of the following arteries were quickly dissected: carotid, coronary, ascending aorta, abdominal aorta, renal proximal mesenteric, distal mesenteric, small mesenteric branches, and femoral. The vessels were dried and defatted as previously described[12]. Collagen and elastin were separated by the method of NEUMAN and LOGAN[13] and hydrolyzed overnight in 6 *N* HCl. Hydroxyproline was deter mined and collagen and elastin calculated as previously described[12].

Results. The composition of the vessels is given in the Table: % collagen, % elastin, and collagen/elastin ratio (C/E). It can be seen that in 3 vessels, the carotid artery, abdominal aorta, and femoral artery, the percent collagen

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