THE EFFECT OF NORADRENALINE ON THE SPINAL CORD CIRCULATION AND ITS POSSIBLE IMPLICATIONS IN THE PATHOGENESIS OF ACUTE SPINAL TRAUMA

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DECLARATION

I declare that this is my own original work and has not, to my knowledge, appeared in thesis form before.

SUMMARY

Acute blunt injury to the spinal cord is an important cause of disability in both man and the domestic animals. The reasons for the formation and spread of the haemorrhagic and necrotic lesion seen initially in the central grey matter of the cord following injury are poorly understood. Evidence has been presented implicating the neurotransmitter substance noradrenaline (NA) as a causative factor in the pathogenesis of the lesion. This thesis contains the results of investigations into the effects of NA on certain aspects of the spinal cord circulation and the histological appearance of the cord.

The effect of intra-arterially administered NA on spinal cord blood flow (SCBF) before and after blood-cord barrier diruption was studied in dogs. Barrier disruption was accomplished with an intra-arterial bolus injection of 2.5M urea. Multiple ligations of branches of the posterior aorta and cannulae placements ensured that the urea was directed to the lumbar and sacral segments of the cord. SCBF was measured by the hydrogen clearance method. Intra-arterial urea by itself had no significant effect on SCBF. The intra-arterial infusion of NA (12 µg/min. & 30 µg/min.) was without overall effect on SCBF. However, if the blood-cord barrier had previously been disrupted with hypertonic urea both concentrations of NA resulted in large reductions in SCBF, the larger dose causing the greater reduction. No such reductions in SCBF were seen with blood-cord barrier disruption and NA if the animals had been pretreated with the α -adrenergic blocker, phenoxybenzamine (1.5 mg/kg.). The evidence thus suggests that NA is capable of causing profound flow reductions in the normal spinal cord and that these reductions are mediated via α-adrenergic mechanisms. With the techniques utilised in these experiments it was not possible to determine whether the α -mediated reductions in SCBF were the result principally of a direct vascular action of NA or due to α-mediated/

α - mediated changes in the metabolic demands of spinal cord tissue.

The effect of NA upon the calibre of spinal cord pial vessels was also tested. Small volumes of various concentrations of NA, dissolved in mock cerebrospinal fluid (CSF) were injected via glass micropipettes into the subarachnoid space adjacent to the vessels under test. Spinal pial arteriolar diameter was measured by a television image-splitting technique. The application of mock CSF alone rsulted in a net vasodilatation of $8.4\pm 6.5\%$ ($\bar{X}\pm SD$). After the effect of CSF had been subtracted from the overall response to NA and CSF, a dose-dependent constriction to NA was seen. A maximum constrictor response of $28.8\pm 5.1\%$ ($\bar{X}\pm SD$) occurred in response to an NA concentration of 5×10^{-3}M (the highest concentration used in the study), but the tendency was for the response to reach a plateau at concentrations greater than 5×10^{-5}M. The inclusion of the α - adrenergic blocker, phentolamine, in the injectate in an equimolar dose, prevented the constrictor response of 5×10^{-5}M, NA and only partially prevented the response to a more concentrated solution of NA. Using the technique of perivascular application of test substance, the blood-cord barrier and possible metabolic effects of the drug can be avoided. Thus the results suggest that the NA mediated decreases in SCBF and vascular calibre are due, at least in part, to a direct vasoconstrictor action of NA mediated via vascular α- adrenergic receptors.

Parts IV & V of this thesis examine the effects of exogenously administered NA upon the histological appearance of the spinal cord. NA was administered in two ways-

1) by direct intraspinal injection.
2) by intra-arterial infusion after disruption of the blood-cord barrier with urea.

The purpose of these investigations was to test if NA could cause lesions of haemorrhagic necrosis as reported by other workers. There was no evidence of haemorrhagic necrosis in cords receiving intraspinal /

intraspinal injections of NA concentrations ranging from 5--35 μg NA dissolved in 20 μl of mock CSF. Likewise, in animals which had been pretreated with the NA uptake blocker desipramine, no haemorrhagic necrosis was seen in response to intraspinal injections of 35 μg NA. The main pathological change seen in these animals (including those injected with CSF alone) was oedema, a feature attributed to the effects of the injectate volume and the time over which the volume was administered.

Similarly, no histological changes were seen in the spinal cords of cats following either intra-arterial administration of 3.0 or 3.5M urea, or NA after blood-cord barrier disruption.

The importance of NA in the pathogenesis of acute spinal trauma is discussed in Part VI in the context of the present studies and those of other workers. It is suggested that while NA, in the doses used in the present study, is capable of producing flow reductions, it is not by itself accountable for the progressive reductions of flow and haemorrhagic necrosis seen in acute spinal injury.

GENERAL INTRODUCTION

Damage to the spinal cord commonly result in loss of motor and sensory function below the level of the lesion, a state which is often irreversible. Traumatic injury to the spinal cord with or without fracture and/or dislocation is an important cause of disability in man and the domestic animals. An improved understanding of this condition would clearly be of great value.

Acute blunt cord injury is characterised pathologically by a progressive haemorrhagic necrosis of central grey matter which may proceed in a longitudinal and radial manner involving both the grey and white matter, resulting in a total functional transection. The reasons for the formation and progressive nature of the lesion are poorly understood. If the pathogenesis of this central lesion can be determined, then rational treatment can be instigated to arrest the progression. By sparing of the white matter the chances of a functional recovery would be greatly increased.

In 1972, evidence was presented which implicated the neurotransmitter substance noradrenaline (or norepinephrine) as a causative factor in the pathogenesis of the central lesion. It was postulated to be elaborated and released from adrenergic neurones in excessive quantities following trauma, leading to toxic vasospasm, hypoxia and subsequent tissue necrosis and haemorrhage (Osterholm 1972).

The aim of this study was to investigate certain aspects of the effect of this amine on the spinal cord.

Parts II and V deal with the effects of noradrenaline on spinal cord blood flow and the histological appearance of the spinal cord. It should be noted that this latter study is not intended to be an exhaustive pathological study. Rather, it briefly looks at whether the administered noradrenaline produces pathological effects similar to those seen in trauma.

Part III/

Part III investigates the effect of noradrenaline on the diameter of spinal pial arteries and arterioles and Part IV is a study of the possible lesion producing capability of noradrenaline in the spinal cord. The results of the studies are discussed in Part VI in the context of available knowledge of the biochemical theory of haemorrhagic necrosis.

P A R T I

LITERATURE REVIEW

The Biosynthesis and Metabolism of Noradrenaline

A knowledge of the formation and degradation of a naturally occurring substance within a particular tissue is necessary when examining the effects of the substance on that tissue. This review presents aspects of these phenomena as they apply to noradrenaline (NA) in the central nervous system.

The following aspects have been considered:-

1) The biosynthesis of NA, including a description of the enzymes and co-factors involved.
2) The regulation of NA biosynthesis.
3) Metabolic degradation of NA.
4) NA uptake mechanisms.

Biosynthesis of Noradrenaline

A pathway for the stepwise biosynthesis of the catecholamines was formulated independently by Blaschko (1939) and Holtz (1939) following the discovery of the enzyme L-dopa decarboxylase in 1938. Blaschko (1957) has reviewed the early work by which the biosynthetic pathway was established. In that year (1957), L-dopa decarboxylase was the only enzyme in the process to have been completely characterised. Since then the biosynthetic scheme has been fully confirmed and elucidated by the description of the enzymes and co-factors responsible for the individual steps that lead from L-tyrosine to adrenaline. (Nagatsu, Levitt & Udenfriend 1964, Udenfriend 1966, Iversen 1967, Molinoff & Axelrod 1971)

Fig. 1 details the biosynthetic pathway of the catecholamines. Essentially this involves the sequential hydroxylation and decarboxylation of the amino acid tyrosine to noradrenaline (NA) with further methylation to adrenaline in chromoffin tissues.

The hydroxylation of the essential amino acid phenylalanine to L-tyrosine by the enzyme phenylalanine hydroxylase in the extracellular fluid, is presented in Fig.1 as the first step in the process. However, although NA can be synthesised from phenylalanine *in vivo* (Gurin & Delluva 1947), this pathway is probably insignificant since L-tyrosine, from dietary sources is abundant/

abundant in animal tissues. The hydroxylation of L-tyrosine to dihydroxy-phenylalanine or L-dopa by the action of the enzyme tyrosine hydroxylase (Nagatsu et al 1964, Udenfriend 1966) takes place within the neurone, the amine being transported across the neuronal membrane by an energy dependent concentrating mechanism. The decarboxylation of L-dopa to dopamine is catalysed by a relatively non-specific aromatic L-amino acid decarboxylase (Lovenberg, Weissbach & Udenfriend, 1962). The enzyme appears to be localised within the neuronal cytoplasm, although Udenfriend (1966) indicated that some of the dopa decarboxylase in the brain was present in the particulate fraction. However, Fahn, Rodman & Coté (1969) and Stjarne & Lishajko (1967) suggested that the particulate fraction of this enzyme may actually be present within synaptosomes in a soluble form.

The copper containing enzyme dopamine-β-hydroxylase catalyses the hydroxylation of dopamine to NA. (Kaufman, 1966, Kolinoff & Axelrod 1971) Dopamine B-hydroxylase appears to be exclusively localised in the storage vesicles of adrenergic nerve organelles. (Kirshner 1959, Stjarne 1966, Stjarne & Lishajko 1967) Approximately 80% of the enzyme present in the organelles is in an insoluble form. (Viveros, Arqueros, Connett & Kirshner 1969, Blaschko 1973) The insoluble form appears to be a major constituent of the membrane proteins. The hydroxylase is present as the fraction known as "chromomembrin A" (Hörtnagl, Winkler & Lochs 1972).

The last step in Fig. 1 shows the methylation of NA to adrenaline by the enzyme phenylethanolamine N-methyltransferase. This enzyme is specifically located in the cells able to form adrenaline such as the chromoffin cells of the adrenal medulla. In mammals, the enzyme is absent from tissues with noradrenergic innervation (Blaschko 1973).

Enzymes & Co-factors involved in the Biosynthesis of Noradrenaline

1. Phenylalanine hydroxylase

This enzyme, catalysing the conversion of phenylalanine to tyrosine/

tyrosine requires oxygen and unconjugated pteridines as co-factors (Goldstein, Gang, Anagoste & Nakajima 1967). Since tyrosine is present from dietary sources the importance of this reaction in catecholamine biosynthesis in the mammal is doubtful.

2. Tyrosine hydroxylase

This enzyme, which catalyses tyrosine to dopa is present in the adrenal medulla (Nagatsu, Levitt & Udenfriend 1964) adrenergic neurones (Levitt, Spector, Sjoerdsma & Udenfriend 1965) and in brain (Iyer, McGeer & McGeer 1963, McGeer, Nagchi & McGeer 1965, Kindwall & Weiner 1966). It has been noted that chronic denervation of adrenergically innervated tissue is associated with complete disappearance of the enzyme, (Potter, Cooper, Willmen & Wolfe 1965) suggesting that the enzyme is totally localised within the adrenergic nerve. The exact intraneuronal distribution is, however, in doubt. Tyrosine hydroxylase appeared largely in the supernatant fraction of homogenates of sympathetic ganglia and bovine splenic nerves (Kueller, Thoenen & Axelrod 1969, Stjarne 1966). Iyer, McGeer & McGeer (1963) and McGeer, Nagchi and McGeer (1965) had previously reported that a considerable fraction of the enzyme in the brain was associated with particles, but Weiner, Cloutier, Bjur, Pfeffer (1972) suggested that much of the particle associated enzyme of brain tissue may be present in soluble form in that portion of the axoplasm trapped within synaptosomes. There have been reports that two types of tyrosine hydroxylase are present in different regions of the neurone, with different kinetic properties (Ikeda, Fahien & Udenfriend 1966, Joh, Kapit & Goldstein 1969).

Tyrosine hydroxylase requires oxygen and a reduced pteridine co-factor for activity (Brenneman & Kaufman 1964, Nagatsu, Levitt & Udenfriend 1964). This co-factor has only recently been identified as 6-methyltetrahydropterin (Lloyd, Mori & Kaufman 1971). Iron also appears to be necessary. (Nagatsu, Levitt & Udenfriend, 1964) The reduced pteridine combines with the inactive oxidised form of tyrosine hydroxylase converting the enzyme to its active reduced form. In turn the pteridine is oxidised to the dihydro form./

form. The latter must be reduced by pteridine reductase to its original tetrahydro form before it is active again.

Many substances are known to inhibit tyrosine hydroxylase, for example, the halogenated compounds such as 3-iodo tyrosine, various chelating agents and derivatives of phenylalamine including the catecholamines themselves. Indeed inhibition of the activity of tyrosine hydroxylase by the catecholamines is regarded as the rate-limiting step in the biosynthetic pathway of the catecholamines. This concept will be examined in greater detail in the relevant section of this review.

3. Dopa decarboxylase

The decarboxylation of dopa to dopamine is catalysed by an aromatic L-amino acid decarboxylase. This enzyme is not as substrate specific as tyrosine hydroxylase, catalysing the decarboxylation steps in the synthesis of other L-aromatic amino acids, such as tyramine and histidine as well as L-5-hydroxytryptophan (Lovenberg, Weissbach & Udenfriend 1962, Iversen 1967, Jonason 1969) It also acts on other compounds such as m-tyrosine, α-methyl-dopa and α-methyl-m-tyrosine (Molinoff & Axelrod 1971). The enzyme is widely distributed in mammalian tissues including the peripheral and central nervous systems (Jonason 1969). Dopa decarboxylase requires pyridoxal phosphate for activity. Molinoff & Axelrod (1971) reported that high concentrations of this co-factor do not retard the activity of the enzyme as was the case with the pteridine co-factor of tyrosine hydroxylase. The enzyme appears to be localised within the cytoplasm of the neurone, though there is some evidence that a fraction of the enzyme may be particle associated. This matter has been discussed earlier.

4. Dopamine-β - hydroxylase

This enzyme catalyses the conversion of dopamine to NA. It is a mixed function oxidase, like phenylalanine hydroxylase and tyrosine hydroxylase, which contains copper and requires oxygen and a reducing agent, without which, it does not display any catalytic activity. The electron donor for this enzyme is ascorbic acid/

acid (Kaufman & Friedman 1965). Moreover, it has been noted that fumarate or acetate, catalase and ATP are required to obtain full activity of the enzyme in vitro (Molinoff & Axelrod, 1971). It has been suggested that the fumarate or acetate may facilitate the formation of the reduced enzyme-oxygen complex and/or accelerate the re-oxidation of the enzyme Cu^{+} to Cu^{++} (Goldstein, Soh & Garney, 1968); catalase, by destroying the peroxides which are formed by auto-oxidation of ascorbate or dopamine, and the ATP function by protecting the enzyme from inactivation (Molinoff & Axelrod 1971).

Dopamine β-hydroxylase has a relatively wide substrate specificity such that it reacts with numerous sympathomimetic amines. (Goldstein 1966). Numerous compounds, most of which act by copper chelation, are known to inhibit the enzyme. (Molinoff & Axelrod 1971).

5. Phenylethanolamine - N - Methyltransferase

This enzyme, which catalyses NA to adrenaline (Iversen 1967, Jonason 1969) is mainly present in the chromaffin tissue of the adrenal medulla but small amounts have been found in the heart and recently, by immuno-histofluorescent techniques, in the brain (Goldstein, Lew, Miyamoto, Battista, Ehstein, Hokfelt & Fuxe, 1974). Methionine and S-adenylmethionine are required as methyl donors for enzyme activation (Axelrod 1966, Molinoff & Axelrod 1971). It is absent from tissues with noradrenergic innervation (Blaschko 1973).

Regulation of Noradrenaline Biosynthesis

NA synthesis was studied in the brains and hearts of guinea pigs treated with a nomoamine oxidase inhibitor (Spector, Gordon, Sjoerdsma & Udenfriend 1967). The amine accummulated in these tissues to a concentration 2 or 3 times that of the untreated animals. In the former, the conversion of radioactive tyrosine to NA was reduced but the formation of NA from radioactive dopa was either not affected or increased. These observations indicated that, in tissues with high NA content, the pathway of NA formation was inhibited at the level catalysed by tyrosine hydroxylase.

The/

The catalysis of tyrosine to L-dopa by tyrosine hydroxylase is regarded as the "rate limiting step" in the synthesis of the catecholamines. The mechanism by which this inhibition occurs is a matter of dispute. Ikeda, Fahien & Udenfriend (1966) suggested that the inhibition represented competition between the catecholamine and the pteridine co-factor for the oxidised form of the enzyme. Udenfriend, Zaltzman-Nirenberg & Nagatsu (1965) demonstrated a 50% inhibition of tyrosine hydroxylase activity in vitro with 1 x 10^{-3}M NA when a similar concentration of synthetic pteridine co-factor was added to the medium. It is likely that much smaller amounts of catecholamine in the axoplasm may severely inhibit the activity of the enzyme since the concentration of co-factor within the neurone must certainly be considerably less than 1 x 10^{-3}M.

Availability of the pteridine co-factor, its rate of formation and rate of conversion of the reduced to oxidised form may also be determinants of the control of tyrosine hydroxylase activity.

The above regulatory mechanism describes changes in hydroxylase activity in response to catecholamine concentration without changes in enzyme levels (ie. "classical" feedback inhibition). This is regarded as the mode of control in the short term. It is thought that changes in the rate of formation of new enzyme are responsible for long term regulation of catecholamine level. (Pletscher 1972). An increase in neuronal activity causes an elevation of tyrosine hydroxylase in the central nervous system and of tyrosine hydroxylase and dopamine β-hydroxylase in sympathetic ganglia. This increase is suppressed by inhibitors of protein synthesis (Pletscher 1972). Increases in activity are not restricted to tyrosine hydroxylase. Other enzymes involved in catecholamine biosynthesis are known to be increased. This has been reviewed by Cotten (1972).

The formation of NA is not only dependent upon the amounts and activities of the biosynthetic enzymes. The uptake kinetics of catecholamine into the storage vesicles, the rate of release of catecholamine from the vesicles, enzymic degradation of intra-neuronal/

neuronal NA and dopamine and neuroual release and re-uptake, all play a part in the ultimate regulation of the biosynthetic process. (Weiner 1970).

Metabolic Degradation of Noradrenaline

There are two pathways for catecholamine catabolism in the central nervous system, namely oxidative deamination mediated by the enzyme monoamine oxidase (MAO) and O-methylation mediated by catechol-o-methyltransferase (COMT). This has been confirmed by several workers (Glowinski, Kopin & Axelrod 1965, Mannarino, Kirshner & Nashold 1963, Matsuoka 1964).

MAO activity was reduced by 30% in the rat salivary gland (Axelrod & Kopin 1969) and by 50% in the rabbit iris (Wattman & Sears 1964), following sympathetic denervation of these structures. COMT activity was not significantly affected. It was, therefore, concluded that MAO was localised, both extra and intra neuronally, while COMT was localised almost exclusively extra-neuronally in both these peripheral tissues and in brain (Axelrod & Kopin 1969, Iversen 1967, Jonason 1969, Bertler, Falck, Owman & Rosengren 1966). Molinoff & Axelrod (1971) reported that COMT was present within the sympathetic neurones of the third eyelid and the vas deferens and Iversen & Jarrott (1970) demonstrated the presence of COMT in the adrenergic neurone and that the affinity of NA to the intraneuronal enzyme was less than to the extraneuronal enzyme.

Within the cell MAO is localised exclusively in the mitochondria mainly at synaptic nerve endings (Rodriguez De Lores Arnaiz and De Robertis 1962) It is also present in brain capillaries where it forms part of the barrier to amines (qv).

The existence of at least two forms of MAO in the brain, designated A and B, has been inferred from *in vitro* and *in vivo* experiments (Johnston 1968, Hall & Logan 1969). The clorgyline sensitive Type A is probably the more important for deamination of NA.

Broch & Fonnum (1972) have shown that the highest concentrations of COMT in the rat brain are found in the hypo-thalamus,/

thalamus, corpora quadrigemina and cerebellum with the lowest concentrations occurring in the hippocampus, corpus striatum and white matter of the corpus callosum and lateral walls of the lateral ventricles. With the exception of the hypothalamus, the enzyme distribution does not correspond to that of the catecholamines. Although COMT is important in the extraneuronal metabolism of NA in the peripheral sympathetic system (Kopin & Gordon 1963) its importance in the central nervous system is much less certain. Glowinski, Kopin & Axelrod (1965) found that intraventricularly injected ^{3}H - NA which was metabolised to normetanephrine and deaminated also formed o-methyl metabolites. In a later study Glowinski, Iversen & Axelrod (1966) found that the ratio of radioactive normetanephrine to ^{3}H - NA was higher after the administration of exogenous NA than after formation of ^{3}H - NA intraneuronally from labelled precursors. These findings indicate that COMT is involved in NA catabolism in the brain and that extraneuronal free NA is metabolised by COMT more readily than intraneuronal catecholamine.

As will be described, the termination of action of the monoamine neurotransmitters is brought about, not by enzymatic degradation but by an avid re-uptake of the amine into the neurone. When one considers this process, the extraneuronal catabolism of NA in the central nervous system becomes much less important in comparison to the catabolic role of intraneuronal MAO.

The metabolic products formed by the actions of MAO and COMT are presented in Fig. 2. NA is deaminated by MAO to 3,4 - dihydroxyphenylglycol (DOPEG) by aldehyde reductase or oxidised to 3,4 - dihydroxymandelic acid (DOMA) by aldehyde dehydrogenase (Leeper, Weissbach & Udenfriend 1958). The pathway to DOMA is thought to be more important in man (de la Torre 1972).

Further methylation of these deaminated products by COMT results in the formation of 3-methoxy - 4 - hydroxyphenylglycol (MOPEG) from DOPEG and 3 - methoxy - 4 - hydroxymandelic acid (VMA) from DOMA (Iversen 1967, Jonason 1969, Molinoff & Axelrod, 1971)./

1971). Normetanephrine (NMN), formed by the o-methylation of NA released from the nerve ending into the extracellular space can be deaminated by extraneuronal MAO to 3 - methoxy - 4 - hydroxyphenyglycoaldehyde. This can be reduced to form the end product MOPEG by aldehyde reductase or oxidised by aldehyde dehydrogenase to form the corresponding end product, VMA. In man 40% of the total urinary metabolites of the catecholamines is VMA (Iversen 1967, Molinoff & Axelrod 1971).

Neuronal Uptake of Noradrenaline

In addition, to the catabolic processes of deamination and methylation, NA is inactivated by a process which involves re-uptake of the released amine into the presynaptic neurone. From a functional point of view this mechanism is most important. Following re-uptake NA is stored in the dense cored granules of the nerve terminals until subsequent release or deaminated by MAO (Axelrod, 1974, Iversen 1967, Molinoff & Axelrod 1971).

Whitby, Axelrod & Weil-Malherbe (1961) demonstrated that the uptake and binding of circulating NA in tissues represented an important mechanism for the inactivation of this substance. In tissues which have a rich adrenergic innervation, this recapture system is the main method of catecholamine inactivation. (Iversen 1967, 1971, 1973).

The mechanism involves two main steps. 1) The transport of NA across the neuronal membrane and, 2) the binding of the amine into the storage sites (Iversen 1967). The transport of NA across the axonal membrane has been designated "Uptake$_1$". Uptake is defined as the transfer of catecholamine across the cell membrane by an active transport mechanism (Dengler, Michaelson, Spiegel & Titus 1964, Iversen 1963). The Uptake$_1$ mechanism appears to have identical properties in the NA containing neurones of the peripheral and central nervous systems (Iversen 1971, 1973).

The avidity of the NA uptake mechanism is perhaps best expressed by a quotation from the Third Gaddum Memorial lecture given by L. L. Iversen (Iversen 1971), "The isolated heart rapidly accummulated/

accummulated labelled NA when perfused with a solution containing a low concentration of (±) - ^{3}H - NA (10ng/ml). The uptake led to concentrations of ^{3}H - NA in the heart which were about 40 times greater than in the perfusion medium after perfusion for 20 minutes. This means that the NA uptake system is capable of clearing the entire extracellular space of the heart in approximately 10 seconds. This performance is even more remarkable when one considers that the uptake sites are restricted to the sympathetic nerve terminals which, in the heart, occupy only a minor proportion of the tissue volume. There is no way of making an accurate estimate of the volume of extracellular space adjacent to the sympathetic terminals but it seems likely that the uptake system could completely clear the NA from this fluid volume in an interval of time measured in milliseconds rather than seconds.

The uptake is restricted to sympathetic nerves as was confirmed by finding that uptake of ^{3}H - NA was reduced by more than 95% in rat hearts, in which the sympathetic innervation was lacking through immunosympathectomy at birth (Iversen, Glowinski & Axelrod, 1966)". If the system is blocked with drugs, such as cocaine and impipramine, prolongation of the physiological response of the catecholamine occurs.

In rat tissues, $Uptake_1$ has been found to be stereo-chemically selective, the affinity for the naturally occurring (-) NA being some five times higher than that for the (+) enantiomer (Iversen, Jarrott & Simmonds 1971, Henley & Snyder 1972).

The uptake process is also temperature sensitive (Iversen 1973) and uptake is markedly reduced if sodium ions are absent from the medium (Bogdanski & Brodie 1969). Several compounds structurally related to NA, for example, tyramine and α-methyl NA can be taken up by the same uptake process (Iversen 1973). This system has the properties characteristic of an active transport system. The property of binding of the accummulated amine into storage granules reduces the amount of free amine in the neuronal cytoplasm and thus reduces the concentration gradient across the synaptic membrane./

membrane.

In addition to the uptake of NA by the neuronal mechanism, uptake can occur into non-neuronal tissues (designated $Uptake_2$, Iversen 1965). This has been reviewed by Iversen (1971) and Gillespie (1973). It is possible that some uptake by smooth muscle cells occurs in the central nervous system. However, this is likely to be negligible in comparison to uptake by neuronal structures.

Methods used in the measurement of Spinal Cord Blood Flow

It can not be denied that the accuracy of measurement of any parameter depends upon the methodology employed to assess it. Several techniques have been used to assess spinal cord blood flow (SCBF) and each has its own particular problems. Since it is important to appreciate limitations of technique in the evaluation of results, this section will examine methods used in the determination of SCBF and comment on the advantages and disadvantages associated with them.

The techniques, for the most part, are modifications of those used in cerebral blood flow (CBF) studies. Some of the methods used in CBF research cannot be used in the spinal cord because of the size and complexity of its vascular supply and drainage. Where these techniques are described, it is because they are important to the development of methods used in the cord.

Freely Diffusible Tracer Techniques

These techniques are based upon the Fick Principle which states that in the steady state the quantity of indicator given out or taken up by a tissue in a specific time is equal to the product of the blood flow and the arteriovenous difference for the indicator, thus $- \frac{dQ}{dt} = F(Ca - Cv)$

where Q = amount of indicator; Ca = arterial concentration; Cv = venous concentration; F = blood flow; and, t = time. Using this relationship Kety & Schmidt (1945) were probably the first workers to successfully quantify flow in the central nervous system. Although their technique cannot be applied to the spinal cord, it is of considerable importance in the development of tracer techniques and as such will be briefly described.

Patients inhaled a 15% Nitrous oxide in air mixture over a 10 minute period, by which time a state of equilibrium between the arterial and venous cerebral blood and presumably the brain tissue was usually reached. During the inhalation period, intermittent arterial and venous cerebral blood samples were taken for/

for analysis of N_2O content. From the integrated arterio-venous difference in N_2O concentration, they could calculate the blood flow of the brain.

In subsequent modifications of the method, N_2O was replaced by the radioactive isotopes, ^{85}Krypton (Lassen & Munck, 1955) or ^{133}Xenon. These modifications, while making the detection and consequent assessment of tracer concentration less difficult, still necessitated the withdrawal of arterial and venous blood samples. An important advance was to administer the radio-isotopes intra-arterially and moniter the isotope in the tissue by means of externally placed counting devices (Lassen & Ingvar, 1961). This had the advantage of eliminating the need for blood samples which needed to be large for the determination of N_2O. Furthermore, flow in regions of brain could now be assessed, the original Kety-Schmidt technique measuring only average flow of the whole brain. In an attempt to measure flow in small regions of the brain Espagno & Lazorthes (1965, 1966) introduced ^{133}Xe into cerebral tissue by direct injection.

The above summary illustrates the 3 methods by which a tracer can be administered to an organ, namely, inhalation, injection or by direct intra-tissue injection.

The methods used in assessment of SCBF using diffusible tracers will now be considered.

^{133}Xenon Clearance

The application of radio-isotope washout studies in the spinal cord presents problems of administration. Workers in the field of cerebral circulation are fortunate that certain species have a main arterial supply to each cerebral hemisphere i.e., the internal carotid artery, into which the tracer can be administered. This is not the case in the spinal cord. The cord is supplied by several arteries along its length whose exact distributions have not yet been accurately defined.

It could be feasible to inject ^{133}Xe in saline into the descending aorta to obtain saturation of the thoracolumbar cord, and/

and monitor the subsequent clearance by means of an externally placed scintillation counter. However, the para-and sub-spinal cord tissues would also receive the isotope. The recorder would receive emissions not only from the cord but also from these tissues. The trauma imparted to the cord by the extensive surgery and lead shielding required to limit this extraneous radiation, renders this approach very unattractive. Moreover, Smith, Pender and Alexander (1969) noted that count rates achieved by this method were so low that satisfactory washout curves were not obtained. This is probably due in a large part to the loss of isotope to abdominal organs and limbs.

They also observed that cannulation of a radicular artery for ^{133}Xe administration, resulted in arterial spasm and distortion of flow. Our own investigations (Griffiths & Crawford, 1976, unpublished observations) are in agreement with this.

The only practicable way that ^{133}Xe can be administered to the cord is by direct intra-parenchymal injection. This has been carried out by 3 groups of workers (Smith, Pender & Alexander 1969, Ducker & Perot 1971, 1972 and Griffiths 1973).

However, it can not be fully ascertained whether the injectate is placed entirely in grey matter, white matter or a variable mixture or both. Smith et al, therefore, analysed their results by calculating the mean weighted flow acknowledging this to be an index of flow rather than a direct measurement. Ducker & Perot, using Smith et al's technique produced similar results.

Griffiths was unable to calculate mean weighted flows, as he observed only 40% of clearances to be bi-exponential. He therefore studied SCBF in relation to the slow component. It is interesting to note that the "average" flows of Smith et al and Ducker & Perot are of a similar magnitude which Griffiths ascribes to white matter, and these in turn are similar to white matter flows determined by Landau, Freygang, Roland, Sokoloff and Kety (1955) and Sandler & Tator (1976) using autoradiography.

The problem of distinguishing the tissue compartment into which the injectate is placed is complicated by the degree of diffusibility/

diffusibility of ^{133}Xe. Espagno & Lazorthes (1965) found, in the brain, that following resection of cortical tissue after injection of ^{133}Xe into that tissue, clearance could still be detected from the underlying white matter. This indicates that ^{133}Xe may diffuse some considerable distance from the exact site of injection. This diffusibility of ^{133}Xe in nervous tissue has been discussed by Rowan (1972).

Further variation was introduced by taking the partition coefficient between cord tissue and blood as unity (Smith et al 1969). It has been shown that the partition coefficients between brain and blood are different for grey and white matter (Veall & Mallett 1965). This approximated value may lead to underestimation of flow where white matter received most of the injectate and overestimation when grey matter received more isotope.

It is evident from the foregoing, that the ^{133}Xe clearance technique can not satisfactorily distinguish flow in grey and white matter compartments. It has the advantage that measurements can be repeated in the same animal. However, there is the undesirable risk of trauma to the cord on each occasion the isotope is administered.

Autoradiography

The method depends upon the detection of the amount of a freely diffusible radioactive tracer in sections of the tissue placed in contact with an X-ray plate. The subsequent degree of darkening of the film reflects the radioactivity of the tissue. If the integrated arterial concentration of the tracer from the start of the infusion until the time of sacrifice of the animal is known, the tissue blood flow can be calculated. The mathematical derivation and assumptions inherent in the calculation have been described by Kety (1951, 1960a, 1960b).

It is fundamental to the accuracy of the technique that the time of infusion should have no effect upon the result. It has been noted that long infusion times (> 1 minute) leads to underestimation/

estimation of the actual flow (Fieschi, Isaacs & Kety, 1968; Eklöf, Lassen, Nilsson, Norberg, Siesjo & Torlof, 1974).

The first measurements of CNS blood flow using the autoradiographic technique was carried out by Landau, Freygang, Roland, Sokoloff & Kety (1955) using the radioactive gas tri-iodofluoromethane ($CF_3{}^{131}I$). Reivich, Jehle, Sokoloff & Kety (1969) pointed out methodological sources of error using this gas and described a technique substituting ^{14}C - antipyrine as the tracer. However, antipyrine has been shown to produce errors at normal perfusion rates in the brain and flows are grossly underestimated in high flow situations such as during hypercapnia. (Eklof et al 1974).

Crone (1964) presented evidence that antipyrine was not freely diffusible in nervous tissue. This has been substantiated by Eckman, Phair, Fenstermacher, Patlak, Kennedy & Sokoloff (1975), who point to its unsuitability as a tracer for autoradiographic studies, due to its low capillary permeability.

Errors in calculated flow values will also result if the tissue under investigation is not homogeneously perfused. However, the degree of error as a result of this is regarded as minimal (Kety 1965) in comparison to the influence of capillary permeability limitations (Eklof et al 1974).

The use of antipyrine in this technique is, therefore, suspect and there is doubt that tritiated water, ^{133}Xenon (Eklof et al 1974), ^{14}C - nicotine (Oldendorf 1975) or ^{14}C - ethanol (Eklof et al 1974, Oldendorf 1975) are any better.

There has been only one specific investigation of SCBF by the autoradiographic technique, (Sandler & Tator 1976a) which utilised ^{14}C - antipyrine as a tracer. The authors base the accuracy of their results on the data of Eklof et.al. (Sandler & Tator 1976b.) However, true evaluation awaits the use of a tracer which suffers none of the disadvantages of those in the gaseous phase and which is truly freely diffusible. It is possible that 4-iodoantipyrine may be superior to antipyrine.

Nonetheless the advantages of the autoradiographic technique are/

are many. It distinguishes flow in grey and white matter and resolution within each of these compartments is high. The method is atraumatic and allows a correlation between flow and histology to be made (Yamaguchi & Waltz 1971). The possibility exists that flow measurements can be combined with assessment of the metabolic state of the tissue as obtained after freezing tissue in situ. The disadvantage of the technique is that only one measurement of flow can be obtained from each animal. Thus time course changes can be investigated only by sacrificing groups of animals serially.

Hydrogen Clearance

The theoretical considerations and evaluation of technique have been described by Aukland, Bower & Berliner (1964). Briefly, the partial pressure of hydrogen in the tissue is proportional to the current generated by its oxidation at a positively polarised electrode. With suitable amplification circuitry and recording apparatus, the change in hydrogen tension during its clearance from the tissue can be monitored.

The use of hydrogen clearance in recording changes of blood flow in the brain was proposed by Misrahy, Clark & Patterson in 1956. Since then, several groups have measured local blood flow in a variety of structures.

For the measurement of CBF, hydrogen has been administered by inhalation (Bozzao, Fieschi, Agnoli and Nardini 1968; Haining, Turner and Pantall 1968; Pasztor, Symon, Dorsch and Branston 1973) injection (Shinohara, Meyer, Kitamura, Toyoda and Ryu 1969; Meyer, Fukuuchi, Kanda, Shimazu and Hashi 1972) or a combination of both. (Fieschi, Bozzao and Agnoli 1965) It is doubtful if injection, which requires arterial puncture, has any advantage over the inhalation technique. Due to its low blood-gas partition coefficient (0.018) hydrogen is cleared very rapidly from the lungs following cessation of administration and thus arterial recirculation is negligible. Stosseck (1974) has described an apparatus which generates hydrogen locally and monitors its subsequent/

subsequent clearance from the tissue.

Spinal cord blood flow using hydrogen clearance technique has been assessed by 2 groups of workers (Kobrine, Doyle and Martin, 1974 and Griffiths, Rowan and Crawford, 1975). Recording flow from the lateral funiculus Kobrine et al reported only monoexponential clearances. However, Griffiths et al found monoexponential and biexponential clearances in both grey and white matter. Biexponential white matter clearances were recorded from those electrodes placed near the periphery of the cord. The fast components of these determinations probably represent clearance into the vasa corona. This statement is supported by the work of Stosseck (1970) who demonstrated rapid clearance of hydrogen by pial arteries in the feline cerebral subarachnoid space. The slow component or monoexponential was taken as the value of flow in the white matter.

In grey matter the majority of clearances were monoexponential. Of those which were biexponential, the fast component values were far in excess of flow values determined for grey matter by the autoradiographic technique (Landau et al 1955, Sandler & Tator, 1976a)and did not correlate with pCO_2 changes. The monoexponentials and slow components of biexponentials were therefore used to calculate flows from electrodes placed in grey matter.

Griffiths et al explained these observations on the basis that electrodes in the grey matter are recording an "average" flow derived from both grey and white compartments. The width of the ventral horn is only about 2 mm at its widest part. Thus the electrodes' "recording area" would include a substantial amount of white matter. Moreover the rapid diffusibility of hydrogen will help "mix the tissue" as the tissue can not support two contiguous areas of different hydrogen tensions.

The above description serves to illustrate a major disadvantage with the hydrogen clearance method in the assessment of SCBF; namely, its inability to distinguish a separate grey matter flow. It is possible that a more accurate assessment of grey matter flow would be obtained if very small electrodes were used.

The/

The necessity of placing a recording electrode into the tissue leads one to consider the possibility of trauma. A prolonged lag phase and plateau between the cessation of administration of hydrogen and onset of clearance represents an injury and diffusion zone around the electrode. However, the eventual clearance will be at the value of the non-injured tissue (Aukland 1965). Of the invasive techniques used in the spinal cord, this method is probably the least damaging as the electrode is placed in the tissue only once and held rigidly in place with cement. Repeated measurements are therefore possible with minimal trauma. Multiple measurements with the intraspinal ^{133}Xe technique in the same site would lead to unacceptable tissue damage. Since measurements can be repeated the animal can act as its own control in experimental studies.

Argon Clearance

This technique is similar to the preceding one. Argon is administered with the respiratory gases, and its eventual clearance is monitored with a vacuum mass spectrometer probe. (Ducker & Garrison 1975 in Sandler & Tator 1976b). The advantages are similar to those with hydrogen. In addition, the mass spectrometer can measure local tissue pO_2 and pCO_2 at the same time. However, the problem of intercompartmental diffusion of argon is likely to be similar to that of hydrogen and there is a definite risk of tissue damage on probe insertion as its diameter is large (1 mm) relative to the diameter of the cord.

Indicator Fractionation Methods

These techniques do not rely upon the Fick Principle. They are based on the indicator fractionation principle as developed by Sapirstein (1956, 1958, 1962) When an indicator is administered intravenously, the fraction of the indicator passing to the tissue is proportional to the blood flow to that tissue. Thus a knowledge of the total amount of indicator given, the amount of indicator in the tissue, and the cardiac output is necessary, in order to calculate the blood flow.

Bingham/

Bingham, Goldman, Friedman, Murphy, Yashon & Hunt (1975) modified Sapirstein's method for the determination of SCBF in rhesus monkeys using ^{14}C-antipyrine as the tracer. They distinguished flows in the anatomical compartments by separating grey from white matter by microdissection prior to tissue tracer determination. Good differentiation between grey and white matter was achieved. However, the complete separation of compartments is unlikely and the possibility of contamination of one by the other is a risk.

Another modification of the original method was the use of isotopically labelled microspheres as the indicator. SCBF has been measured using ^{131}I-macro-aggregated albumin particles (Flohr, Brock & Poll 1969; Flohr, Poll & Brock 1971). These workers did not distinguish between grey and white matter but measured flow in the whole cross section of cord. Their results are in close agreement with the weighted mean flows of Smith, Pender and Alexander (1969) using ^{131}Xe injection. Separation of grey and white matter was achieved with the microsphere technique by Marcus and his associates (1977).

It is evident that the microsphere technique can distinguish grey and white matter flows and the method is atraumatic. A major disadvantage, though, is only one determination of flow can be made in each animal. Discrepancies could arise from rheological factors, for example, preferential streaming of microspheres. However, this is reported to be insignificant. (Neutze, Wyler & Rudolph 1968)

Heat Clearance

The method of measurement of local blood flow by means of heat transfer was introduced by Gibbs (1933). The principle involves the ability of the blood to convect heat between the heated and non-heated thermojunctions of a thermoprobe placed in or on a tissue. If the heat conductivity of the tissue remains constant, the temperature difference between the two thermojunctions is a reflection of blood flow.

This/

This technique was probably the first method used in the detection of blood flow in the spinal cord (Field, Grayson and Rogers 1951). Since then it has been used by several groups in investigation of the factors regulating SCBF (Palleske & Hermann, 1968, Wullenweber 1968, Kindt, Ducker & Huddlestone 1970 and Ducker & Kindt 1971).

Although the method gives a continuous estimate of flow, allowing the effect of blood gases, trauma, etc. on SCBF to be assessed, it is a purely qualitative method and valid comparisons between animals can not be made.

In those cases where a probe is implanted into the organ, tissue damage is a distinct possibility as the probe is of a relatively large size. Moreover, thermocouples placed on the surface of the cord will record the flow only in the superficial cord.

Another criticism of this method is, the requirement that head conduction of the tissue remains constant. In states of increased tissue metabolism around the thermocouple, tissue temperature would rise and thus heat conduction of the tissue would be affected.

Vascular Outline Methods

Flow changes have been observed qualitatively by the intravenous injection of fluorescent indicators such as fluorescein (Wagner, Taslitz, White & Yashon 1969) and thioflavine S (Dohrmann, Wick & Bucy 1973) after experimental trauma. These techniques are relatively simple to perform and can depict patent versus non-patent vessels *in vivo*, but as they are by nature qualitative they can provide only very limited information.

The Structural Basis of the Blood-Brain Barrier

Before the idea of a blood-brain barrier (BBB) was formed, pathologists had observed that in cases of jaundice all the tissues of the body were stained a yellowish green, except those of the brain and spinal cord. The concept of a BBB arose from the classic studies of Ehrlich (1882, 1885) who noted that after the intravenous injection of acidic vital dyes, the central nervous system was, unlike other body tissues, unstained. These studies were confirmed and extended by Goldmann (1900, 1913). He injected trypan blue into the cerebrospinal fluid and found that the brain stained rapidly but no dye entered the bloodstream. Tschirgi (1950) noted that the acidic dyes used in the earlier experiments were strongly bound to serum albumin. The experiments therefore demonstrated the impermeability of the barrier to blood-borne proteins.

More detailed information on the nature of the barrier awaited the development of the electron microscope and suitable tracer substances. Graham & Karnovsky (1966) introduced the tracer horse radish peroxidase (HRP). This has a particle diameter of 40-60 Å and a molecular weight of 40,000 which is to some extent smaller than that of albumin (particle size $\simeq$ 75Å). The localisation of HRP is enhanced by its enzymatic reaction so that a few molecules produce a large number of electron dense particles which can be readily seen with the electron microscope.

Reese & Karnovsky (1967) investigated the permeability of cerebral vessels to HRP. They found that intravascular HRP did not pass into brain substance, although it entered the extra-vascular spaces of other tissues. They attributed this lack of permeability to HRP in the brain to certain morphological specialisations of the cerebral endothelium. Firstly, they noted that adjacent endothelial cells were joined by continuous, true tight junctions, thereby preventing the intercellular passage of HRP. Moreover, these cells contained very few pinocytotic vesicles in comparison to the endothelia of other vascular beds. A few vesicles were seen to contain HRP but the authors found no evidence/

evidence of vesicular discharge of the tracer to the abluminal side of the endothelium. These findings have been given considerable support by a number of other workers (Becker, Hirano & Zimmerman, 1968, Bodenheimer & Brightman 1968, Brightman & Reese, 1969, Brightman, Klatzo, Olsson & Reese 1970, Brightman, Reese & Feder, 1970 and Pappas, 1970).

The above description applies to the situation in the higher vertebrate. There is considerable species variation in the morphology of the BBB. For example, open clefts between cerebral endothelial cells have been observed in the nurse shark, guitar fish and numbfish (Brightman, Reese, Olsson & Klatzo 1971) and intracellular channels have been seen in the cerebral endothelium of Japanese sharks (Hashimoto 1972). Brightman, Reese & Feder (1970) have noted in studies utilising microperoxidase, that the barrier in the shark results from fused glial end feet surrounding relatively porous capillaries.

It was contended by some workers that the barrier in the higher vertebrate was situated at the level of the perivascular astrocytic end feet. This theory was proposed by Schaltenbrand & Bailey (1928). However, Brightman & Reese (1969) have demonstrated that the astrocytes are connected by "gap" junctions and not tight junctions and molecules the size of lanthanum can pass through these junctions into the extracellular space of the brain.

The Blood Brain Barrier to Noradrenaline

Specific enzymatic barrier mechanisms have been shown to exist within the endothelial cells of the central nervous system. Using a combination of chemical and histochemical procedures, Owman and his co-workers (Owman 1966, Bertler, Falck, Owman & Rosengren 1966, Owman & Rosengren, 1967, Owman, Edvinsson, Falck & Nielsen 1974)found that the cerebral endothelia contained high levels of the enzymes monoamine oxidase and DOPA-decarboxylase. These enzymes are absent in peripheral capillaries and in capillaries of brain areas such as the median eminence and area postrema which are known to lack a BBB to acidic dyes. Their work indicates that the amine precursors/

precursors DOPA and 5 - hydroxytryptophan are prevented from passing into the neural parenchyma by a mechanism whereby the precursors are decarboxylated in the capillary endothelium by DOPA-decarboxylose after which the amines formed are metabolised by local monoamine oxidase. Blood-borne noradrenaline entering the endothelial cell would also be metabolised by MAO. Oldendorf (1971) measured the brain uptake of a variety of radiolabelled amino acids, hexoses and amines including noradrenaline relative to that of tritiated water (which he took to be completely extracted). The brain uptake index but for noradrenaline was about 5%. The true value is even lower than this since water is not as freely diffusible as was once supposed (Eichling, Raichle, Grubb & Ter-Pogossian 1974). Nonetheless this low BUI for noradrenaline correlates well with the concept of an enzymatic blood-brain barrier to noradrenaline.

Situations in which blood-brain barrier function may be altered.

Hyperosmolarity

The effect of hypertonic solutions on the BBB was investigated in 1945 by Broman and Lindberg-Broman. They showed that various chemical and physicochemical agents in hypertonic concentrations could open cerebral vessels to intra-vascular tracers. These solutions were thought to cause increased barrier permeability by a direct toxic action on the blood vessels. This theory was questioned by Rapaport (1970) who tested the effect of concentrated solutions of Na NO_3, NaCl, urea and ethanol applied to pial vessels. The integrity (or otherwise) of the barrier was indicated by the acidic response on the cortical surface to intravenous Na HCO_3. These experiments led him to postulate that concentrated solutions opened the BBB by shrinking the endothelial cells, thereby widening the tight junctions by the tension exerted upon them by the retracted cell membranes.

Rapaport, Hori & Klatzo (1972) extended this work by testing a large selection of concentrated solutions. The solutions were applied to cortical pial vessels or given by intracarotid infusion. Their effect on barrier integrity was demonstrated by the ability of/

of the solution to cause extravasation of an Evans-blue albumin complex (EBA). The authors stated four criteria necessary for an agent to act in accordance with the hypothesis of barrier disruption by hyperosmotic cell shrinkage. Namely, a) the solute should act reversibly, b) it should not act on the barrier by specific drug action, c) its damaging effect should increase with increasing concentration and d) its effect should be related inversely to its ability to penetrate the cell membrane. Concentrated solutions of highly lipid soluble, non-polar solutes were found to cause irreversible barrier damage, whereas polar, lipid-insoluble substances were found to satisfy the criteria outlined above. A concentration of 2M urea was found to be the threshold dose at which the dye-protein complex escaped from the vessels.

Rapaport and his co-workers demonstrated reversibility of barrier opening by the lack of EBA leakage when the dye was given 30 mins. after application of the test substance. However, isotope studies have shown that increased permeability to albumin may remain for up to 4 hours (Studer, Welch & Siegel, 1974).

It has been shown that an infusion of 2M urea or lactamide via the lingual artery, when the external and common carotid artery are temporarily occluded, will induce osmotic opening of the BBB in the ipsilateral cerebral hemisphere of the monkey, without producing gross neurological deficits (Rapaport & Thompson, 1973). Moreover, in baboons osmotic barrier disruption by urea has no effect upon CBF or the responses of flow to hypercapnia or acute hypertension (Pickard, MacKenzie, Durity, Welsh, Langfitt, Jennett & Harper 1975).

The distribution of EBA and HRP was investigated, following an intracarotid infusion of 3M urea in rabbits (Brightman, Hori, Rapaport, Reese & Westergaard, 1973). Both the dye and peroxidase crossed some vessels. Capillaries were mainly affected, although some arterioles and possibly venules were also involved. Peroxidotic reaction product was demonstrated within extracellular pools between successive tight junctions. The endothelial cells did/

did not appear to be shrunken but the authors point out rehydration may have taken place during fixation or during the interval between urea infusion and fixation.

Tracer could possibly be delivered across the vessel wall by vesicular transport. The aforementioned authors noted that cytoplasmic vesicles containing HRP were few in number. Large calyciform cisterns, some containing HRP, were observed in the endothelium. Whether these can transport the tracer to the abluminal surface is yet to be determined.

Contrast media are known to have a deleterious effect upon barrier function (Broman & Olsson 1948, 1949).
This may be a result of an osmotic effect on the barrier or the divalent ion content of the medium may be of importance. The rate of pinocytosis is reported to be elevated by divalent ions. (Job, 1971).

Hypertension

Acute arterial hypertension may damage the BBB. It has been shown that an abrupt rise in blood pressure of more than 90 mm.Hg, induced by the intravenous injection of Aramine, results in the extravasation of previously administered EBA (Johansson, Li, Olsson & Klatzo 1970). Johannson & Linder (1974) acutely raised blood pressure in dogs by clamping the thoracic aorta and attributed the resulting barrier disruption to increased intra-luminal pressure and not to the direct action of the pressor drug given in the previous study. Haggendal & Johansson (1972) found that stepwise increases in blood pressure induced by Aramine did not cause EBA extravasation.

The extent of barrier disruption in acute hypertension has been shown to be more widespread than has been indicated by EBA. Studies involving the administration of EBA and HRP before Aramine induced hypertension have shown that increased permeability as evidenced by HRP extravasation occur outwith as well as within those delineated by EBA (Brightman & Broadwell, 1976).

Chronic hypertension also increased the permeability of cerebral/

cerebral vessels to tracer substances. Giacomelli, Wiener & Spiro (1970) reported enhanced permeability of cerebral vessels to HRP in rats submitted to renal hypertension of 2 to 5 weeks duration.

The increased intraluminal pressure in both acute and chronic hypertension is thought to cause overdistension of endothelial cells leading to a pathologically increased permeability. Hansson, Johansson & Blomstrand (1975) have shown that in acute hypertension, peroxidase can penetrate both through and between endothelial cells in arterioles, capillaries and occasionally venules.

It can be seen from the above selection of the literature that there is strong evidence to link hypertensive states to increased barrier permeability. The situation in the spinal cord is much less certain. No studies relating the spinal barrier to hypertension have been reported in the literature. Our own (unpublished) preliminary observations (Griffiths & Crawford, 1977) have indicated that Aramine induced acute hypertension, which produced multiple gross foci of EBA extravasation in the brain, failed to produce similar lesions in the spinal cord. Further, no evidence of EBA fluorescence was seen in the cord on microscopical examination. Whether barrier damage could be demonstrated with the more sensitive tracer HRP remains to be determined.

Tumours

The permeability of the barrier is known to change in association with neoplastic conditions. In human malignant gliomas, contiguous endothelial cells are not joined by tight junctions (Long, 1970). This abnormality is seen also in choroid plexus papillomata induced by the intracerebral injection of SV_{40} virus in newborn Syrian hamsters and EBA and HRP can escape from the vessel lumen to the pericapillary environment. Endothelial fenestrae are also seen. Each fenestra is covered by a thin diaphragm which vary or may not be permeable to protein (Brightman & Broadwell 1976).

In metastatic tumours, new capillaries may resemble those of their tissue of origin. Intracerebral metastatic lymphomas exhibit endothelial cells characteristic of a normal lymph node, rather than

of/

of cerebral capillaries (Hirano, Ghatak, Becker & Zimmerman, 1974).

Other Situations

Many other states can be responsible for altered brain permeability. The following illustrate some examples. Marked extravasation of labelled serum protein occurs in connection with experimental brain concussion (Rinder & Olsson 1968). Following acute spinal trauma induced by the weight-dropping technique, alterations in the integrity of the endothelial tight junctions were reported by Goodman, Bingham & Hunt (1976). Griffiths & Miller (1974) noted that EBA leakage occurred in experimental spinal injuries, predominantly in veins and venules.

Increased barrier permeability to EBA occurs as a realatively late event to cerebral ischaemia (Hossman & Olsson 1971, Ito, Go, Walker, Spatz & Klatzo 1976). Following cerebral ischaemia in gerbils induced by occlusion of the left common carotid artery, Westergaard, Go, Klatzo & Spatz (1976) assumed that transendothelial vesicular transport was responsible for the extravasation of blood-borne HRP, as no endothelial or tight junction damage was evident.

Severe hypercapnia has been shown to increase barrier permeability (Cutler & Barlow 1966) and induced seizures can cause transient barrier disruption (Lorenzo, Shirahige, Liang & Barlow, 1972).

Hudgins & Garcia (1970) have shown that even surgical manipulations, such as exposure of the brain and retraction of cerebral tissue and the use of local electrocautery can increase barrier permeability. The importance of these latter findings on investigations of barrier integrity is obvious.

The Effect of Noradrenaline on Blood Flow in the Central Nervous System

Little work on the effect of vasoactive substances on SCBF has been performed (Palleske 1968). This section, therefore, presents a brief review of experiments performed to determine the influence of NA upon CBF. It illustrates the controversy, comments upon some of the results obtained and provides a basis for comparison with results of the effect of NA on SCBF presented in this thesis.

The question as to whether there is a functional noradrenergic control of blood vessels within the CNS is of great importance when one considers the effect of this amine on haemodynamics of the central nervous system. A great deal of work has been carried out in an attempt to answer this question and while it is not intended to review this matter in depth, comment upon it must be made.

Edvinsson (1975) has investigated and reviewed this subject extensively. Using histofluorescence microscopy techniques, a well developed supply of noradrenergic nerves has been found in cerebral pial vessels, the arterioles receiving a richer supply than the veins. Intracerebral blood vessels are relatively less well supplied, the density of innervation decreasing with decreasing vessel size. However, it has been noted by Owman, Edvinsson and Nielsen (1974) that the density of innervation is not only related to the number of nerve terminals present but also the amount of smooth musculature supplied by the nerves. The nerve terminals were related to the outer layer of smooth muscle in a manner expected for true functional innervation. These authors have also shown that the nerves originate from the superior cervical ganglion. There is, however, controversy regarding the functional significance of the innervation of the intraparenchymal vessels. Harper, Deshmukh, Rowan and Jennett (1972) have suggested that there is no autonomic control of these vessels and that they are influenced only by metabolic factors, sympathetic stimulation resulting in the constriction of extracerebral vessels only.

Intracerebral/

Intracerebral adrenergic nerve fibres, thought to originate from the locus coeruleus of the brain stem were postulated to innervate parenchymal vessels (Hartman, 1973). The influence of this system upon the vessels is however a matter of debate.

Thus, two separate noradrenergic components occur in the brain, a central monoaminergic system originating in the brain stem and a component arising from the superior cervical ganglion. There appears to be structural basis for a relationship between the cerebral circulation and noradrenaline. Although the situation has not been examined in detail, there seems to be an analagous situation in the spinal cord. The cord is supplied by a descending noradrenergic bulbospinal system and by noradrenergic fibres originating from the sympathetic chain (Ohgushi, 1968). It has not been determined if bulbospinal neurones innervate spinal vessels but a relationship has been shown between cord vessels and peripherally originating sympathetic fibres (Ohgushi, 1968). Moreover, Edvinsson has demonstrated by histofluorescent technique, noradrenergic nerves in close association with spinal cord blood vessels. Some examples of this association are illustrated in Fig.3.

Many studies have been performed to elucidate the action of noradrenaline on CBF. The results, however, have differed widely, ranging from a decrease, to no change, to an increase in flow.

In 1952, King, Sokoloff and Wechsler examined the effect of NA on CBF in man, measured by the N_2O clearance technique and found a reduction in flow. Essentially similar results were reported by Sensenbach, Madison and Ochs (1953) and Moyer, Morris and Snyder (1954). These studies reported the effect of intravenously administered NA in awake man. In such a situation NA causes hyperventilation. The concomitant reduction in $paCO_2$ values reported by these authors corresponds approximately to the decrease in CBF noted. The lowered CBF values in response to NA administration can, therefore, be explained on the basis of hypocapnic alteration of flow caused by drug induced hyperventilation.

Greenfield & Tindall (1968), investigating the effect of NA administered by the intracarotid route, found no effect of the amine/

amine on internal carotid blood flow as measured by electromagnetic flowmetry. Likewise, Olesen (1972) measuring regional CBF by the ^{133}Xe clearance technique, could detect no change in flow to an intracarotid infusion of NA.

Thus, on closer examination of the human studies, there appears to be little difference in the results. The findings from animal studies are, however, more contradictory. Intravenous NA administration has been reported to decrease CBF in anaesthetised dogs by 40% (Haggendal, 1965). In these studies CBF was measured by radioactive inert gas clearance. The results, however, would be rendered inaccurate, due to contamination from the extracranial tissues which were not removed in these experiments. Ekstrom-Jodal, von Essen, and Haggendal (1974) produced similar results but again extracranial tissues were left intact.

Lluch, Reimann and Glick (1973), investigating the effect of several concentrations of NA adminstered intra-arterially, noted a dose dependent decrease in CBF in the unanaesthetised goat. Flow was measured with an electromagnetic flowmeter previously implanted on the internal maxillary artery, which in this species provides the sole blood supply to the ipsilateral cerebral hemisphere. Extracranial structures are also supplied by the internal maxillary artery in the goat via the rete mirabile. The effect of this structure upon the results obtained by Lluch et al was shown by Edelman, Epstein, Cherniak & Fishman (1972), who noted that NA administered proximal to the rete mirabile caused a decrease in internal maxillary blood flow, whereas no reduction occurred following NA infusion distal to it.

Green & Denison (1956) investigated the effect of NA on flow in the common carotid artery in dogs whose intracranial circulation had been surgically isolated. No change in flow was demonstrated. The experimental design in this study could be criticised on the basis of the scale of the surgery required. The possibility of traumatic alteration of cerebral haemodynamics could, therefore, be suspected. Ito (1970) attempted to distinguish CBF and extracranial flow by measuring flow in both the internal and common carotid/

carotid arteries. The response of this preparation to NA essentially agreed with those of Green and Denison.

Harper & Gabriclian (1969) found no difference in CBF measured by Krypton clearance after intravenous NA at normotension. However, the intracarotid injection of a large dose of NA caused a 30% reduction. This effect was not noted in subsequent injections in the same animal.

Meyer, Lavy, Ishikawa & Symon (1964) found an increase in intracarotid blood flow, in response to intravenous NA, which they interpreted to be secondary to changes in systemic arterial blood flow.

Intra-arterially administered NA was noted to have a minimal effect on CBF in the baboon during normocapnia but had a significant vasoconstrictory action during hypercapnia when the intraparenchymal vessels were near maximally dilated (Harper, Deshmukh, Rowan, Jennett 1972, MacKenzie, McCulloch & Harper, 1976). MacKenzie, McCulloch, O'Keane, Pickard & Harper (1976) postulated that the absence of the response of the cerebral circulation to NA at normocapnia could be due to the presence of a blood-brain barrier to NA. Following disruption of the barrier with hypertonic urea, a 49% increase in CBF was demonstrated. Circumventing the barrier by injecting NA intraventricularly, they again demonstrated an increase in flow. Rosendorff and Cranston (1971) have demonstrated a dual, dose-dependent response of blood flow in the hypothalamus in response to NA after intrahypothalamic injection of the amine. The smaller doses caused a rise in hypothalamic blood flow and the larger doses caused flow reduction. The responses could be abolished by the appropriate β-or α-noradrenergic antagonist, respectively.

Biogenic Amine Levels and Acute Spinal Trauma
(with special reference to noradrenaline)

The histopathological changes of progressive haemorrhage and necrosis which occur following severe blunt injury to the spinal cord have been well described. (Ducker, Kindt and Kempe, 1971; Wagner, Dohrmann and Bucy, 1971)

In an attempt to explain the aetiology of the progression of the lesion Osterholm and Mathews, (1972a) propounded a biochemical theory. Their hypothesis was based on elevated levels of NA within impacted cords which was 5 times that of the control values within 1 hour of an injury which should produce irreversible paraplegia. They suggested excessive quantities of NA are synthesised by spinal fibres in response to severe trauma. This accummulated NA is released from the neurones into the extracellular space, inducing toxic vasospasm, secondary hypoxia and subsequent tissue necrosis and haemorrhage.

Following these original observations a number of investigations into the relationship between biogenic amines and spinal trauma were carried out with widely differing conclusions. Using an identical injury technique, de la Torre, Johnson, Harris, Kajihara and Mullan (1974) were unable to demonstrate a change in NA or 5-hydroxytryptamine (5-H.T.) levels one hour after injury, although there was a non-significant increase in dopamine (D.A) levels. Hedeman, Shellenberger and Gordon (1974) observed NA levels to fluctuate considerably becoming subnormal at 45 mins. after injury, attaining normal levels at 1½ hours and returning to subnormality by 3 hours. In the same animals DA levels were dramatically increased, particularly in the region 1 cm above the lesion site reaching a peak at 45 mins post-trauma. DA levels at the site of injury were also elevated, though not to the same extent. The levels declined steadily over the period 45 mins to 3 hours post-injury, although they were still above control values. Naftchi, Demeny, Decrescito Tomasula, Flamm and Campbell (1974) reported no change in NA or 5-HT levels 1 hour after impact injury. DA levels, however, approximately doubled. The investigations of Rawe, Roth, Boadle-Biber/

Boadle-Biber and Collins (1977) likewise reveal no significant change in NA levels at 1 hour post-trauma.

In contrast, Bingham, Ruffolo and Friedman (1975) found a progressive decrease in NA activity, following trauma, which demonstrated the kinetics of a first order process. No significant changes in DA levels were discovered at the injury site. However, a significant increase was seen 4 hours post-trauma at a level of 5 thoracic segments caudal to the impact site. Zivin, Doppman, Reid, Tappaz, Saavedra, Kopin and Jacobowitz (1976) measured biogenic amine levels selectively in grey and white matter. NA decreased in the centre of the lesion within 5 mins of a traumatic insult. In surrounding white matter its level was unchanged or decreased. 5-HT also decreased in the lesion centre but increased at the lesion edges. They reported no change in DA activity.

It is apparent that the above studies provide little support for the hypothesis of Osterholm and Mathews. However, Tsubokawa, Nakamura, Hayashi, Taguma, Sugawara, Goto and Moriyasu (1975) noted a rise in spinal cord NA from a control level of 0.6 ug NA/gm of spinal cord tissue (wet weight) to 0.88 µg/gm at 30-60 mins post-trauma. (These authors specifically point out that their experimental procedure paralleled that of Osterholm and Mathews). Moreover, they demonstrated a threefold rise in 5-HT content in the same site and stated that while NA levels were not markedly elevated in the presence of marked central necrosis, the severity of the lesion had a direct relationship to the level of 5-HT (rather than NA as suggested by Osterholm and Mathews). Shoultz (1977) noted a twofold increase in the level of NA at the site of impact at 1 hour following trauma and a return to control levels by 2 hours. The two letter reports are the only publications describing an increase in NA and in neither case did the NA content of the spinal cord tissue reach the dramatic heights described in Osterholm and Mathews' studies.

It can thus be readily appreciated that a great disparity of opinion is present, not only with regard to the behaviour of NA/

NA after injury but also to 5-HT and DA. A satisfactory explanation of these widely differing results is difficult. However, the following factors may be of importance:-

1) The technique of amine determination

This is arguably the most important factor. As can be seen from Table 1 fluorometric technique produced a wide range of results. In one study a range of 0.33 - 0.75 ug NA/gm spinal tissue wet weight was produced (Osterholm and Mathews, 1972a). These values are in marked contrast to that of Zivin et al (1976) who, utilising a radiometric enzymatic assay method, gave the resting NA content of a corresponding segment of spinal cord to be 0.148 ug/gm. Clearly, the sensitivity of the technique utilised is of prime importance.

2) Segmental level

It has been shown that NA concentration varies with segmental level. Bingham, Ruffolo and Goodman (1975) have noted that the highest NA levels were found in lower cervical and lumbosacral regions, correlating directly with the amount of grey matter present in the segment. An essentially similar situation was reported by Rawe et al (1977).

3) Segmental Rhythms

Reis and Gutnick (1970) described mutually independent segmentally specific rhythms of NA in the spinal cord. C1-2 and sacrococcygeal segments showed a circadian rhythm and a biphasic cycle was seen in segments T9 - 13. Clearly both the segment and the time of sample collection must be taken into account for accurate comparison.

4) Method of producing spinal injury

The most widely used technique of producing a measurable and reproducible injury is based on the method of Allen (1911). Basically, a known weight (in gms) is dropped a known distance (in cms) on to an impounder made of some lightweight substance resting on the intact dura of the surgically exposed spinal cord. The magnitude of the injury is calculated as the product of the weight and the distance the weight travels before impact (gm. cms.). This/

This latter expression relates to energy and not force. Force is defined as energy exerted per unit area. Thus, the cross-sectional area of the impounder must be taken into account. It is reasonable to suppose that following injury, the reaction of a particular biogenic amine in the spinal cord may vary with the magnitude of injury. Thus, accurate comparison of the reported studies is complicated by the various impact energies and impounder dimensions which have been used (Table 2).

In one study, injury was produced by the rapid inflation of a Fogarty balloon inserted into the enclosed spinal column via an intervertebral foramen, making comparison with results utilising the weight dropping technique even more difficult.

5) Species difference

Many different species have been employed in these studies. Resting values of the amines may be different in corresponding spinal cord segments of different animals. Moreover, there is great species variation in damage produced by Allen's weight dropping method. Rats and rabbits are severely wounded by a 50 gm.cm. energy impact (Howitt and Turnbull, 1972), yet an impact of approximately 500 gm.cm. is needed to produce a corresponding injury in larger mammals, such as the dog. Tables 1 & 2 list the experimental animals used in the reported studies.

Vise, Yashon and Hunt (1974) offered an alternative explanation of the increased NA levels in injured spinal cords observed by Osterholm and Mathews. Using histofluorescent techniques they suggested that NA accumulated in the cord from exogenous blood-borne sources, gaining entry via the damaged blood-cord barrier. This was not confirmed by Bunegin, Albin and Jannetta (1976), who found no difference in (^{3}H) - NA content of control and traumatised cords after its intravenous administration.

It can be seen from the above brief review of the literature of biogenic amines and spinal trauma, that a great deal of confusion abounds. Clarification of the behaviour of these amines awaits the careful application of amine turnover studies in spinal injury situations.

PART II

The Effect of Noradrenaline on Spinal Cord Blood Flow before and after blood-cord barrier disruption with Urea

METHODS

Surgical Preparation

Unselected dogs of either sex, weighing between 10 and 20 Kg. were used in these experiments.

Anaesthesia was induced with thiopentone sodium (25 mg/Kg) and maintained on a semi-closed system with a 50% nitrous oxide/oxygen mixture and 0.5% Halothane. The gas mixture was delivered through a respiratory pump, its rate and volume adjusted to maintain normocarbia during the surgical preparation and subsequent measurements. When surgery was complete, the halothane was discontinued and anaesthesia maintained with a 1% solution of alphachloralose (60 mg/Kg) administered intravenously. To aid surgery and prevent reflex movements during the intraspinal placement of blood flow electrodes, muscle paralysis was produced with intravenously delivered succinylcholine (1 mg/Kg). Additional doses were given as necessary.

A femoral artery and vein and one brachial artery were cannulated, the latter for the continuous electromanometric recording of arterial blood pressure and the withdrawal of blood for the analysis of arterial pCO_2 ($paCO_2$), pO_2 (paO_2) and pH. Base deficit was corrected where necessary with an 8.4% ("standard") sodium bicarbonate solution using the correction formula of Astrup, Siggard-Anderson, Jørgenson and Engel (1960): -

viz., $Na\,HCO_3$ = Base deficit x Body weight (Kg) x 0.3 (empirical correction factor)

The femoral artery cannula was used for the administration of test substances. Pharyngeal temperature was measured with a mercury thermometer and heating lamps were used to maintain the temperature between 37 - 38°C.

Via a midline laparotomy incision, the abdominal aorta was located and the following branch arteries ligated - the posterior mesenteric artery, an external iliac artery, that part of the aorta just proximal to the bifurcation of the internal iliac arteries, both deep circumflex iliac arteries, and in male dogs/

dogs the testicular arteries were also ligated. The femoral artery cannula was adjusted so that its tip was positioned in the aorta at the level of the 4th lumbar arteries. A ligature was then tied round the external iliac artery of that side. Prior to closure of the abdominal incision, the 5th left lumbar artery was cannulated retrogradely for the continuous recording of blood pressure. This lumbar artery was chosen because it has been shown by Doppman and Ramsey (1971) that, at this level, the arterial supply to the spinal cord is derived mainly from the 4th left or 5th right lumbar arteries. Thus cannulation of the 5th left lumbar artery should give an indication of arterial blood pressure in the lumbar bed without disturbance of spinal cord blood supply. The pattern of ligations and cannulations is shown schematically in Fig 4.

The animal was then placed in sternal recumbancy and a 3-level dorsal laminectomy was performed to expose the L_2, L_{5-6} and S_{1-2} segments of the spinal cord. The bone was sealed with bone wax, epidural fat removed and the dura mater incised and reflected. Perspex laminae were inserted to replace the removed bone and these were held in place with dental cement. The dog was then suspended by four Steinman intramedullary pins inserted through appropriate dorsal spines and attached to retort stands bolted to the operating table. By this means, the respiratory movements to the spinal cord were effectively eliminated. Hydrogen sensitive electrodes, for the determination of SCBF, were inserted into the cord through holes drilled in the perspex laminae and held in place with dental cement.

Calculation of SCBF

SCBF was measured by the hydrogen clearance technique. This method, utilising positively polarised needle electrodes inserted into spinal cord tissue, and hydrogen as the freely diffusible indicator, is based upon the Fick principle. This principle has been dealt with in the relevant review section but as it is of paramount importance in the derivation of a formula to calculate SCBF, it will be restated here.

Assuming a single homogenously perfused tissue, the Fick principle/

principle states that the amount of gas extracted or released by a tissue is equal to the quantity conveyed to it by the arterial blood less the quantity removed by venous blood,

i.e., $\frac{dQ}{dt} = F\ (Ca - Cv)$ .....(1)

Where Q = quantity of hydrogen taken up or given out by a tissue in time t.

F = blood flow

Ca = arterial concentration of gas

Cv = venous concentration of gas

If Ci is taken to be the concentration of gas in a tissue of weight W (gms).

$Q = Ci \times W$ .....(2)

Substitution of(2)in equation(1)and rearrangement reveals.

$\frac{dCi}{dt} = \frac{F}{W}\ (Ca - Cv)$ .....(3)

For a highly diffusible and lipid soluble gas such as hydrogen, it may be assumed that the tissue is in instantaneous diffusion equilibrium with venous blood throughout the whole saturation (or desaturation) period.

i.e., $Ci = \lambda Cv$ or $Cv = \frac{Ci}{\lambda}$ .....(4)

where λ = tissue/blood partition coefficient.

Substitution of (4) in (3) gives:-

$\frac{dCi}{dt} = \frac{F}{W}\ (Ca - \frac{Ci}{\lambda})$ .....(5)

In a desaturation process, the arterial concentration can be taken to equal zero.

Thus $\frac{dCi}{dt} = -\frac{F}{W\lambda}\ Ci$ .....(6)

In a homogenously perfused tissue $\frac{F}{\lambda W}$ is a constant (k)

Thus $\frac{dCi}{dt} = -kCi$ .....(7)

Integration of (7) gives

$Ci = Ci_o e^{-kt}$ (where Ci_o = initial concentration of hydrogen in the tissue)

This last formula describes a simple monoexponential curve. When the concentration is plotted against time on semi-logarithmic paper/

paper the resultant graph is a straight line. The time taken for the concentration to reach half of its initial value ($t_{\frac{1}{2}}$) is expressed by $\log_e 2 \frac{W\lambda}{F}$

Therefore flow is determined by the formula.

$$\frac{F}{W} = \frac{\log_e 2 \lambda}{t_{\frac{1}{2}}} = \frac{0.693 \lambda}{T_{\frac{1}{2}}}$$

Thus, with a knowledge of the $t_{\frac{1}{2}}$ and λ the weighted flow in a homogenous tissue can be calculated. λ used in the calculation of SCBF is taken to be I. (Auckland, Bower and Berliner 1964).

The same formula can be used to calculate flow in heterogeneously perfused tissue provided the following assumptions are taken into consideration, viz., 1) that the arterial concentration of gas during desaturation is zero; 2) that the partial pressure of hydrogen in each type of tissue is the same as in the venous blood which drains it; 3) that the initial concentration of gas in each tissue is the same; and 4) that the partition coefficient is the same for each tissue.

If the clearance of gas from a heterogenously perfused tissue is plotted on semi-logarithmic paper, the slope is not a straight line but decays with time, due to gas being more rapidly cleared from areas with higher flow rates than from tissues which are more slowly perfused. The "tail-end" of the curve reflects flow in these latter areas. The curvecan be resolved,by compartmental analysis into fast and slow compartments, the initial part of the curve reflecting the average flow of the compartments being detected.

It has been noted by Griffiths, Rowan and Crawford (1975) that only 40% of the clearances recorded from spinal grey matter were biexponential. The SCBF values in this section are therefore presented as flows calculated from monoexponential clearances or from the slow component of biexponential clearances.

Electrodes and Recording Circuits

The tissue electrodes were constructed from 0.2 mm. grade 2 platinum wire. These were thinly coated with araldite and the terminal/

terminal 1 mm. scraped bare, care being taken to ensure that the araldite/platinum junction was smooth and the naked platinum tip was sharp. This procedure was carried out under a dissecting microscope. Insulation provided by the araldite, was checked, both visually and electrically in saline using a Multimeter. The electrode tips were cathodised in a 5% platinum chloride solution.

A silver/silver chloride EEG electrode implanted subcutaneously in the animal's back was used as a reference electrode and a positive polarising voltage of 700 mv. was employed.

The current generated from the oxidation of hydrogen at the tip of the recording electrode was amplified using a six-channel system. Analog Devices amplifiers, type 233K which have low input bias drifts of less than 1pA/°C were employed. These were set so as to give an output of 1 volt for an input of 1 microamp. The system is shown schematically in Fig. 5 and the amplifier circuit diagram is shown in Fig. 6. The output from each amplifier was displayed on either single or dual Servoscribe recorders. Each amplification channel was provided with an input balance control so a zero base line on the recorder could be set to correspond with a zero hydrogen concentration in the tissue. Moreover, each recorder was provided with a variable gain control allowing the hydrogen concentration equilibrium plateau level to be set at full scale deflection without affecting the original zero concentration setting. The amplifier bandwidth was restricted to 0-1 Hz to limit the effect of high frequency noise.

Administration of the Indicator

Hydrogen gas was introduced with the respiratory gases into the anaesthetic circuit, before the respiratory pump. During the inhalation of hydrogen, the N_2O was discontinued and the O_2 increased so that an approximate 60/40% mixture of O_2/H_2 was delivered. The recorders registered an increasing concentration of hydrogen in the tissue and, when a plateau was reached, saturation was deemed to be achieved. The hydrogen was then turned/

turned off, the original N_2O/O_2 mixture resumed and the hydrogen clearance curve was monitored. SCBF was calculated using the formula derived previously and the results expressed in ml/100g/min.

The Effect of urea on SCBF

Six dogs with a total of 16 electrodes were used to determine the effect of urea on SCBF. Five electrodes were placed in the L_2 segments, seven in L_{5-6} and four in S_{1-2}. In each experiment at least three determinations of flow were performed to establish baseline values. Following the control measurements 1.5 ml/Kg of 2.5M urea was administered intra-arterially over approximately ten seconds via the femoral cannula, and a series of three clearances were made after temporary disturbance in blood pressure had subsided (see results).

In order to demonstrate that urea had disrupted the blood-spinal cord barrier, the following procedure was carried out. At the end of the experiment a 2% solution of Evans Blue with 5% albumin (EBA) made up in normal saline was administered by slow intravenous injection at a dosage of 10 ml/Kg and a further intra-arterial injection of urea was given. The animal was killed shortly after, the electrode positions marked by diathermy and pieces of cord removed for fluorescent microscopy and histological examination. Histological sections were stained with haematoxylin and eosin, Martius scarlet blue and cresyl violet.

The Effect of Noradrenaline on spinal cord blood flow before and after disruption of the blood cord barrier.

Thirteen dogs were used for the experiments. The animals were prepared as previously described. No measurement of SCBF was performed until at least one hour after electrode placement to allow complete polarisation of the electrodes.

At least three control measurements were made. NA was then continuously infused into the aorta using a slow infusion pump set at a constant rate (1ml/min) and three further measurements of SCBF made during the infusion of NA. Flow determinations commenced once arterial blood pressure had stabilised. On completion/

completion of the third measurement the NA infusion was discontinued briefly while the blood-cord barrier was disrupted by the intra-arterial injection of urea as previously described. The infusion was then continued and three further measurements of flow performed.

The NA was prepared freshly as a solution of noradrenaline hydrochloride* (as base) made up in normal saline. A small quantity of ascorbic acid was added to the solution to improve the stability of the drug. Infusion concentrations of either 12 µg/ml or 30 µg/ml were used. The results presented are from the L_2 and L_{5-6} segments of the cord. The pattern of response at S_{1-2} was similar but there was insufficient data at this level for statistical analysis.

Following the experiments, electrode positions were marked by diathermy and samples of spinal cord taken for histological examination.

The Effect of α-Adrenergic Blockade

The effect of α-adrenergic blockade on SCBF during infusion of 30 µg NA/min after blood-cord barrier disruption was studied in three dogs. Three control measurements of flow were followed by the intravenous injection of phenoxybenzamine** (PBZ; 1.5 mg/Kg). After blood pressure stabilisation two more flow measurements were made. The barrier was then disrupted by injection of urea, NA (30 µg/min.) was infused intra-arterially as described and three further measurements of flow made. Values were obtained from L_{5-6} segments of the spinal cord.

*L-Arterenol: Sigma, London Chemical Co.Ltd., Kingston-upon-Thames

** Dibenyline: S, K & F Laboratories Ltd.

RESULTS

The control flows in these experiments were similar to those obtained previously with the hydrogen clearance technique. (Griffiths, Rowan and Crawford, 1975). Moreover, a similar value was obtained with regard to the ratio of monoexponential to biexponential clearances. In the present study 62.5% of clearances were slow monoexponential curves.

The effect of urea on SCBF The ligation procedure described in the section on surgical preparation had no effect upon resting arterial blood pressure. After injection of urea, however, the mean systemic arterial blood pressure rose by approximately 30 mm. Hg. This was accompanied by a corresponding fall in 5th left lumbar arterial pressure. These parameters returned to resting levels within 20-30 seconds (Fig. 7).

The results of the blood flow measurements are tabulated in Table 3 and presented in graphic form for the L_{5-6} level of the cord in Fig. 8.

The histological and fluorescence features are examined in greater detail in a later section. However, it can be stated here, that sections of cord examined by fluorescence microscopy revealed EBA in vessel walls confirming the disruption of the blood-cord barrier. The sections of the paraffin-embedded material appeared normal.

The effect of noradrenaline on spinal cord blood flow after disruption of the blood-cord barrier.

12 µg/min infusion: The effect of this concentration of NA upon SCBF at the L_{5-6} level of the cord before and after blood-cord barrier disruption with urea is shown in absolute values in Table 4 and Fig.9 and expressed as the percentage change of control in Fig. 10. Before disruption of the barrier, NA was without overall effect on SCBF with the exception of the first measurement, which was significantly increased ($p < 0.05$). However, this flow figure is not significantly different from control when expressed in absolute values using the same statistical test. (Students t-test for paired data)

Following/

Following disruption of the barrier SCBF was reduced significantly in the second and third clearances (Table 4: Fig.9). The large scatter in Fig. 10 is due to the results from two electrodes (one experiment) where blood flow doubled in response to NA, otherwise the tendency was for flow to decrease.

The effect of a 12 µg/min. infusion of NA upon flow at the L_2 segment of the cord is shown in Table 4 and compared with the same infusion at the L_{5-6} level in Fig. 9. NA given prior to barrier disruption had no significant effect on SCBF. Following disruption, a significant decrease in flow was observed on the third measurement.

30 µg/min infusion: The results at the L_{5-6} level are shown in Table 5 and Figs. 9 and 11. Although the first flow following NA infusion was significantly increased ($p < 0.02$) the two subsequent flows showed no significant difference from control values.

After blood-cord barrier disruption there was a marked and progressive decrease in SCBF in response to NA infusion. In three of these experiments (6 electrodes), there was a complete cessation of flow following disruption of the barrier.

At the L_2 level a similar but less marked reaction occurred in response to an infusion of 30 µg NA/min. Again, the first flow following NA infusion was significantly increased ($p < 0.05$) and after barrier disruption a progressive decrease in flow occurred. This flow reduction was significant in the last two measurements. The results are presented in Table 5 and Fig. 9.

Neither blood pressure nor pCO_2 before and after blood-cord barrier disruption in both the 30 µg and 12 µg experiments differed significantly from control. The results for blood pressure are presented in Table 6 and for CO_2 in Table 7.

The effect of ∝ adrenergic blockade

∝-adrenergic blockade was by itself, without significant effect/

effect upon SCBF, although mean arterial blood pressure was significantly reduced from 139 $\pm$ 9 mm. Hg. (mean $\pm$ SD) to 87 $\pm$ 13 mm.Hg. However, it abolished the pronounced vasoconstrictor action of an infusion of 30 μg NA/min after blood-cord barrier disruption; the response being significantly different from that with NA and barrier disruption alone ($p < 0.001$ by grouped analysis). The results are presented in Table 8 and the responses of the individual clearances are in Fig. 12.

DISCUSSION

Blood-cord barrier disruption

The blood-brain barrier consists anatomically of tight junctions between the adjacent endothelial cells of venules, capillaries and arterioles. In addition these cells exhibit a low rate of pinocytotic activity (Reese & Karnovsky, 1967, Westergaard & Brightman 1973). This arrangement, together with large amounts of degradativ enzymes, especially monoamine oxidase within the endothelial cells (Pardridge, Conner & Crawford 1975) effectively prevents the entry of amines from the blood into the brain and spinal cord parenchyma. To study the effect of a systemically administered, barrier-limited, pharmacologically active agent on SCBF, it is necessary to disrupt this barrier, preferably in a manner that will minimally affect other parameters.

Rapoport, Hori & Klatzo (1972) have described various methods by which the blood-brain barrier may be disrupted and have investigated in detail the ability of intra-arterially administered solutions of urea to reversibly open the barrier (Rapoport, Bachman & Thompson 1972, Rapoport, Hori & Klatzo 1971, Rapoport & Thompson 1973). Brightman, Hori, Rapoport, Reese & Westergaard (1973) have produced electron microscopic evidence that a hyperosmotic solution of 3M urea may result in the opening of endothelial junctions through which horse-radish peroxidase can pass from the blood to the extracellular fluid of the brain. Pickard, McKenzie, Durity, Welsh, Langfitt & Jennett (1975) have reported that intracarotid infusion of hypertonic urea opened the blood-brain barrier to Evans blue albumin and penicillin G but did not affect cerebral perfusion, metabolism, autoregulation or CO_2 reactivity.

The present study demonstrates that 2.5 M urea will effectively disrupt the blood-cord barrier, as evidenced by the presence of Evans blue albumin complex within vessel walls and perivascular spaces and the urea itself does not alter SCBF.

The technique of multiple arterial ligations was chosen in preference to selective arterial catheterisation, as this latter method/

method involves the injection of contrast materials which may themselves alter barrier permeability. Selective arterial catheterisation could also compromise blood supply to the level of cord being investigated. The ligations themselves do not alter SCBF as control measurements in these experiments are within the normal limits for SCBF as previously determined by the hydrogen clearance method (Griffiths, Rowan & Crawford, 1975).

The effect of noradrenaline on SCBF

The effects of NA on cerebral blood flow have been the subject of intensive study for many years (see relevant review in this thesis) and reported effects have ranged from vasoconstriction, to no change, to vasodilatation. Intra-arterially administered NA has a minimal effect on cerebral blood flow in the baboon during normocapnia, but has a significant vasoconstrictory action during hypercapnia, when the intraparenchymal vessels are near maximally dilated (Harper, Deshmukh, Rowan & Jennett 1972, McKenzie, McCulloch & Harper 1976). McKenzie postulated that the inability of NA to alter cerebral blood flow at normocapnia was possibly due to the presence of a blood-brain barrier to NA. If NA was administered following barrier disruption, large increases in cerebral blood flow were observed, a response which may be secondary to changes in cerebral metabolism, rather than a primary vascular action. (McKenzie, McCulloch, O'Keane, Pickard & Harper (1976))

In contrast, Raichle, Hartman, Eichling & Sharpe (1975) reported that stimulation of ascending noradrenergic cerebral pathways by carbachol resulted in a decrease in cerebral perfusion. Support for both views can be found in the work of Rosendorff & Cranston (1971) who demonstrated a dual, dose-dependent flow response to NA, smaller doses causing a rise in hypothalamic blood flow and larger doses a decrease in flow. At the larger dose, inclusion of phenoxybenzamine in the injectate prevented the vasoconstrictor action of NA. Rosendorff & Cranston suggested that at this dosage NA acted directly on vascular receptors but a metabolically/

metabolically linked event cannot be excluded. (Weight & Salmoiraghi 1967) In these experiments the blood-brain barrier was by-passed as test substances were injected directly into the hypothalamus.

In the present experiments, both concentrations of NA significantly reduced SCBF at the L_{5-6} segments of the cord following osmotic blood-cord barrier disruption, the higher dose producing the greater reduction. The same pattern was observed at the L_2 cord level but the degree of flow reduction was not as great as was produced by the same concentration at the L_{5-6} level, (Fig. 9). This could be explained on the basis of the relative blood supply to the two cord levels by the 4th lumbar arteries. The L_{5-6} level, receiving the greater proportion of flow from this source, would in turn receive a higher concentration of NA than the L_2 segment.

Prior to disruption of the barrier NA was without effect upon SCBF, with the exception of the first clearances, following the commencement of infusion of NA when increases in flow were seen. Significant increases were seen with a 30 μg/min infusion at both the L_2 and L_{5-6} levels, the increase at the latter level being more marked. The 12 μg/min infusion produced a non-significant increase at the L_{5-6} level and no effective change at the L_2 segment. The degree of changes can be related to the dosage administered and the relative blood supply to the cord segments, as outlined above.

These increases, however, were unexpected and are difficult to interpret. McKenzie et al (1976) using a non-invasive method of cerebral blood flow determination, demonstrated an increase in flow to NA following hyperosmotic blood-brain barrier disruption. They combined the flow studies with cerebral metabolic investigations and noted increases in the cerebral metabolic rates for oxygen and glucose in association with the flow increase. The present study used intraparenchymally placed blood flow electrodes, which necessarily are invasive. It is possible that the electrodes produced a localised damage of the blood-cord barrier/

barrier around the recording tip of the electrode, thereby allowing enough NA entry to the cord parenchyma to stimulate local neuronal metabolism and cause the increase blood flow in that area. Unfortunately it is not yet possible to apply the metabolic studies used in the brain to the spinal cord because of the complexity of its blood supply and drainage. The return of flows to more normal levels could then be explained on the grounds of a developing tachyphylaxis to the response.

Pre-treatment of animals with PBZ, which by itself was without effect on SCBF, prevented the marked decrease in flow caused by the higher dose of NA following barrier disruption. The evidence suggests that flow reductions are mediated via α-adrenergic receptors. With the present techniques it is not possible to determine whether these α-mediated reductions in SCBF are the result principally of a direct vascular action of NA or due to α-mediated changes in the metabolic demands of spinal cord tissue.

The present study does not give any indication of the amount of active NA needed to decrease flow as an unknown "dilution" occurs. The intra-arterially administered drug is directed to extraspinal tissue as well as to the cord itself. Only a small percentage of the amine which reaches the spinal cord will actually cross the barrier. It has been shown in the brain that after osmotic disruption of the blood-brain barrier with urea, there is a fourfold increase in permeability to NA (Hardebo, Edvinsson, McKenzie & Owman, 1977). In normal tissue the active concentration will be further reduced by neuronal uptake. Thus, an estimate of the true concentration of NA causing the flow reductions can not be made. However, considering the initial large concentrations of NA used to depress flow in the present experiments and taking into account the above diluting factors, it is likely that the active NA concentration is still in excess of that used by McKenzie et al (1976). The possibility therefore exists, as suggested by Rosendorff & Cranston (1971), that small doses could increase flow through metabolic stimulation, higher doses/

doses producing α-mediated reductions in flow.

The relevance of the present findings in the pathogenesis of acute spinal trauma will be discussed separately.

PART III

The Effect of Noradrenaline on the diameter of spinal pial arteries and arterioles

MATERIALS and METHODS

Cats weighing between 2 and 4 Kg. were used for these experiments. Anaesthesia was induced with a minimal dose of Thiopentone and maintained with a 1% solution of alphachloralose (60 mg/Kg I.V.). Endotracheal intubation was performed and 100% oxygen delivered via a respiratory pump whose rate and volume were adjusted to maintain normocapnia ($paCO_2$ = 32 mm.Hg.). Additional doses of alphachloralose were administered as necessary.

One femoral vein was cannulated for the administration of anaesthetic and other drugs. A femoral arterial cannula was used for the electromanometric measurement of arterial blood pressure and for the removal of blood samples, which were analysed for $paCO_2$, paO_2, pH and base deficit. Any base deficit was corrected with 8.4% sodium bicarbonate given by slow intravenous injection. End tidal CO_2 concentration was monitored continuously with an infra-red analyser* and displayed on a Devices chart recorder. Body temperature was maintained between 37-38°C with a homeothermic blanket and where necessary with the addition of heating lamps.

Following a paraspinal incision extending from behind the last rib to the iliac crest, the musculature covering the right lateral aspect of the lumbar spine was removed. A high speed dental drill was used to perform up to three hemilaminectomies in each animal. During drilling, the burr was cooled by a jet of saline. The hemilaminectomy measured approximately 1 x 0.5 cm. in each case. The dura in these areas was removed, special care being taken to leave the underlying arachnoid mater intact, as tears would lead to leakage of cerebrospinal fluid. The cut dural margins were sealed by bipolar diathermy. Throughout the surgical procedure strict haemostasis was observed. The skin was sutured to a tubular metal frame supported by a retort stand, which was heavily weighted to prevent movement of the frame. This arrangement formed a bath for heated mineral oil which constantly irrigated the operation site./

* Capnograph Godart

site. The surface spinal cord temperature was maintained at 38°C by controlling either the flow rate or the temperature of the oil.

Spinal pial arteriolar calibre was measured by a modification of the television image-splitting technique of Baez (1966). The vessels were visualised with a triocular Bausch & Lomb stereomicroscope at a magnification of x40 or x70. The image was passed through an image-splitting device (Vickers) situated on the vertical eyepiece of the microscope to an attached television camera (Grundig FA70) and displayed on a video screen. The shearing screw of the image-splitter was connected to a potentiometer and thence to a Servoscribe pen recorder. Thus not only could the degree of shear be visualised directly on the video moniter but a permanent record of the measurement could be obtained. The degree of shear necessary to split the image of the vessel is directly proportional to the calibre of the vessel. Illumination of the area was provided with the use of a cold, fibre-optic light source (Schott).

Before measurements began, the system was calibrated against nylon filaments of known diameter. Thus the absolute diameter in um could be calculated.

The solutions under investigation were drawn into sharpened glass micropipettes which had a tip diameter of approximately 8 μm. (The manufacture of the pipettes is described in Appendix 2). The tops of these were sealrd with oil and the tips submerged in the bulk of the solution, again under oil; thus minimising any change in pH of the solution in the pipette. These pipettes were used as soon as possible after preparation.

Application of solution to a spinal arteriole was carried out in the following way - the pipette was inserted into a pipette holder, which was connected to a hydraulic syringe and the holder mounted on a micromanipulator (Leitz). By careful manoevring, the pipette tip could be placed into the subarachnoid /

subarachnoid space adjacent to the vessel under test. Judicious application of pressure to the syringe resulted in the release of approximately 2 µl of solution into the perivascular space.

After each application of test solution, succesive image-shearing manipulations were carried out until the arteriolar diameter had returned to its previously determined base-line. The maximum response to the injected solution was compared to those control observations.

In these experiments, not only were the effects of several concentrations of NA upon spinal arterioles examined, but the effect of NA in the presence of the α-adrenergic blocking agent phentolamine was assessed. The effect of mock cerebrospinal fluid (CSF) alone was also examined.

Test solutions were made by dissolving noradrenaline hydrochloride in artificial CSF containing Na^+ 156 mM, K^+ 3 mM, Ca^{++} 1,5 mM, Cl^- 151 mM and HCO_3^- 11mM.* The solution was covered by a layer of mineral oil and a final pH of 7.15 achieved by bubbling a 5% CO_2/95% O_2 gas mixture through it. The osmolarity of the mock CSF was 300 m.osm/litre.

NA was applied in concentrations between 5×10^{-8} and 5×10^{-3} M. The reaction of spinal pial vessels to two concentrations of NA (5×10^{-5} and 5×10^{-3}M) was investigated when phentolamine was was added to the injectate at a concentration of 5×10^{-5}M.

In most experiments, due to the small number of arterioles per hemilaminectomy site, vessels were examined more than once. However, where possible different sites on that vessel were assessed. Where the same site was examined, at least half an hour was allowed to elapse before re-investigation.

The method of perivascular application of test substances and the measurement of vessel calibre are essentially similar to the technique performed on the brain cortex by Wahl, Kuschinsky, Bosse and Thurau (1972).

* Artificial CSF made with double - distilled water.

RESULTS

The pial arterioles investigated had resting diameters in the range 50 μ to 240 μ.

All perivascular applications were performed at normocapnia (31.9 ± 1.2 mm. Hg.) and normotension (111.7 ± 20.7 mm.Hg.). Mock CSF alone, applied to spinal pial vessels, elicited a dilatatory response of 8.4 ± 6.5% ($\bar{x}$ ± SD). Because of this reaction the effect of CSF alone in a particular experimental animal was subtracted from the subsequent results obtained for NA, phentolamine and NA + phentolamine. The significance of the response to CSF will be discussed later.

Significant decreases in vessel wall diameter were seen with all the concentrations of NA, with the exception of 5×10^{-8} M, NA. At this dosage, a non-significant constriction was noted. Constrictor responses to NA were seen only at the injection site. During micro-application of test substance there was no change in either systemic blood pressure or end-tidal CO_2. Moreover, no correlation between initial vessel size and the degree of response to NA was noted.

Fig. 13 presents the effect of the various concentrations of NA upon the pial arterioles. Although a maximum constriction of 28.8 ± 5.1% ($\bar{x}$ ± SD) occurred in response to an NA concentration of 5×10^{-3}M (the highest concentration used in the study). Fig. 13 reveals a tendency for the response to reach a plateau at concentrations higher than 5×10^{-5}M.

The effect of phentolamine upon the constrictor action of 5×10^{-5} and 1×10^{-3}M, NA is shown in Fig. 14. An equimolar concentration of NA and phentolamine prevented the constrictor response of 5×10^{-5}M, NA. However, 5×10^{-5}M phentolamine only partially prevented the response to the more concentrated solution of NA.

Phentolamine applied alone produced a non-significant dilatation of 1.0 ± 3.2% ($\bar{x}$ ± SD).

DISCUSSION

The effect of NA on SCBF need not be due to direct effect on the blood vessels but alterations in blood flow could be secondary to changes in spinal cord metabolic/neuronal activity. For example, it has been shown by Weight & Salmoiraghi (1967) that microiontophoretic application of NA causes depression in the firing rates of α-motoneurones in the spinal cord. It was therefore decided to investigate the effect of NA on spinal cord arteries and arterioles by the direct application of the amine on to the pial vessels. Using this method, the blood-cord barrier and possible metabolic/neuronal effects could be avoided.

Early work utilising the 'pial window' technique in the brain (Forbes, Finley & Nason 1933, Fog 1939) involved either dripping the test substance on to the brain surface or flushing the entire field of observation with the test solution. It is possible that in such situations, especially with high concentrations of catecholamines changes in neuronal function, with secondary changes in blood flow and vessel diameter could have occurred. The present experiments, using small volumes of very locally applied test solution to the perivascular environment makes this possibility much less likely. Moreover, Wahl, Kuschinsky, Bosse, Olesen, Lassen, Ingvar, Michaelis & Thurau (1972) noted that repeated application of the same concentration of NA to a vessel resulted in a decreased response. By utilising the vessels in the manner described in the Methods section, the problem of a developing tachyphylaxis to NA was minimised.

The net dilatory effect of mock CSF alone, when applied to spinal pial arterioles, was unexpected and is difficult to explain satisfactorily. The CSF employed was prepared in a manner identical to that of Wahl et al (1972). In their experiments CSF of pH 7.15, containing 11 mEq/l HCO_3^- induced no change in arteriolar calibre. In a subsequent study by the same group (Kuschinsky & Wahl 1975) a dilatation of 2.5% to the same CSF was noted. In the laboratory where the present work was carried out,/

out, cerebral pial microapplication studies revealed an overall change of $0 \pm 1.4\%$ ($\bar{x} \pm SE$) to mock CSF (Harper & Mckenzie 1977). The CSF used by these authors was prepared and administered in a manner identical to that of the present study. Thus the difference in response cannot be explained on methodological grounds. It is possible that the dilatation is related to a difference in the natural CSF content at the surface of the lumbar cord compared to that at the cortical surface. No reliable studies of the ionic content of lumbar CSF have been carried out for the cat. However, Wei, Raper, Kontos & Patterson (1975) compared the composition of "normal" cat brain CSF as detailed by Levasseur, Wei, Raper, Kontos & Patterson (1975) to the mock CSF used by Wahl and his colleagues. Two major differences can be seen. The pH of the former is more alkaline with a HCO_3^- concentration of 25 mEq/l versus 11 mEq/l in the case of Wahl et al. If such a situation pertained to the cord, then application of mock CSF with a lower pH & HCO_3^- content could modify the local perivascular environment to elicit a dilatory vascular response. Regional differences in CSF content have been inferred by Kuschinsky, Wahl, Bosse & Thurau (1972) who demonstrated a CSF pH gradient between the cisterna magna, cerebral subarachnoid space and perivascular space.

The situation is even more complex as not only have HCO_3^- and H^+ been implicated in the regulation of pial arteriolar tone, but other ions present in naturally occurring CSF are involved. The importance of K^+ (Knabe & Betz 1972) and Ca^{++} (Betz, Brandt & Csornai 1975) has been investigated and it is possible that other ions such as Mg^{++} and Cl^- also play a part. Thus a complex series of interactions take place in the maintenance of resting vessel tone. Clearly if artificial CSF with different ionic concentrations from naturally occurring CSF is applied to a perivascular site, the resultant modification of the milieu could result in a vessel response.

Vasoconstriction of cerebral pial arterioles to topical adrenaline was demonstrated in 1933 by Forbes, Finley & Nason and/

and by Fog in 1939. Similarly, Fraser, Stein, Barrett & Pool (1971) found a constrictor response of the basilar and vertebral arteries on irrigation of the subarachnoid space with NA. In contrast to this, Raper, Kontos, Wei & Patterson (1972) found no reaction of pial vessels when artificial CSF containing the amine was flushed through a cranial window preparation. Likewise no vessel response to sympathetic stimulation was observed. They explained this lack of response on the absence of sufficient vascular receptors for NA. However, using a microapplication method Wahl et al (1972) demonstrated a dose-dependent constriction to NA. The difference in the last two reports can be explained by the finding of Wahl et al that CSF containing 22 mEq/l of HCO_3^- causes a 20% constriction in vessel diameter. The HCO_3^- content of Raper et al's CSF was 25 mEq/l. They were apparently applying NA to vessels already constricted by HCO_3^-, thereby masking any NA induced effect. A subsequent study by Wei, Raper, Kontos & Patterson (1975) revealed constriction of pial arteries $>$ 100 u in diameter to topically applied NA.

There is adequate evidence that in the brain NA produces a constriction of pial arteries and arterioles. The present results indicate that the same situation occurs in the spinal cord. When the effect of the CSF is subtracted the present results (Fig. 13) are similar to those of Wahl et al (1972) ie. a dose-dependent constriction of spinal pial vessels occurs in response to microapplication of NA.

Furthermore α-adrenergic blockade by phentolamine gave similar results to that of Kuschinsky & Wahl (1975). The manner in which phentolamine blocked NA-induced constriction (Fig. 14) is suggestive of competitive antagonism. The results indicate that the response of the vessels to NA is mediated via α-adrenergic vascular receptors.

Thus an analgous situation to the brain occurs also in the spinal cord. Noradrenergic innervation of spinal pial and intra-parenchymal vessels has been shown to exist. (Ohgushi 1968, Edvinsson, personal communication 1977) and vasconstrictory responses to topically applied NA occurs. Although no experiments investigating/

investigating the effect of sympathetic stimulation on SCBF have been carried out, it would seem that the arguments pertaining to the possible neurogenic control of the cerebral circulation apply also to the spinal cord.

The results of this study lend support to the promise that the NA mediated decrease in SCBF reported in the previous study are due, at least in part, to a direct vasoconstrictor action of NA, mediated via vascular α-adrenergic receptors.

PART IV

The effect of intraspinal injections of Noradrenaline on the histological appearance of the cord.

MATERIALS and METHODS

Eight unselected cats weighing between 2.4 and 4.5 Kg. were used in the experiments. Five cats were used to study the effects of several concentrations of NA injected intra-medullary upon the histological appearance of the spinal cord. The effect of the highest concentration of NA used in this study (35 µg/20 µl) upon spinal cord histology in three cats pretreated with the uptake blocker, desipramine, was investigated.

Anaesthesia was induced with a minimal dose of thiopentone and maintained with a 1% solution of alphachloralose (60 mg/Kg IV). Endotracheal intubation was performed and a 50% nitrous oxide/ oxygen mixture was delivered through a respiratory pump. The rate and volume of the pump was adjusted to maintain normocarbia ($paCO_2 \simeq$ 32 mm.Hg.) during surgery and the subsequent manipulations. Muscle relaxation was achieved with succinylcholine (1 mg/kg IV).

One femoral vein was cannulated for the administration of anaesthetic and other drugs. A femoral arterial cannula was implanted to allow the electromanometric measurement of arterial blood pressure and for the removal of blood samples which were analysed for $paCO_2$, pO_2, pH and base deficit. Any significant base deficit was corrected with 8.4% sodium bicarbonate given by slow intravenous injection. Rectal temperature was measured with a mercury thermometer and heating lamps were used to maintain the temperature between 37 - 38°C.

The animal was placed in sternal recumbency and a 3 - level dorsal laminectomy was performed to expose the L_2, L_{3-4} and L_{5-6} segments of the spinal cord. The bone was sealed with bone wax and epidural fat removed.

The cat was then suspended in the following way. Two Steinman pins were inserted through appropriate dorsal spines anterior and posterior to the operation site. These pins were attached to 2 steel bars, one on each side of the animal, arranged parallel to its longitudinal axis. The bars were supported by retort stands bolted to the operating table. This arrangement effectively/

effectively eliminated the transmission of respiratory movements to the spinal cord.

The skin margins of the original incision were sutured to the bars to create a bath for heated mineral oil which constantly irrigated the operation site. Surface spinal cord temperature was maintained at 38°C by controlling either the flow rate or the temperature of the oil.

Small, laterally placed incisions were made in the exposed dura by gently and repeatedly stroking a small area of the dura with the tip of a scalpel blade. These incisions were performed under the bath of warmed mineral oil.

When surgery was complete, a 2% solution of Evans blue dye with 5% Fraction V bovine albumin (EBA) made up in normal saline was administered by slow intravenous injection at a dosage of 3 ml/Kg.

Spinal injections were performed using sharpened glass micropipettes. These were similar to those described in an earlier section of this thesis, with the exception that the present study utilised pipettes with a tip diameter of approximately 15 µm. (Their manufacture is described in Appendix 2) The pipettes were graduated so that the volume of solution injected could be assessed at any time during the injection period.

Test solutions were prepared by dissolving noradrenaline hydrochloride in artificial CSF. The ionic content of CSF, preparation of NA solutions and method of charging and sealing the pipettes has been described earlier.

The volume of CSF employed in each injection was 20 µl, containing either 5, 12, 15, 30 or 35 µg NA. The effect of 20 µl CSF alone on spinal cord histology was also assessed.

The injections were carried out as follows - a pipette was inserted into a pipette holder attached to a hydraulic syringe and the holder mounted on a micromanipulator (Narashigi). The pipette tip was carefully manoevred through a dural incision and inserted into the grey matter of the spinal cord. 20 µl of the test/

test solution was injected, the rate of injection being controlled manually. Not more than 2 injections were performed at each laminectomy site.

Desipramine pretreated cats were prepared in an identical manner to that described above, with the exception that following EBA administration, desipramine was administered at a dosage of 2 mg/Kg, given by slow intravenous infusion over 30 minutes. The effect of injection of 35 μg NA in 20 μl was investigated in these animals. Injections in these cats commenced 30 minutes after termination of desipramine infusion.

Two hours after the last injection was made, the animals were given 1,000 units of Heparin intravenously. The left thoracic wall was incised and the aortic arch was cannulated in an orthograde direction. A two-stage perfusion was performed utilising a weak and a strong paraformaldehyde-glutaraldehyde mixture. The weak fixative was administrered at a temperature of 37°C and the strong at approximately 4°C (Karnovsky 1965). A cannula placed in the right ventricle allowed release of blood and excess fixative. Perfusion pressure was maintained at or up to 10 mm. Hg. above the mean arterial blood pressure measured through the arterial cannula.

The cords were removed 2 hours after perfusion and further fixed in 2.5% glutaraldehyde overnight. 1 mm^2 blocks of spinal grey matter were taken from each injection site and control blocks were removed from caudal thoracic segments. These were osmicated, dehydrated and embedded in Epon. 1 μm sections were cut and stained with toluidine blue.

RESULTS

a) A total of thirty-two intraspinal injections were performed. Nine injections were made with CSF alone, five with 5 μg NA, nine with either 12 or 15 μg NA and nine with either 30 or 35 μg NA included in the injectate. The mean injection time was 132 ± 37 secs ($\bar{x}$ ± SD) with a range 50 - 216 secs. Mean arterial blood pressure was 124 ± 7 mm. Hg ($\bar{x}$ ± SD). Intraspinal injection had no effect upon blood pressure. There was no evidence of gross EBA extravasation when the injection sites were examined prior to preparation for microscopy except in one case where haemorrhage and EBA extravasation had occurred as a focal circular area within white matter.

Sections of caudal thoracic segments were taken to serve as controls. The microarchitecture of the spinal grey matter was normal in these areas (Fig. 15). Occasional erythrocytes could be seen in some blood vessels but in general the clearing of vessel lumina by the perfusion fixation technique was good. Occasional artifactual enlargements of perivascular spaces were observed otherwise no abnormalities were present.

Effect of CSF injections: The effect of 20 μl injections of CSF on the histological appearance of the cord was variable. In three injection sites no abnormalities were observed. In 5 injection sites the most common appearance was that of oedema. There was generalised looseness of the neuropil with perivascular and perineuronal astrocytic swelling. Where the injections were made at grey/white matter junctions, the white matter also showed evidence of oedema (Fig. 16). A few neurones were pale staining with loss of peripheral Nissl substance and occasionally darkly-stained shrunken vacuolated neurones were observed. The areas where the described changes occurred were focal and well defined. Outwith these areas the spinal cord architecture was normal. In one case where an injection was made into white matter, haemorrhage and oedema was observed (Fig. 17).

Effect of 5 μg NA injections:/

Effect of 5 µg NA injections: Injection sites receiving CSF containing 5 µg NA showed no observable differences from sites receiving CSF alone. Three areas appeared normal and two showed evidence of oedema as described above.

Effect of 12 - 15 µg NA injections: Three injections with 12 µg NA in CSF were made. Two sites showed no difference from control sections. In the remaining injection, a volume of 40 µl CSF was injected into the spinal cord parenchyma in 50 secs. The effect was to create an area of cavitation at the injection. There was compression of cord tissue at the periphery of the cavity and the surrounding grey matter showed evidence of gross oedema with swelling of neuroglial elements and shrunken, vacuolated, degenerating neurones (Fig. 18). Fig. 19 illustrates the severe perivascular and perineural astrocytic swelling which was typical of the grey matter appearance surrounding the cavity.

Of the six injections made, utilising a concentration of 15 µg NA in 20 µl CSF, three sites showed no evidence of histological change. The remaining three sites showed perivascular and perineuronal swelling with swelling of neuroglial processes evident within the neuropil. Fig. 20 illustrates this appearance. Neuronal changes of the kinds described earlier were minimal. As with the previous injections, these areas of oedema were well defined, focal areas surrounded by normal spinal cord tissue.

Effect of 30 - 35 µg NA injections: The effects of injections of 30 or 35 µg NA were essentially similar to those of the preceding groups. Of the three sites into which 30 µg NA in CSF was injected, two appeared histologically normal (Fig. 21) and one showed moderate oedema with pale staining neurones exhibiting loss of peripheral Nissl substance.

Six sites were injected with 35 µg NA in CSF. Four appeared normal and two showed the oedematous picture previously described for the injections involving CSF alone. Fig. 22 illustrates a typical example.

b)/

b) Effect of CSF and 35 µg NA in CSF injections on the spinal cords of Desipramine pre-treated animals.

A total of 16 injections were made in these animals. Six injections utilised the injection of 20 µl of CSF alone and ten injections were made with 35 µg NA in 20 µl CSF. The mean time for injection of this volume was 118 ± 10 secs ($\bar{x}$ ± SD) with a range of 90 - 130 secs. Mean resting arterial blood pressure before the intravenous administration of desipramine was 128 ± 11 mm.Hg. ($\bar{x}$ ± SD). Following administration, the mean pressure did not differ significantly from this mean (122 ± 9 mm.Hg).

Control sections, taken from caudal thoracic segments of the cord, were histologically normal. Of the six injections made with CSF alone, four sites showed a normal histological appearance. The remaining two sites showed evidence of oedema with perivascular and perineuronal astrocytic swelling as previously described for CSF injections in the non-treated animals. Neuronal changes were minimal.

The effect of injections of 35 µg NA in CSF was also variable. Of ten sites in which injections were performed, seven showed no sign of histological abnormality (Fig. 23), while in three sites oedema with perineuronal and perivascular swelling was evident. (Figs. 24 a), b) & c)).

DISCUSSION

In the course of experiments designed to test the hypothesis that NA was an important factor in the pathogenesis of the central haemorrhagic lesion typical of acute injury to the spinal cord. Osterholm & Mathews (1972a) and Hill & Osterholm (1973) injected NA directly into the cord and examined the effects histologically. They noted that injections of 35 µg and 100 µg NA given in a volume of 10 µl saline over 5 mins caused lesions of a size 4.6% and 8.0% of the cross-sectioned cord respectively. Areas of haemorrhage and necrosis, localised about the needle tip (35 µg) and spreading 2 mm over grey matter (100 µg), were found in specimens injected with NA. Control animals injected with saline alone were found to exhibit some oedema and laking but no central haemorrhages. The technique of intraspinal microinjection utilised was that developed by Sinha, Ducker & Perot (1971, 1973).

In the present study, injection of NA over a range of 5 - 35 µg did not produce the central haemorrhage and necrosis obtained by the above authors. The most prominent change was oedema, which was also present in control areas injected with CSF alone. In only one case was haemorrhage seen. This was most likely due to direct trauma to intraspinal vessels by the pipette tip. The presence of oedema in the present study is most probably a function of the volume of injectate given and the time over which it was administered. Osterholm (1974) stated that if > 10 µl/min of solution is given, injection trauma results in histopathological changes similar to those following blunt injury. This was not found to be the case in this study. Indeed, in one site where 12 µg NA was given in a volume of 40 µl CSF over 50 secs. no haemorrhagic necrosis occurred. Rather, an area of cavitation with surrounding severe oedema was seen. It could be postulated that the excess interstitial fluid interfered with the normal passage of oxygen from the capillaries to the neurones, thus leading to gradual deterioration of the neuronal elements, (Blackwood 1967) a feature seen in this site. Osterholm (1972a) found oedema and laking in his control animals/

animals 2 hours after the injection of 10 µl saline over a 5 minute period. The formation of oedema in cords in the present study is therefore most probably the effect of excessive injection times and volumes.

The great disparity in results between the present work and those of Osterholm & Mathews (1972a) and Hill & Osterholm (1973) is difficult to explain satisfactorily. Kakari, De Crescito, Tomasula, Flamm & Campbell (1973) performed intraspinal injections of NA in cats and noted that haemorrhagic necrosis appeared not only in cords receiving 35 µg NA in saline but also in those receiving an equal volume of saline alone. The extent of the haemorrhagic necrosis was however greater in the former group. Two points of difference exist between these studies and the present one. Sharpened glass micropipettes of 15 um OD were used in the present study, compared to the much larger steel needles utilised in the other studies. The likelihood of traumatic damage to the cord by the larger needle is greater. Moreover, the spine must be held absolutely rigidly or respiratory movements of the cord would exacerbate the trauma of injection. In such a situation it would not be surprising if a potent vasoconstrictor substance like NA increased the extent of damage elicited by needle trauma.

Secondly, the vehicle used for NA injection in the other studies was saline. In the present experiments artificial CSF was used. Following addition of NA to the CSF, the pH of the solution was adjusted to 7.15 to eliminate possible effects of extreme pH. In the other studies no mention of buffering the NA/saline solution was made. The solution injected in these studies must therefore have been very acidic. The cumulative effect of trauma, extreme low pH and NA could possibly explain the results obtained in these studies.

The lack of histopathological change to injected NA in the present experiments would suggest that either NA does not cause the development of haemorrhagic necrosis or that the exogenous NA is rendered inactive by avid neuronal uptake. No evidence of haemorrhagic necrosis was seen 2 hours after intrapsinal injection of 35 µg NA in animals in which neuronal uptake blockade by desipramine had been induced.

It is, therefore, concluded that NA in the doses utilised is not/

is not by itself responsible for the development of haemorrhagic necrosis typical of acute blunt injury to the spinal cord.

PART V

The Effect of Noradrenaline on the histological appearance of the spinal cord after blood-cord barrier disruption with urea.

MATERIALS and METHODS

6 unselected cats weighing between 2 and 4 Kg were used for the experiments. Anaesthesia was induced with a minimal dose of thiopentone and maintained during surgery with a 50% nitrous oxide/oxygen mixture and 0.5 - 1% Halothane. The mixture was delivered through a respiratory pump whose rate and volume were adjusted to maintain normocarbia ($paCO_2 \simeq$ 32 mm.Hg.). On completion of the operative procedures, the Halothane was discontinued and anaesthesia maintained with 1% alphachloralose (60 mg/Kg IV).

One femoral vein was cannulated for the administration of anaesthetic and other drugs. Both femoral arteries were cannulated, one for administration of urea, or urea and NA and the other for the continuous electromanometric recording of arterial blood pressure and the removal of blood for the analysis of $paCO_2$, paO_2 and pH. Any significant base deficit was corrected with an 8.4% solution of sodium bicarbonate administered intravenously.

Rectal temperature was measured with a mercury thermometer and the temperature maintained within 37 - 38°C by heating lamps.

Following midline laparotomy incision, all branches of the posterior aorta caudal to the renal arteries, with the exception of the lumbar arteries, were ligated. The abdominal surgery was identical to that described in the dogs and illustrated in Fig. 4 except that, in this series, the 5th left lumbar artery was not cannulated. The femoral artery cannula through which test substances were administered was adjusted so that its tip was at the level of the 4th lumbar arteries. The abdominal incision was then sutured and the animal placed in lateral recumbency.

Following surgery and injection of alpha chloralose, a 2% solution of Evans blue dye with 5% Fraction V bovine albumin (EBA) made up in normal saline was administered by slow intravenous injection at a dosage of 3 ml/Kg.

a) 3 cats prepared as described above were used to determine the effect of urea on the histological appearance of the spinal cord. 30 minutes after the administration of EBA, 6 ml/Kg of 3 or 3.5 M urea/

urea was steadily given over a period of 5 mins. via the femoral cannula. The animals were then left for 2 hours after which perfusion fixation was carried out (see below).

b) 3 cats were used to determine the effect of NA upon spinal cord histology after blood-spinal cord barrier disruption induced by the intra-arterial infusion of urea. Immediately following the administration of urea at a dose and rate described in a), 2 µg NA/Kg/min. was delivered via the arterial cannula. (This value approximates closely by weight to the higher dose of NA given to the dogs in the blood flow studies) Two hours after the termination of NA infusion, perfusion fixation was carried out.

Perfusion fixation in each of the above sections was preceded by the intravenous injection of 1,000 units of Heparin. Fixation in each animal was carried out as follows. The left chest was opened and the aortic arch cannulated in an orthograde direction. A two stage perfusion was performed using 0.5 L weak (at 37°C) and 1.5L strong (at 4°C) paraformaldehyde-glutaraldehyde mixture (Karnovsky 1965). Perfusion pressure was maintained at or up to 10 mm. Hg. above the mean systemic arterial blood pressure as measured through the femoral arterial cannula. The total perfusion time was about 15-20 mins. Following perfusion, the cords were removed after 2 hours and further fixed in 2.5% glutaraldehyde overnight. 1 mm^2 blocks were taken from lumbar segments of the cords at areas where barrier disruption had occurred (as indicated by blue staining of the cord with EBA) and from control blocks (non-stained) taken from caudal thoracic segments. These were osmicated, dehydrated and embedded in Epon as outlined in Appendix 3. 1 µm sections were cut and stained with Toluidine blue.

Other blocks were processed in the histokene and embedded in paraffin wax. Sections cut at 8 u were stained with haematoxylin and eosin (H/E), cresyl violet and Martius scarlet blue.

Further blocks were frozen in liquid nitrogen, mounted on chucks and sections cut at 10 µ on a cryostat. The sections were mounted/

mounted in 50% glycerol and examined in a fluorescent microscope using incident illumination. Excitor and barrier filters were combined with a chromatic beam splitter. The excitor filter allowed passage of UV light of λ below 500 nm and the barrier filter allowed λ of above 528 nm to pass.

c) An additional cat was used to determine the distribution of intra-arterially injected substances in animals ligated as described above. 1 ml. of Conray was injected intra-arterially via a femoral cannula adjusted such that its tip was at the level of the 4th lumbar arteries. The animal, lying in lateral recumbency, was radiographed, during the injection, utilising a Puck rapid film exchanger to take serial films over 1 minute.

RESULTS

a) The effect of urea on blood pressure, blood-cord barrier and histological appearance of the spinal cord.

Following urea administration, mean arterial blood pressure was 139 $\pm$ 21 mm.Hg ($\bar{x} \pm$ SD), a value which was not significantly different from the resting pre-urea administration value (137 $\pm$ 16 mm.Hg).

EBA extravasation was seen microscopically in the feline spinal cords extending from the sacral to approximately the L_2 segments. Leakage occurred, predominantly in the grey matter, although it was occasionally observed in the white matter as scattered, focal, punctate areas. Some staining was also visible on the pial surface ventrally and ventrolaterally. On fluorescence microscopy, vessels with fluorescent walls were seen. These were mainly arteries, arterioles and capillaries, though small venules affected. (Figs. 25a & b). The degree of extravasation was small, the indicator being confined mainly to the vessel wall and immediately perivascular neuropil.

Urea had no effect on the histological appearance of the cord. The sections of paraffin embedded material appeared normal. Similarly the 1 µm sections stained with Toluidine blue were normal in appearance. Fig. 26 shows the appearance of a control section from a non- EBA stained block taken from the 12th thoracic segment. Fig. 27 illustrates the histological appearance from an EBA stained block obtained from the lumbar cord following urea administration. No abnormalities are detectable.

b) The effect of NA on blood pressure and the histological appearance of the spinal cord after blood-cord barrier disruption.

Administration of NA following barrier disruption with urea significantly increased blood pressure from a mean of 128 $\pm$ 15 mm.Hg. ($\bar{x} \pm$ SD) to 172 $\pm$ 13 mm.Hg ($p < 0.01$). This peak was reached within 2-3 mins of starting the NA infusion. The blood pressure gradually declined to a mean value of 145 $\pm$ 13 mm.Hg during the NA infusion/

NA infusion time.

Of the three animals given NA after blood-cord barrier disruption by urea, only one showed evidence of pathological change. Generalised oedematous swelling of cell processes was the prominent feature (Fig. 28) A number of blood vessels were also observed to contain erythrocytic clumps which were mainly confined to capillaries and arterioles. In this animal considerable difficulties were encountered in the perfusion-fixation process. A high perfusion pressure was necessary to infuse a reasonable volume of fixative.

Fig. 29 illustrates the normal histological appearance of the spinal cords following NA administration, typical of the other two animals.

c) The distribution of the injectate as demonstrated by angiography.

Fig. 30 illustrates the distribution of contrast media injected intra-arterially. The ventral spinal artery can be clearly seen. Ligation of the vessels referred to in an earlier section prevents loss of injectate to abdominal organs (with the exception of the kidney).

DISCUSSION

a) The effect of urea on blood pressure, blood cord barrier and histological appearance of the cord

Certain differences in the responses of cats to the administration of urea occurred. In pilot studies, (not reported here) the bolus injection of 2.5 M urea led to a severe and abrupt rise in blood pressure which was unacceptable. Moreover, on examination of the cord, both grossly and by fluorescent microscopy, barrier disruption was either absent or very minimal. It is probable that excessive pressure was used during the injection and much of the urea was lost to other tissues. However, a slower infusion of 2.5 M urea such that blood pressure was unaffected, did not always cause disruption of the barrier. A slow infusion of 6 ml of 3 or 3.5 M urea given over a period of 5 mins was found adequate to cause barrier disruption in all cases. However, the extent of the barrier disruption as demonstrated by gross extravasation of EBA was highly variable even within 1 - 2 segments of the cord.

The urea had no effect on the histological appearance of the cord and appears a useful method for the investigation of the effects of arterially administered barrier-limited, pharmacologically active agents on spinal cord function.

b) The effect of NA on blood pressure and the histological appearance of the spinal cord after blood-cord barrier disruption

The blood pressure rose approximately 40 mm.Hg. in each animal as the NA was infused. This is in contrast to the situation in the dog where there was no significant change in pressure. The blood pressure in the cats returned to resting values during the course of the NA infusion. The cat appears much more sensitive to intra-arterially administered NA than the dog.

In the one animal in which there was evidence of pathological change/

change in the spinal cord, difficulty was encountered in the perfusion fixation. It is possible that delayed fixation, due to the vasoconstriction induced by NA, resulted in the oedematous appearance of cell swelling.

However, there was no evidence of haemorrhage and/or necrosis of the type that Osterholm and Mathews (1972a) produced by intraspinal injections of NA.

c) The radiograph (Fig. 30) shows the distribution of a bolus injection of contrast medium administered intra-arterially. The outline of the ventral spinal artery is clearly seen showing that the preparation allowed the intra-arterially administered substances to reach the spinal cord. The test solutions used in the experimental situation were given at a much slower rate. There would not be as great a loss of test substance to the kidney, as occurred in the angiogram.

PART VI

A discussion of the possible involvement of noradrenaline in the pathogenesis of acute spinal trauma.

Both the light and electron microscopic appearance of spinal cords of various species after experimentally produced trauma have been reported. (Goodkin & Campbell, 1969, Ducker, Kindt & Kempe, 1971, Wagner, Dohrman, Bucy, 1971) Stepwise sequential changes were observed when cords were examined at various times after injury and a classical picture of progressive central haemorrhagic necrosis is generally accepted. In association with the development of this lesion, changes in blood flow occur. In the grey matter there is a progressive decrease in SCBF from normal pre-trauma values. (Kobrine, Doyle & Martins, 1975, Bingham, Goldman, Friedman, Murphy, Yashon & Hunt, 1975, Griffiths, 1976).

Osterholm and his colleagues have proposed a biochemical theory to explain the events following injury. Their catecholamine hypothesis states that excessive quantities of NA are synthesised by noradrenergic spinal nerve fibres in response to severe blunt trauma. The accummulated amine is progressively released from neurones into wounded tissue, inducing a maximal toxic vasospasm which impedes circulation, diminishes local oxygenation and produces necrosis of vessels and neurones, leading to the classical picture of haemorrhagic necrosis. These workers investigated the effect of NA in the following ways:

a) by measuring spinal NA concentrations before and after experimental injury to the cord;

b) by investigating the capability of NA to produce spinal lesions; and,

c) by evaluation of the possible modification of injury response by anti-catecholamine therapy.

These points will be discussed in relation to the findings of other workers and the results of the present experiments.

Osterholm and Mathews' discovery of increased NA levels after experimental spinal cord injury, stimulated others to investigate this phenomenon. The results of these studies have been reviewed elsewhere in this thesis and will, therefore, not be repeated here. However, it should be reiterated that some of these investigations can not be directly compared with one another/

another, due to differences in experimental design. If a broad conclusion can be inferred from the studies *in toto*, it is that the tissue concentration of NA in the spinal cord at the site of injury is unchanged or decreased following a traumatic insult.

Under physiological conditions NA within CNS tissue is stored almost entirely within neuronal granules and its main termination of action is by a neuronal uptake mechanism (Iversen 1973). While under normal circumstances one would expect that the neuronal uptake mechanism for catecholamine would remove NA from the interstitial fluid, in the hypoxic and ischaemic situation created by trauma this mechanism may be severely hampered. The possibility of local increases of NA in the interstitial fluid has been discussed by Wurtman and Zervas (1974).

However, tissue levels of amines before and after trauma are not the critical parameters from a functional point of view, in that it may be the active extracellular component which is important rather than the total tissue concentration. Thus investigations into the differential demonstration of active versus inactive NA in the cord after acute trauma could provide important information as to the role of NA in the pathophysiology of the lesion. In this context, 35 µg NA given by direct intraspinal injection after neuronal uptake blockade with desipramine failed to produce any haemorrhagic necrosis.

The second method by which Osterholm and co-workers examined the NA theory was by testing the amine's possible lesion-producing capacity (Osterholm & Mathews, 1972, Hill & Osterholm 1973). They reported that intraspinal injections of 35 and 100 µg NA in 10 µl of saline given over 5 mins produced lesions of haemorrhage and necrosis 4.6% and 8.0% of the cross-sectioned area of the cord, respectively. Kakari, DeCrescito, Tomasula, Flamm and Campbell (1973) also reported lesions after the intraspinal injection of 35 µg NA in cats. Injected NA is known to cause haemorrhagic sloughing of many body tissues (Oglesby & Baugh, 1968; Sethbhakdi, Pfeiffer & Roth, 1970) and Alksne & Greenhoot (1974) presented evidence that injection of NA into the prepontine cistern of the monkey/

monkey produced acute and delayed intracranial arterial spasm. Electron microscopic examination of the basilar artery removed during the second phase of spasm revealed mycnecrosis of the media, with fragmentation of myofibrils, dissolution of the sarcolemma and interstitial oedema. The animals remained clinically normal until sacrificed. The present author examined the effect of intraspinally injected NA over a wide range of concentrations (qv) but found no evidence of haemorrhagic necrosis even after neuronal uptake blockade of NA. The main pathological change seen was oedema formation with occasional dark, shrunken neurones, probably indicative of injection trauma. The presence of oedema in these specimens has been discussed. Light microscopical examination revealed no evidence of pathological change in blood vessels. Similarly, no sign of haemorrhagic necrosis was seen in the cords of cats in which intra-arterially administered NA had been given in a concentration equivalent to that which produced a profound decrease of SCBF in dogs.

Investigations into the modification of injury by anti-catecholamine therapy has been carried out by several groups. Osterholm and Mathews (1972b) reported that administration of α- methyltyrosine (a drug which prevents or diminishes the conversion of tyrosine to Dopa by inhibition of the enzyme tyrosine hydroxylase, and thereby significantly alters NAsynthesis) to experimental animals 15 mins. after cord injury, protected against haemorrhagic necrosis, reducing the area of the lesion by a factor of 8. However, Hedeman and Sil (1974) reported that α - methyl-tyrosine had no protective effect either on the extent of the central grey lesion or the clinical status. They noted that a high degree of protection was conferred by treatment with phenoxybenzamine, an α - adrenergic receptor blocker, but again this drug had no effect upon the extent of the lesion. Rawe, Roth & Collins (1977) likewise found no evidence of reduction of grey or white matter haemorrhages after either α - methyltyrosine or reserpine treatment but found a marked reduction in those animals pretreated /

pretreated with phenoxybenzamine. They concluded that the absence of the pressor response to spinal trauma (a result of phenoxybenzamine pretreatment) was of importance. Shoultz (1977) analysed the histopathological changes to trauma in catecholamine-intact cats and in cats depleted of catecholamines either by reserpine administration or by adrenalectomy. The sequential nature of the development of haemorrhagic necrosis was similar in both groups.

As with NA assay before and after trauma, a great deal of disparity is present in the literature. Much of it may be due to differing experimental methodologies, for example drug dosage, time of administration before or after trauma etc. However, the weight of evidence points to the possibility that the role of NA in the genesis of the spinal lesion is not as important as was once thought.

Co-existent with the sequential pathological changes after experimental cord trauma is a progressive decrease in SCBF. The effect of exogenously administered NA on SCBF was therefore examined. The present results show that NA in the doses utilised can adversely affect SCBF in the cord after barrier disruption. This study does not give an indication of the amount of active NA needed to decrease flow as an unknown "dilution" of exogenous NA occurs.(qv) However, considering the large concentrations of NA used to depress flow in the study and taking into account the diluting factors, it is unlikely that the quantity of NA present in the injured cord could by itself account for the progressive and profound reductions of flow seen in trauma.

While NA alone may not be the important factor in the genesis of traumatic spinal lesions, recent evidence points to the possibility of interaction between NA and other substances present in spinal tissue. Rosenblum (1975) has offered evidence that NA plus prostaglandin $F_2\alpha$ produces a significantly greater contractile response in cerebral pial arterioles than either agent can cause when applied alone. Jonsson & Daniell (1976) demonstrated that prostaglandin F is present in spinal tissue and levels dramatically/

dramatically increase following traumatic injury. Moreover, it has been shown that the contractile response of cerebral arteries to both NA and 5-H.T. is increased in the present of blood. (Svengaard, Edvinsson & Owman, 1977) It could, therefore, be postulated that amines in the presence of haemorrhage in the cord may play a role in the development of the arterial spasm.

5-H.T. may also play a role in the pathogenesis. Although platelets are known to adhere to damaged endothelium, it has also been shown that they can cause endothelial damage (Jørgensen, Hovig, Rowsell & Mustard, 1970). Raised tissue levels of 5-H.T. in thermal injury of the cortex has been attributed to release from platelet aggregates (Costa, Ito, Spatz & Klatzo, 1973, and Zivin, Doppman, Reid, Tappaz, Saavedra, Kopin & Jacobowitz, 1976) have demonstrated platelet aggregates in the spinal grey matter after injury.

In an investigation of the early vascular changes in spinal grey matter, following impact injury, Griffiths, Burns & Crawford (1978) have suggested that the initial events of perivascular haemorrhage, protein extravasation and some disruption of the neuropil, are a result of mechanical forces. It could be postulated that in this situation a number of endogenous substances may act in concert to produce vasospasm, continuing hypoxia and progressive haemorrhagic necrosis, typical of severe trauma to the cord. However, it is felt that NA by itself is not the most important factor in the development and progression of the lesion as was first suggested by Osterholm's group.

GENERAL CONCLUSIONS

The results of this thesis indicate:

1) 2.5M urea will effectively disrupt the spinal blood-cord barrier in dogs without alteration of SCBF.

2) NA in the doses utilised is capable of reducing SCBF following barrier disruption.

3) The degree of reaction of the spinal vasculature to NA is dose related.

4) The reduction in SCBF and vascular calibre to NA is mediated via α- adrenergic receptors.

5) NA does not cause lesions of haemorrhagic necrosis in the spinal cord either when infused intra-arterially after blood-cord barrier disruption or when directly injected into the cord.

6) It is suggested that despite the profound flow reductions which NA caused, it can not be held to be solely responsible for the propogation of the lesion seen in acute spinal trauma.

APPENDIX

1) The Measurement of the Acid-Base Status of Blood

pH, pCO_2, pO_2, HCO_3^- and base-excess levels were routinely measured from blood samples of experimental using a Corning pH Blood Gas Analyser 165C*. This machine contains a Clark type oxygen electrode, a Severinghaus-Stow type CO_2 electrode and a flow-through, glass capillary pH electrode with reference assembly. HCO_3^- and base-excess values were presented as calculated functions. The machine is calibrated such that each parameter can be read off directly.

a) The principle of pH measurement - pH is the negative logarithm of the chemical potential (or roughly the concentration) of the hydrogen ion. Chemical potential is analogous to partial pressure in gases, both being the measure of the force exerted by a concentration gradient. The pH electrode is a thin glass membrane which is permeable to H^+ ions but not to other ions. When a sample containing H^+ is exposed to the membrane, a potential is generated. In order to measure this potential, an Ag/AgCl reference electrode is placed in a stable reference solution surrounding the H^+-sensitive glass membrane and is directly connected to an electrometer. The other connection is formed between the sample in which the pH is to be measured and a saturated KCl solution (a liquid junction) which is in turn connected to a calomel reference electrode. The calomel electrode forms the other connection to the electrometer. Because of the high resistance of the glass membrane, the current flow caused by the relatively small potential difference generated by the system is very low, and a very sensitive stable electrometer must therefore be used.

* Corning Scientific Instruments, Medfield, Mass.

b) The principle of pCO_2 measurement - The CO_2 electrode consists basically of a membrane which separates the sample being measured from a bicarbonate solution in which is placed a glass electrode. The membrane is freely permeable to dissolved CO_2 but not to HCO_3^-. Thus, CO_2 diffuses from the sample into the bicarbonate solution, which is held in a thin layer next to the H^+-sensitive glass membrane. At equilibrium the change in pH of the bicarbonate solution is proportional to the pCO_2 of the sample, and this is measured by the glass electrode with a suitable reference electrode. The pH of the bicarbonate solution is given by the Henderson-Hasselbalch equation. Since the bicarbonate concentration can be considered to be constant, the equation can be simplified thus:

$$\log pCO_2 = \text{constant minus pH}$$

The analyser is calibrated such that the pCO_2 can be read off directly.

c) The principle of pO_2 measurement - The pO_2 electrode is based upon polarographic principles. It is made up of a negatively charged platinum wire covered with a polyethylene membrane. The end product at the cathode is OH^- which accumulates at the rate of 4 moles / mole of O_2. The current passing from the platinum electrode to the Ag / AgCl electrode in saturated KCl is directly related to the pO_2 at its surface.

2) The Manufacture of Micropipettes

Micropipettes are constructed from commercially made pipettes 15 cms. long, with an outside diameter of 1.0 mm. and a wall thickness of 0.16 mm. The pipette tips are drawn on a custom-made pipette puller (Sperre). A pipette is placed with its mid-point over a platinum heating element, whose temperature is controlled electrically. Each end of the pipette is clamped to a mounting which is connected to a spring. The springs are arranged in such a manner that when the /

the mid-point of the pipette is heated, and the glass begins to melt, the two springs release in opposite directions, separating the pipette into two sections. As the pipette separates, the heated part is drawn into a fine tip. The tip is further prepared by grinding it at an angle on a water-cooled grinding stone of the finest carborundum. This procedure is performed under a microscope. The result is a sharp, bevelled tip. The final diameter of the tip is measured under a microscope utilising a graduated eyepiece. By judicious grinding the required tip diameter can be obtained.

3) Processing of Spinal Tissue for Microscopical Examination

Following perfusion fixation of experimental animals, removal of spinal cord and overnight fixation in glutaraldehyde, $1mm^2$ blocks of tissue were cut under a dissecting microscope with a razor blade and processed as follows:

a) 3 rinses of 2 mins. each in phosphate buffer.

b) 2 hrs. post-fixation in osmium tetroxide*.

c) Brief rinse in phosphate buffer.

d) Dehydration in 50%, 70%, 80%, 90% and absolute alcohol respectively. The tissues spent 15 mins. in each concentration of alcohol. 2 changes of absolute alcohol are made within the last 15 mins. of dehydration.

e) Clearing with propylene oxide - 30 mins. with 2 changes.

f) Overnight in a 50/50 solution of propylene oxide+ and Epon.

g) 6 hrs. or overnight in 100% Epon.

h) Embedding in Epon in capsules for 48 hrs. at 60°C.

i) Following trimming of Epon blocks, 1 µm sections were cut, placed on microscope slides and stained with 1% Toluidine blue & 1% borax in distilled water over a hot-plate.

j) The sections were mounted in DPX mountant+.

* Polaron Equipment Ltd., Watford, England.

\+ BDH Chemicals Ltd., Poole, England.

Phosphate Buffer

Phosphate buffer contained:

A) $^{M}/_{15}$ Disodium hydrogen phosphate.

& B) $^{M}/_{15}$ Potassium dihydrogen phosphate.

For pH = 7.38, 80ml. of A) is added to 20 ml. of B).

Osmium Tetroxide solution

2% osmium tetroxide in distilled water is mixed with phosphate buffer in a 50/50 solution by volume.

Epon

The Epon was made up as follows:

A) DDSA (dodecenylsuccinicanhydride) - 186.2 gms.

& Epon (Epikote 812) - 160.0 gms.

B) MNA (methylnadicanhydride) - 155.6 gms.

& Epon - 200.0 gms.

A) & B) are mixed in equal proportions and 15 drops/10 mls. of final solution of the accelerator DMP 30 (2, 4, 6, - tri(dimethylaminomethyl)phenol) are added.

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TABLES & FIGURES

T A B L E 1

Authors (year)	Assay technique	Species	Spinal level	Resting NA content ($\mu g/gm$) ($\bar{x} \pm$ SD unless stated otherwise)
Osterholm & Mathews (1972)	Spectrofluorometric method of Fleming	cat	mid-thoracic	0.33 0.75
de la Torre et al (1974)	Cation-exchange method of Barchas et al	dog	T11 - 12	0.227 ± 0.022
Hedeman et al (1974)	Spectrofluorometric method of Shellenberger & Gordon	dog	T10	0.144 ± 0.012
Naftchi et al (1974)	Fluorometric assay	cat	T7 - 10	0.150 ± 0.015
Rawe et al (1977)	Fluorometric method of Von Euler (as modified by Roth & Stone)	cat	T5	0.077 ± 0.013
Bingham et al (1975)	Modification of spectro-fluorometric method of Shellenberger & Gordon	rhesus monkey	average of T1, T5 & T10	0.237 ± 0.16
Zivin et al (1976)	Enzymatic radiometric assay	rabbit	L2 - 3	0.148
Tsubokawa et al (1975)	Spectrofluorometric method of Fleming	dog	T9 - L3	0.6
Reis & Gutnick (1970)	Fluorometric method of Von Euler	cat	T9 - T13 (1 pm)	0.217 ± 0.034 (SE)
Shoultz (1977)	Fluorometric method of Weil-Malherbe	cat	L6	0.126

T A B L E 2

Authors (year)	Species	Method of Injury*		Response of amine		
		gm.cm.energy	impounder dimension	NA	DA	5-HT
Osterholm & Mathews (1972)	cat	500	not stated	increase	-	-
de la Torre et al (1974)	dog	500	not stated	no change	no change	no change
Hedeman et al (1974)	dog	420	not stated	decrease	increase	-
Naftchi et al (1974)	cat	400	not stated	no change	increase	no change
Rawe et al (1977)	cat	500	7.06 mm^2	no change	no change**	-
Bingham et al (1975)	rhesus monkey	300	5 mm D.	decrease	nc change	-
Zivin et al (1976)	rabbit	Fogarty Balloon		lesion centre GM: decrease	no change	decrease
				WM: decrease or no change	no change	-
				lesion edge -	no change	increase
Tsubokawa et al (1975)	dog	300 or less	not stated	no change	-	increase
		500 or more	not stated	increase (slight)	-	increase
Shoultz (1977)	cat	400	8 mm D.	increase	-	-

* In all but one series, injury was produced by the weight dropping method of Allen (1911)

** No dopamine was detected in either control or traumatised samples.

T A B L E 3

The effect of intra-arterial hyperosmotic urea on SCBF

Spinal segmental level		L_2	L_{4-5}	S_{1-2}
	n (electrodes)	5	7	4
SCBF (ml/100g/min) ($\bar{X}$ + SD)	Control values	13.5 $\pm$ 7.4	12.8 $\pm$ 3.2	9.7 $\pm$ 2.5
	0 - 10 mins post urea	12.7 $\pm$ 6.5	11.5 $\pm$ 3.9	8.8 $\pm$ 3.2
	11 - 20 mins post urea	12.2 $\pm$ 5.6	10.8 $\pm$ 3.0	7.9 $\pm$ 1.9
	21 - 30 mins post urea	15.7 $\pm$ 7.5	12.4 $\pm$ 4.7	10.2 $\pm$ 5.8

Flows do not vary significantly from controls with respect to time or segmental level.

T A B L E 4

Effect of 12 µg/min infusion of noradrenaline on SCBF before and after blood-cord barrier disruption with urea.

Spinal level	Control values	Noradrenaline Infusion					
		Pre-urea (mins)			Post-urea (mins)		
		30	20	10	10	20	30
L_2	SCBF 16.6 ± 4.9	17.9 ± 6.2	16.3±3.8	17.4± 5	15.2±5.4	14.8±8.3	11.5±5.8
	n (electrodes) 10	8	8	8	10	10	8
	p -	NS	NS	NS	NS	NS	0.01
L_{5-6}	SCBF 15.3 ± 4.7	17.8 ± 7.0	13.5±4.9	13.4±4.0	13.0±10.3	7.9±6.9	8.8±5.8
	n (electrodes) 15	13	13	8	15	12	9
	p -	NS	NS	NS	NS	0.01	0.02

Blood flow figures are expressed as $\bar{x}$ ± SD ml/100g/min.

TABLE 5

Effect of 30 μg/min infusion of noradrenaline on SCBF before and after blood-cord barrier disruption with urea.

Spinal level	Control values	Noradrenaline Infusion					
		Pre-urea (mins)			Post-urea (mins)		
		30	20	10	10	20	30
L_2	SCBF 15.3±4.7	17.8±7.0	13.5±4.9	13.4±4.0	13.0±10.3	7.9±6.9	8.8±5.8
	n (electrodes) 15	13	13	8	15	12	9
	p -						
L_{5-6}	SCBF 13.4±2.1	20.9±8.4	15.5±8.0	17.4±8.4	3.4±4.8	1.5±2.4	1.1±1.8
	n (electrodes) 10	10	9	6	10	10	10
	p -	0.02	NS	NS	0.001	0.001	0.001

Blood flow figures are expressed as $\bar{x} \pm$ SD ml/100g/min.

T A B L E 6

Mean systemic arterial blood pressure (mm.Hg.) during infusions of 12 µg or 30 µg/min noradrenaline before and after blood-cord barrier disruption.

Infusion rate/spinal level	Control values	Noradrenaline Infusion	
		Pre-urea	Post-urea
12 µg/min L_2	137 ± 21	141 ± 18	141 ± 16
12 µg/min L_{5-6}	144 ± 21	146 ± 15	149 ± 17
30 µg/min L_2	135 ± 17	148 ± 20	139 ± 21
30 µg/min L_{5-6}	137 ± 11	144 ± 15	145 ± 15

In no case was blood pressure significantly different from control. (Analysed by Student's grouped t-test)

TABLE 7

pCO_2 values (mm.Hg.) during infusions of 12 µg or 30 µg/min noradrenaline before and after blood-cord barrier disruption.

Infusion rate/spinal level	Control values	Noradrenaline Infusion	
		Pre-urea	Post-urea
12 µg/min L_2	40.0 ± 1.9	40.4 ± 2.2	40.2 ± 1.8
12 µg/min L_{5-6}	39.9 ± 2.1	40.5 ± 2.4	40.4 ± 2.1
30 µg/min L_2	39.7 ± 1.7	40.2 ± 2.0	40.4 ± 1.0
30 µg/min L_{5-6}	39.4 ± 1.5	39.7 ± 2.0	40.3 ± 1.2

In no case was pCO_2 significantly different from control.
(Analysed by Student's grouped t-test)

TABLE 8

The effect of α-blockade on SCBF and of 30 µg/min NA on SCBF after blood-cord barrier disruption at the L_{5-6} level of the spinal cord.

Experimental Conditions	n	SCBF (ml/100g/min) $\bar{x} \pm$ SD
Control	21	24.3 ± 12.5
α- blockade*	14	21.9 ± 9.8
NE infusion** α- blockade blood cord barrier disruption	21	27.1 ± 13.5

NS (Control vs α- blockade)

NS (Control vs NE infusion** α- blockade blood cord barrier disruption)

* Two clearances were performed with α- blockade alone. The results were pooled to give a single value.

** Three estimations of flow were made in each experiment within a 30 minute period. The value is the mean of the total results.

n = no. of electrodes.

Fig. 1 Intermediate stages in the formation of the catecholamines.

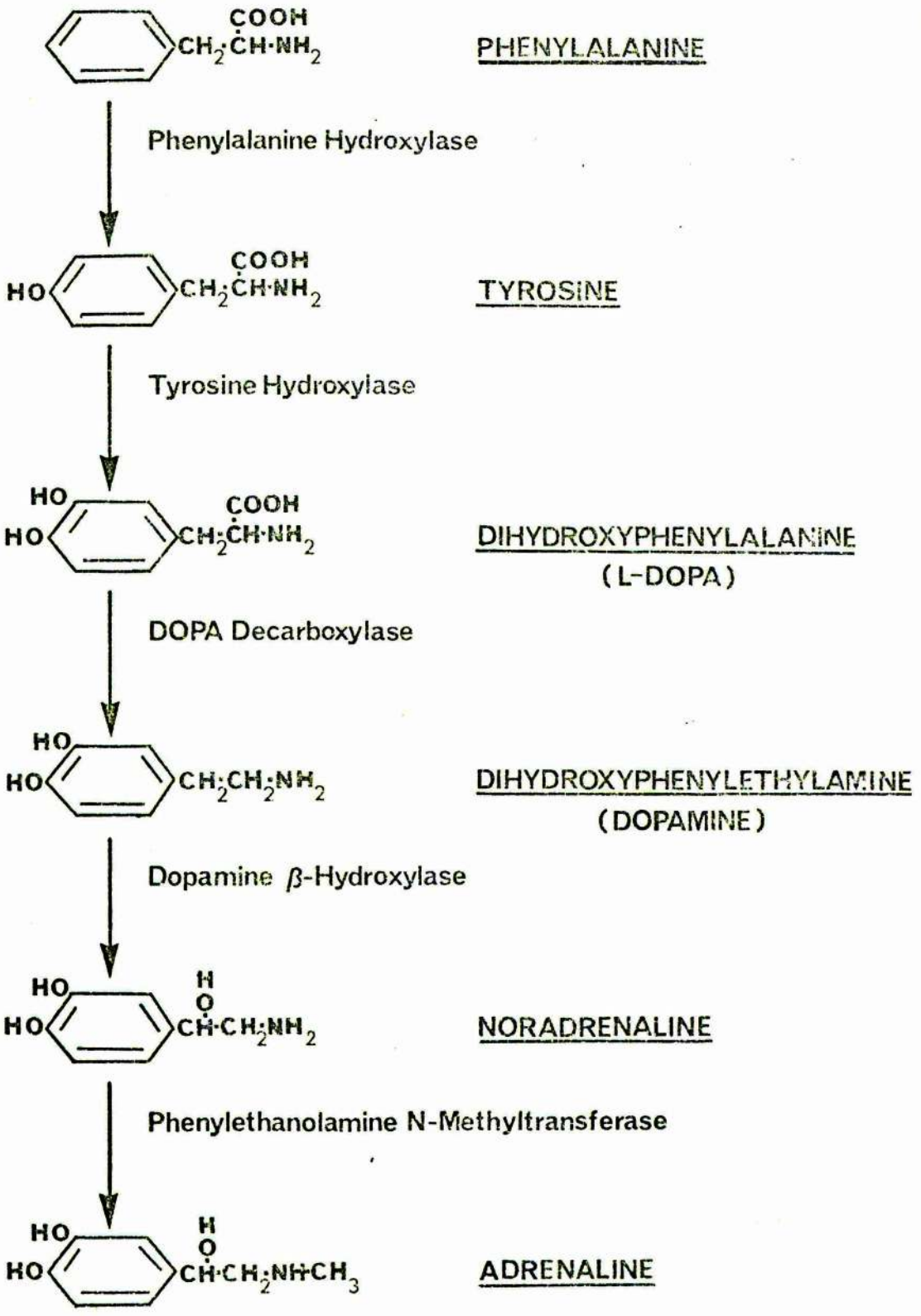
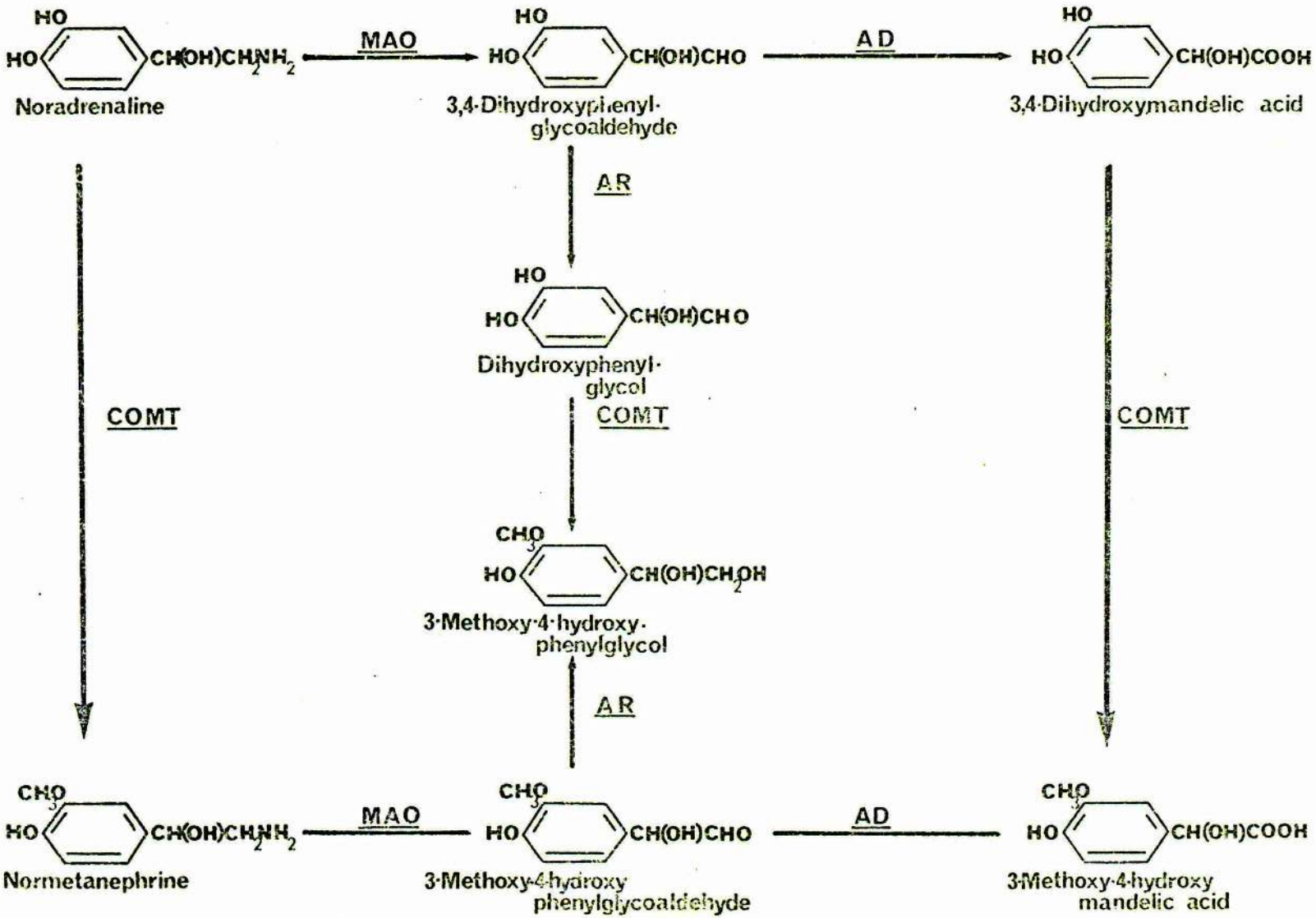

PHENYLALANINE
Phenylalanine Hydroxylase
TYROSINE
Tyrosine Hydroxylase
DIHYDROXYPHENYLALANINE
(L-DOPA)
DOPA Decarboxylase
DIHYDROXYPHENYLETHYLAMINE
(DOPAMINE)
Dopamine β-Hydroxylase
NORADRENALINE
Phenylethanolamine N-Methyltransferase
ADRENALINE

Fig. 2 The metabolic degradation of noradrenaline.

MAO = Monoamine oxidase
COMT = Catechol O - methyltransferase
AD = Aldehyde reductase


HO
HO
CH(OH)CH2NH2
Noradrenaline
MAO
HO
HO
CH(OH)CHO
3,4-Dihydroxyphenyl-glycoaldehyde
AD
HO
HO
CH(OH)COOH
3,4-Dihydroxymandelic acid
AR
HO
HO
CH(OH)CHO
Dihydroxyphenyl-glycol
COMT
COMT
COMT
CH3O
HO
CH(OH)CH2OH
3-Methoxy-4-hydroxy-phenylglycol
AR
CH3O
HO
CH(OH)CH2NH2
Normetanephrine
MAO
CH3O
HO
CH(OH)CHO
3-Methoxy-4-hydroxy phenylglycoaldehyde
AD
CH3O
HO
CH(OH)COOH
3-Methoxy-4-hydroxy mandelic acid

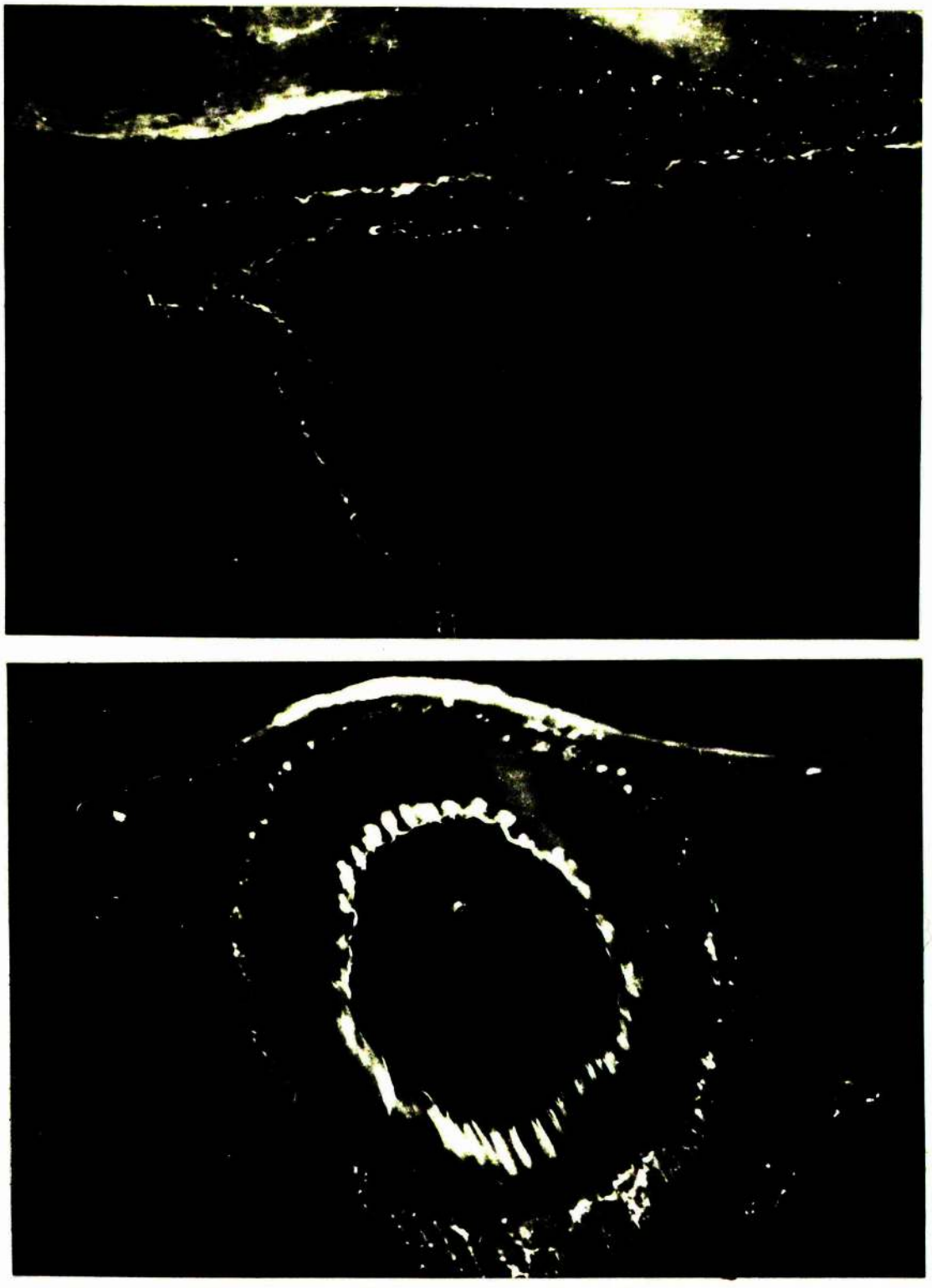


Fig. 3 Histofluorescent preparations of spinal cord prepared to demonstrate noradrenaline containing nerve fibres. The upper photograph depicts a spinal pial vessel and the lower one an intraspinal vessel. Fluorescent noradrenergic fibres are seen in close association with the vessels. Magnification unknown. (Courtesy of Dr. Lars Edvinsson).

FIG. 4 Schematic diagram of the posterior aorta and branches showing the pattern of ligations and cannulations employed in the experiments.

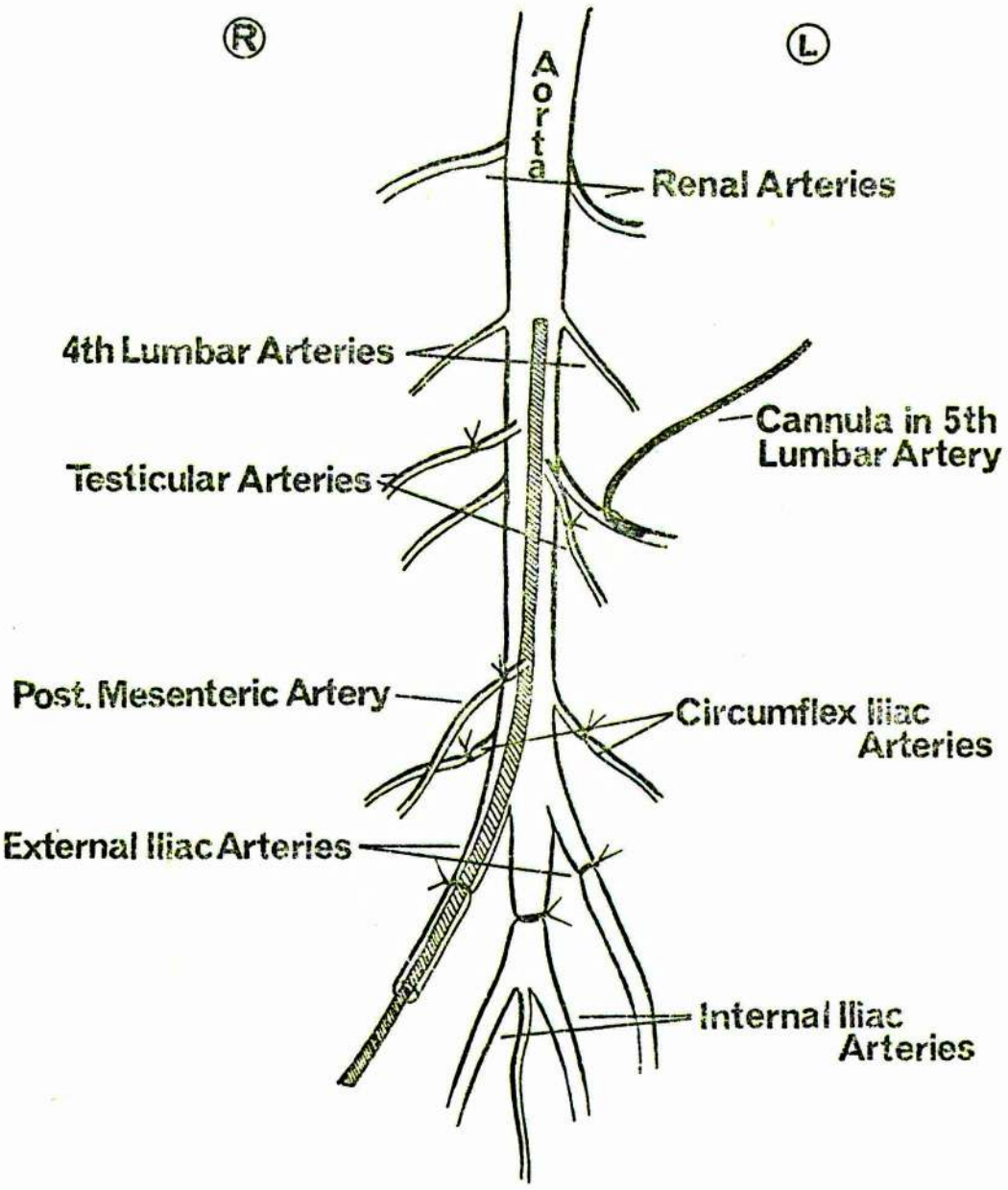

®
Aorta
Ⓛ
Renal Arteries
4th Lumbar Arteries
Cannula in 5th Lumbar Artery
Testicular Arteries
Post. Mesenteric Artery
Circumflex Iliac Arteries
External Iliac Arteries
Internal Iliac Arteries

FIG. 5 Schematic diagram of the 6 channel amplification system used in determination of spinal cord blood flow by the hydrogen clearance technique.

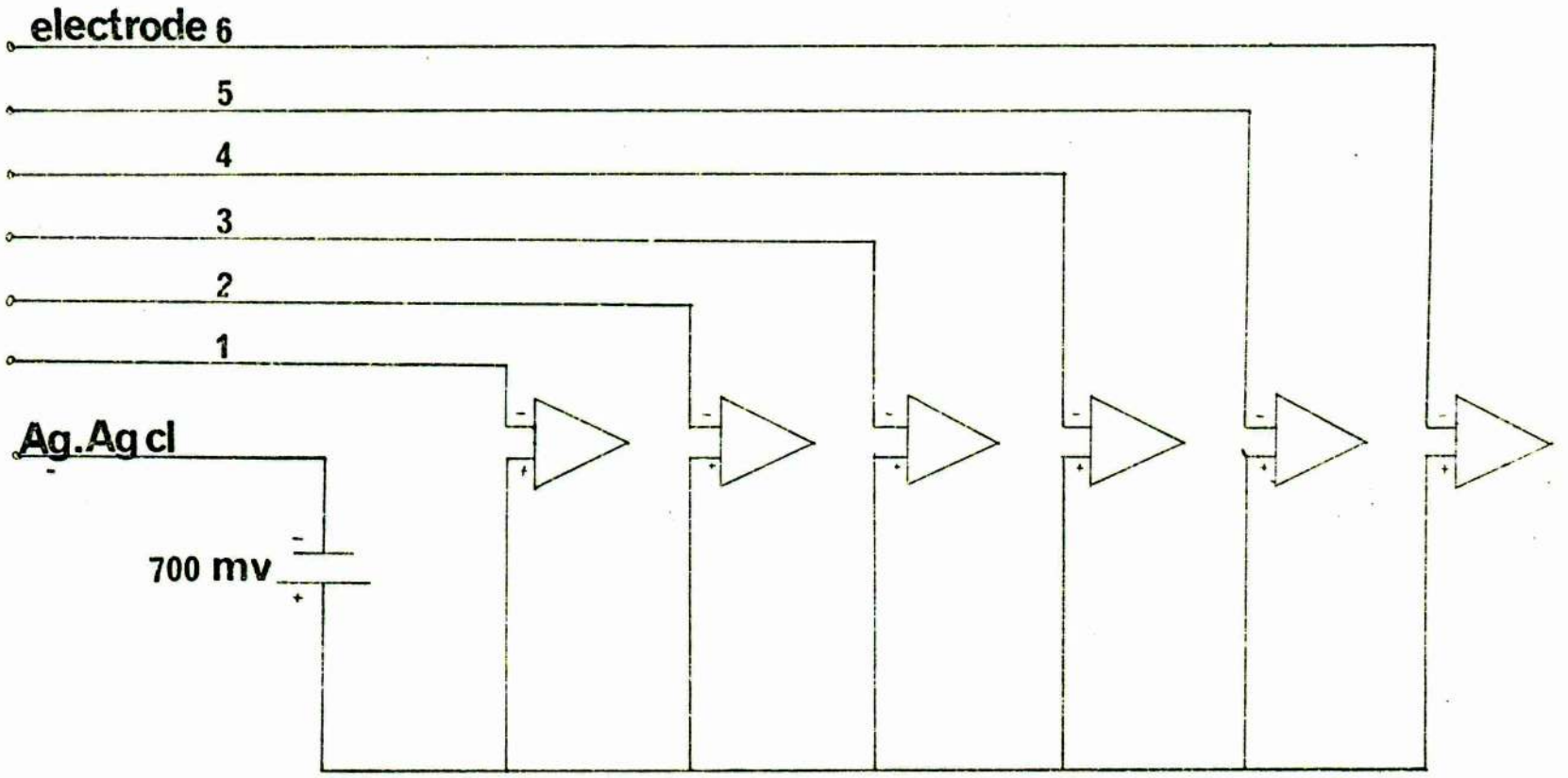

electrode 6
5
4
3
2
1
Ag.Ag cl
700 mv

FIG. 6 Circuit diagram of hydrogen electrode current amplifier.

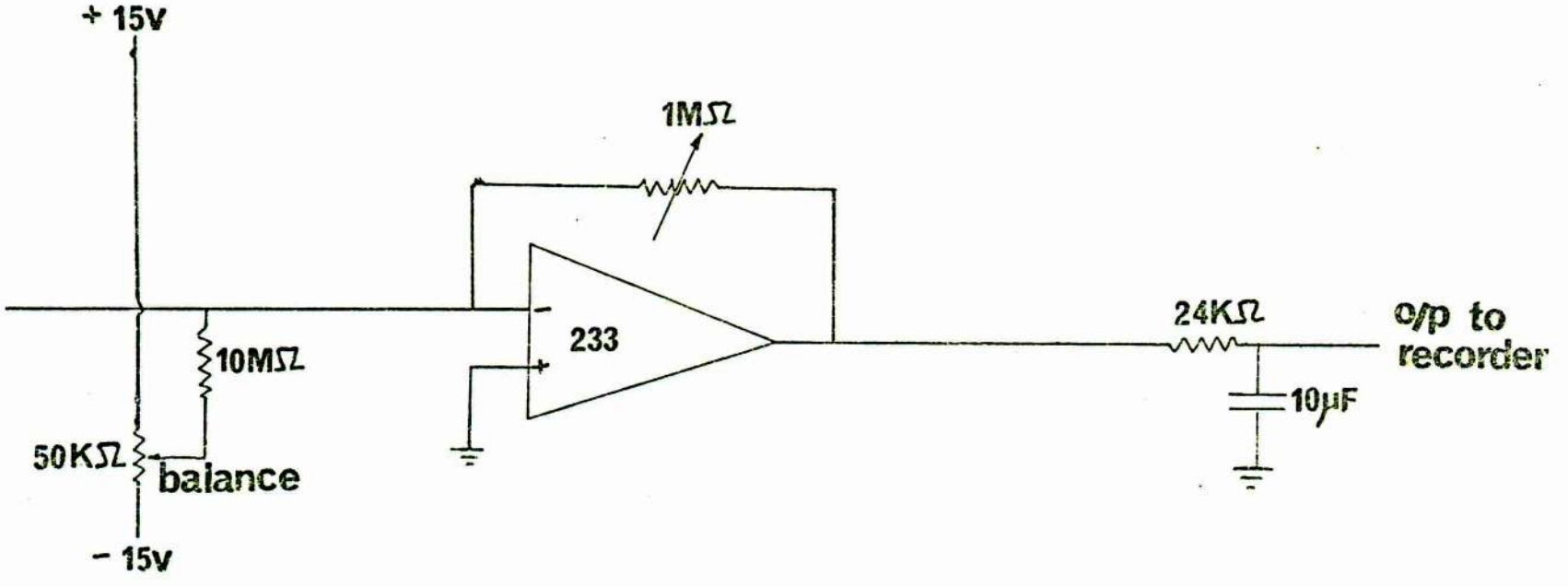

+ 15V
1MΩ
10MΩ
50KΩ
balance
- 15v
233
24KΩ
o/p to
recorder
10μF

FIG. 7 The effect of intra-arterially administered hyperosmotic urea on systemic arterial blood pressure (ABP) and 5th lumbar artery pressure.

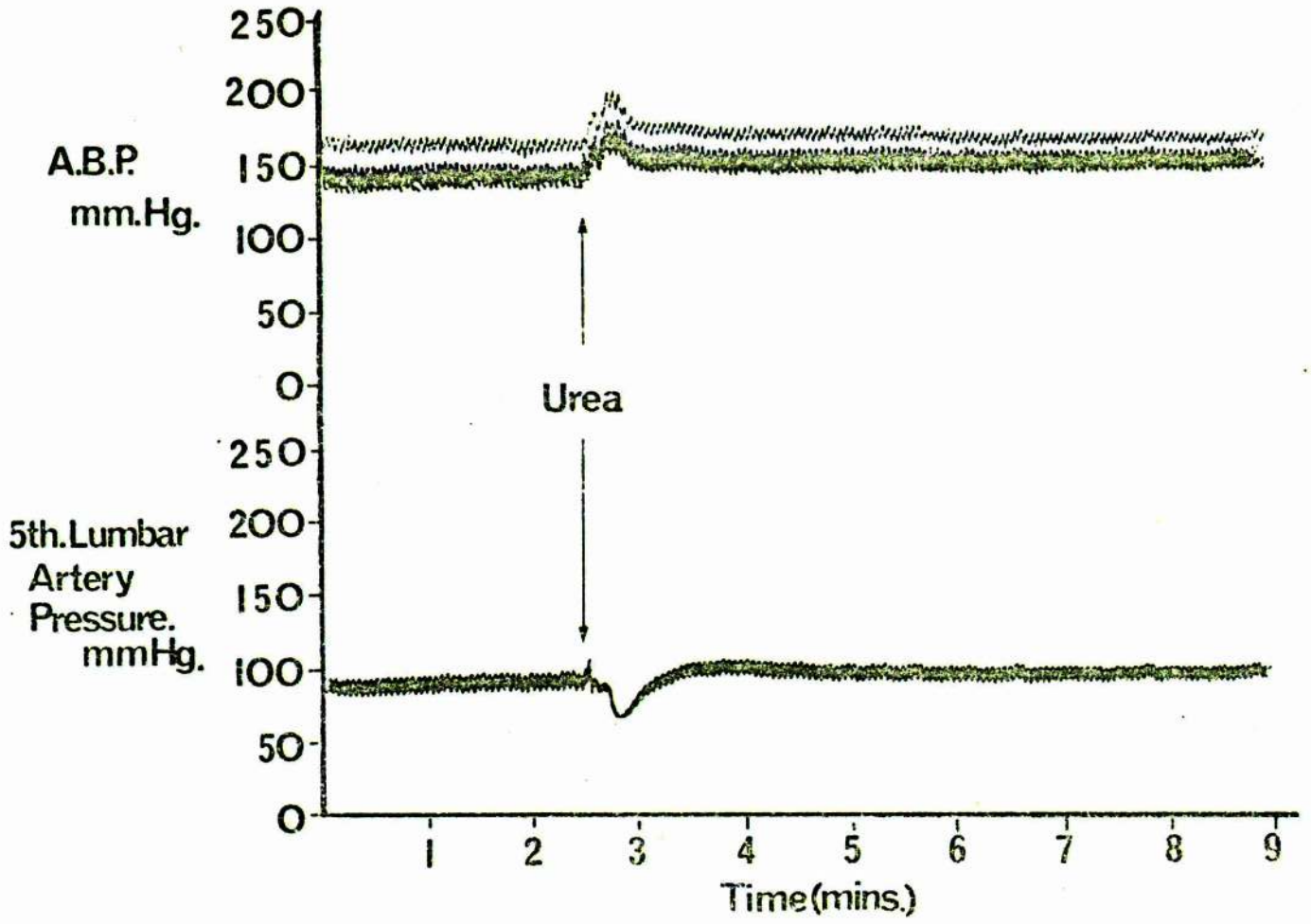

A.B.P.
mm.Hg.
250
200
150
100
50
0
Urea
5th.Lumbar
Artery
Pressure.
mmHg.
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time(mins.)

FIG. 8 The effect of urea on SCBF at the L_{5-6} segment of the spinal cord. No significant differences in flow occurred following the intra-arterial administration of urea.

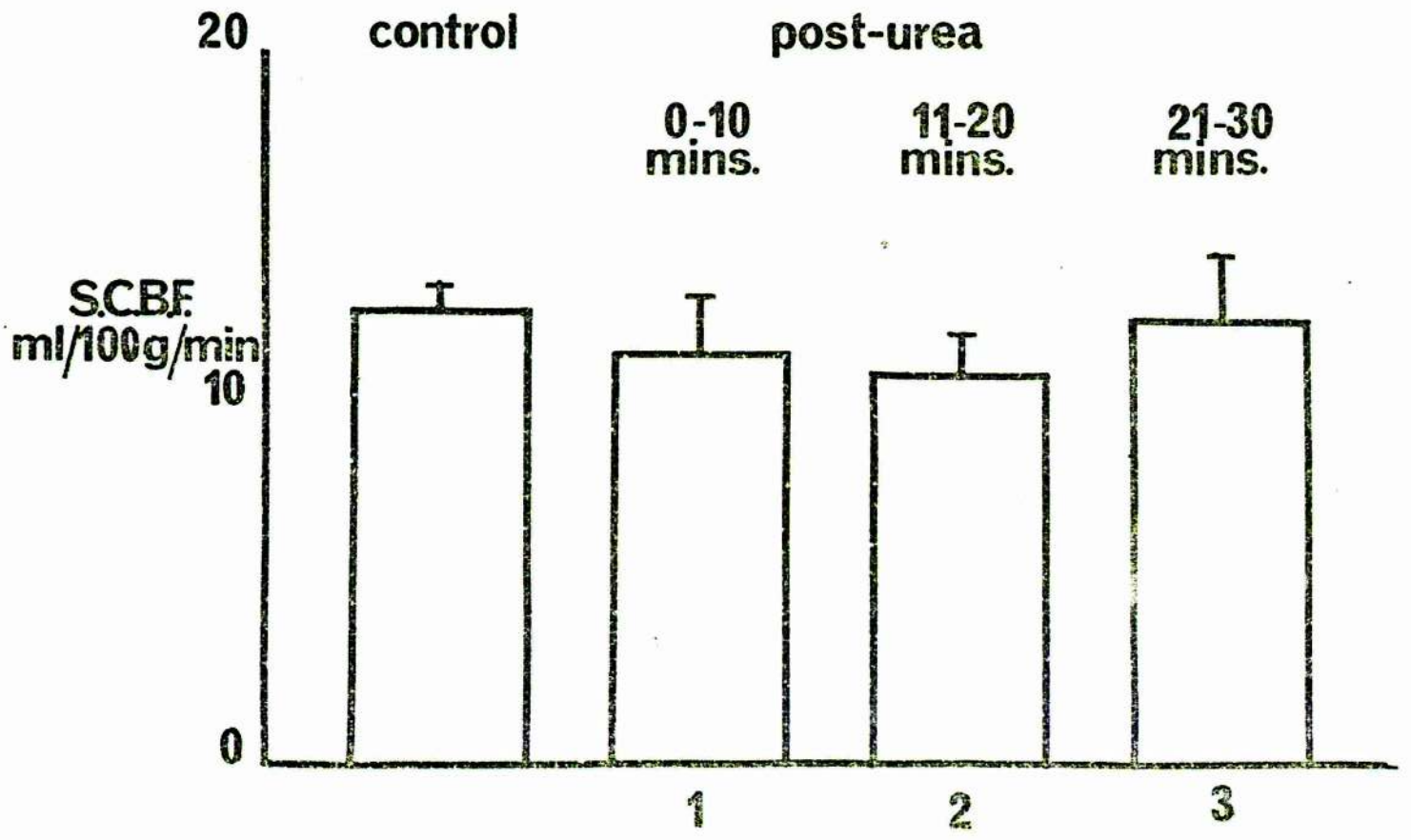

20
control
post-urea
0-10
mins.
11-20
mins.
21-30
mins.
S.C.B.F.
ml/100g/min
10
0
1
2
3

FIG. 9 Graphical representation of SCBF results during an infusion of ether 12 µg/min or 30 µg/min noradrenaline before and after blood-cord barrier disruption with urea at L_2 and L_{5-6} levels of the spinal cord.

c = control values

Runs 1, 2 & 3 are 30, 20 & 10 mins pre-urea administration respectively.

Runs 4, 5 & 6 are 10, 20 & 30 mins post-urea administration respectively.

■ = $p < 0.01$

● = $p < 0.001$

Δ = $p < 0.05$

▲ = $p < 0.02$

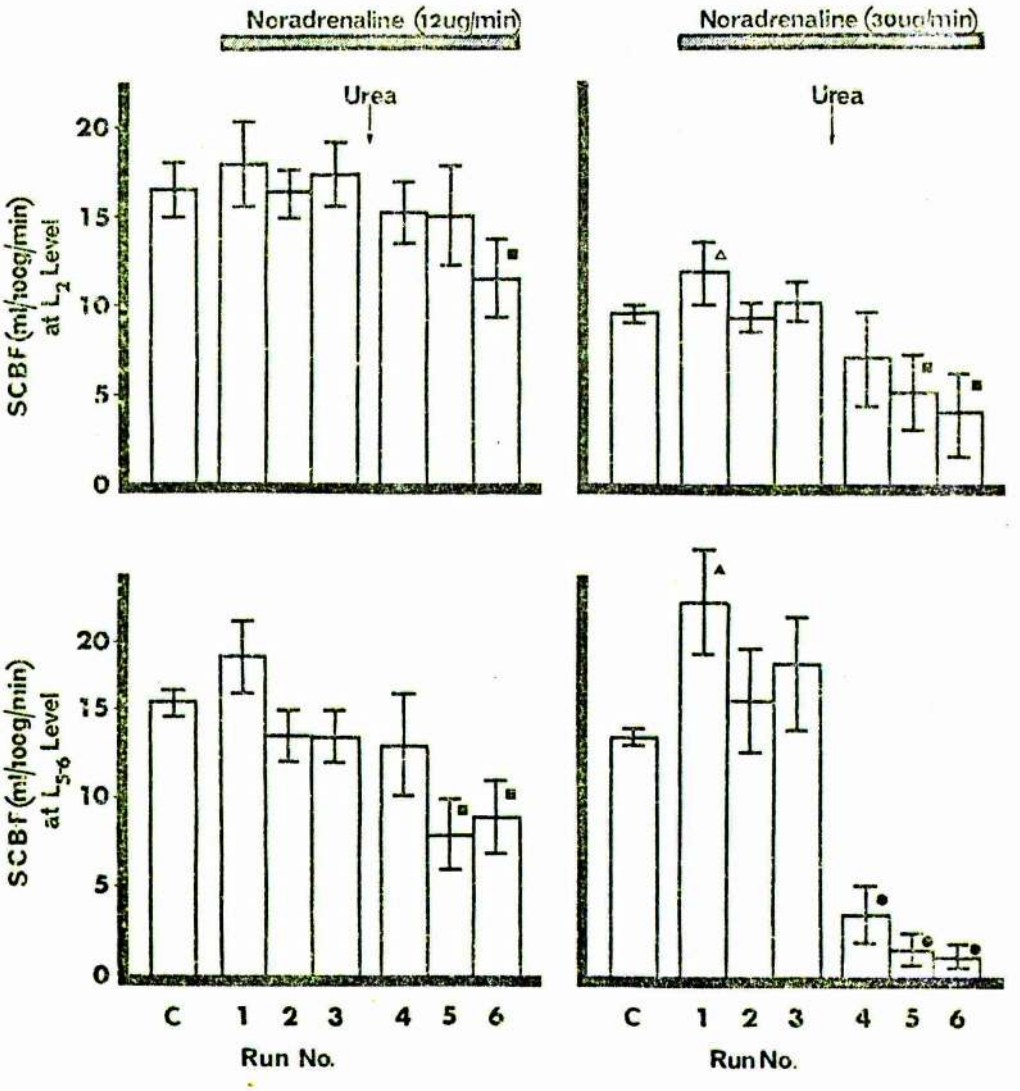
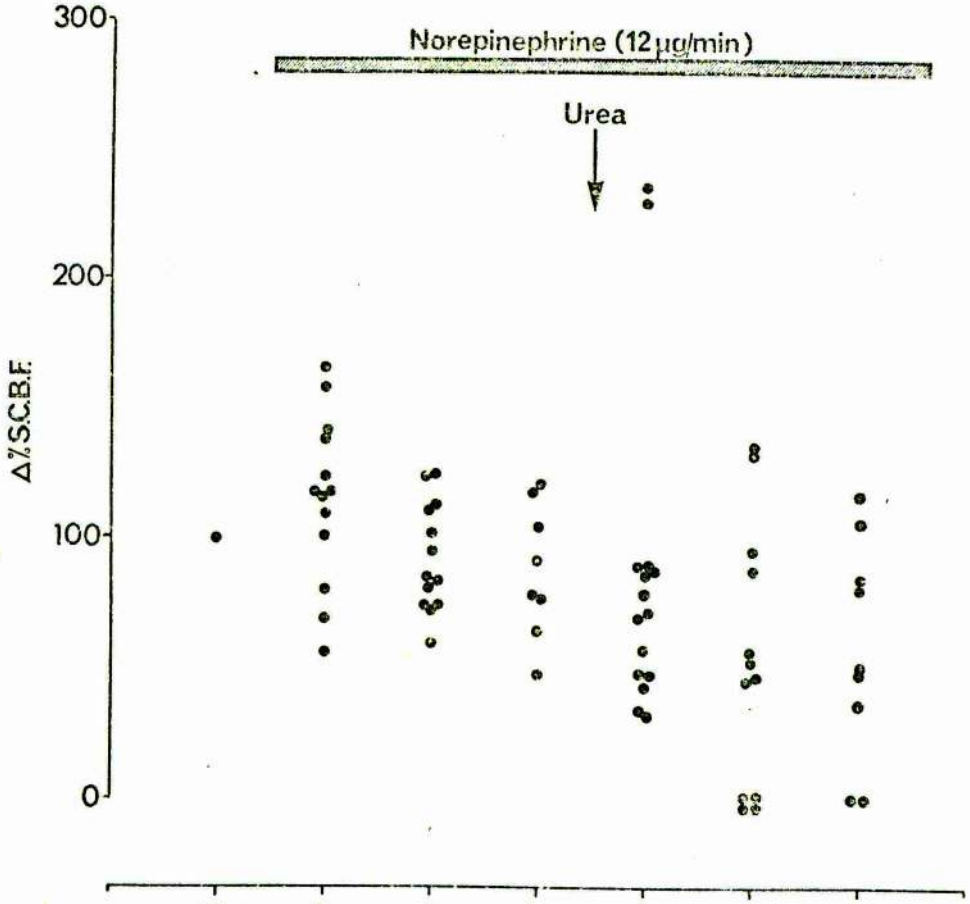
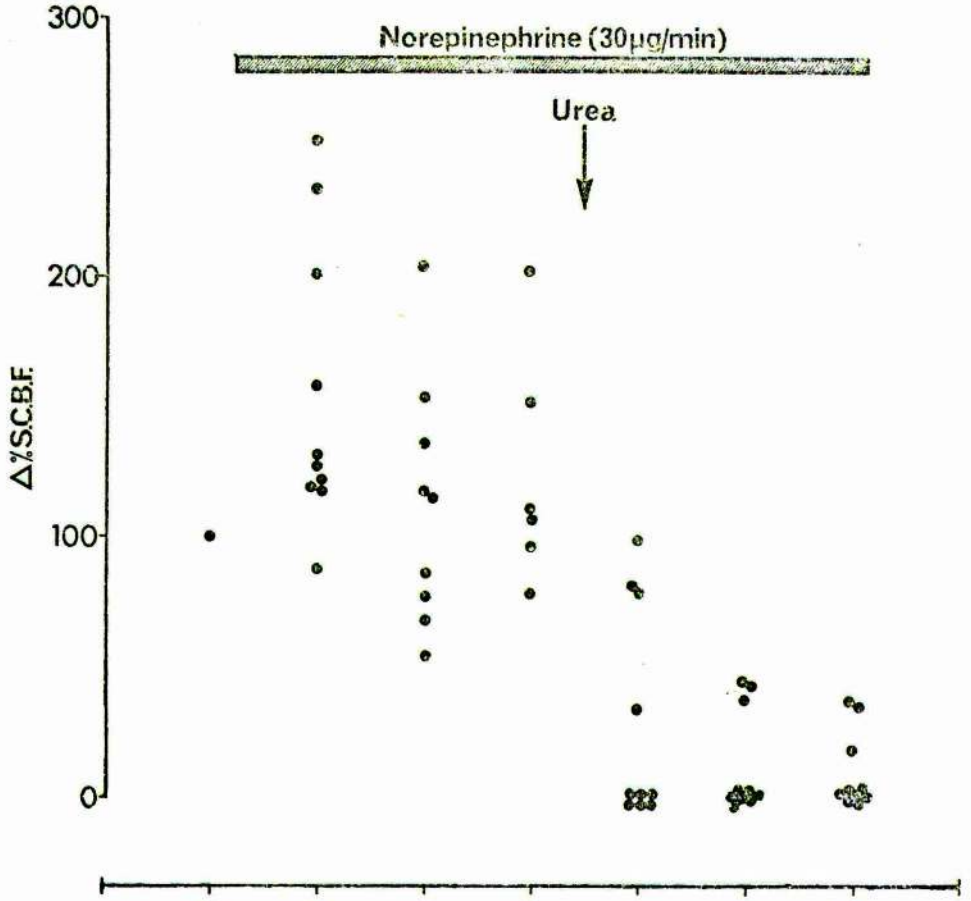

Noradrenaline (12ug/min)
Noradrenaline (30ug/min)
Urea
Urea
SCBF (ml/100g/min) at L_2 Level
SCBF (ml/100g/min) at L_{5-6} Level
20
15
10
5
0
C 1 2 3 4 5 6
Run No.
Run No.

Fig. 10 Scatter graph of percentage change of flow in response to a 12 µg/min intra-arterial infusion of noradrenaline (norepinephrine) before and after blood-cord barrier disruption. Flow estimations were performed at approximately 10 minute intervals. C = control (100%).



$\bar{x}$ (%)	100	125.0	92.9	68.1	86.0	54.7	58.5
s.e. (%)	-	11.5	6.1	9.9	16.7	15.2	15.0
p	-	<0.05	n.s.	n.s.	n.s.	<0.01	<0.02
n.	15	13	13	8	15	12	9

Fig. 11 Scatter graph of percentage change of flow in response to a 30 µg/min intra-arterial infusion of noradrenaline (norepinephrine) before and after blood-cord barrier disruption. Flow estimations were performed at approximately 10 minute intervals.



$\bar{x}$ (%)	100	155	112.8	124.7	29.4	12.6	9.2
s.e.(%)	-	18.4	16.7	20.3	13.7	6.8	5.2
p	-	<0.02	ns	ns	<0.001	<0.001	<0.001
n.	10	10	9	6	10	10	10

FIG. 12 The effect of α-blockade by phenoxybenzamine (PBZ) on SCBF and of 30 μg/min noradrenaline (NA) on SCBF after blood-cord barrier disruption during α-blockade. Clearances were made at approximately 10 min. intervals.

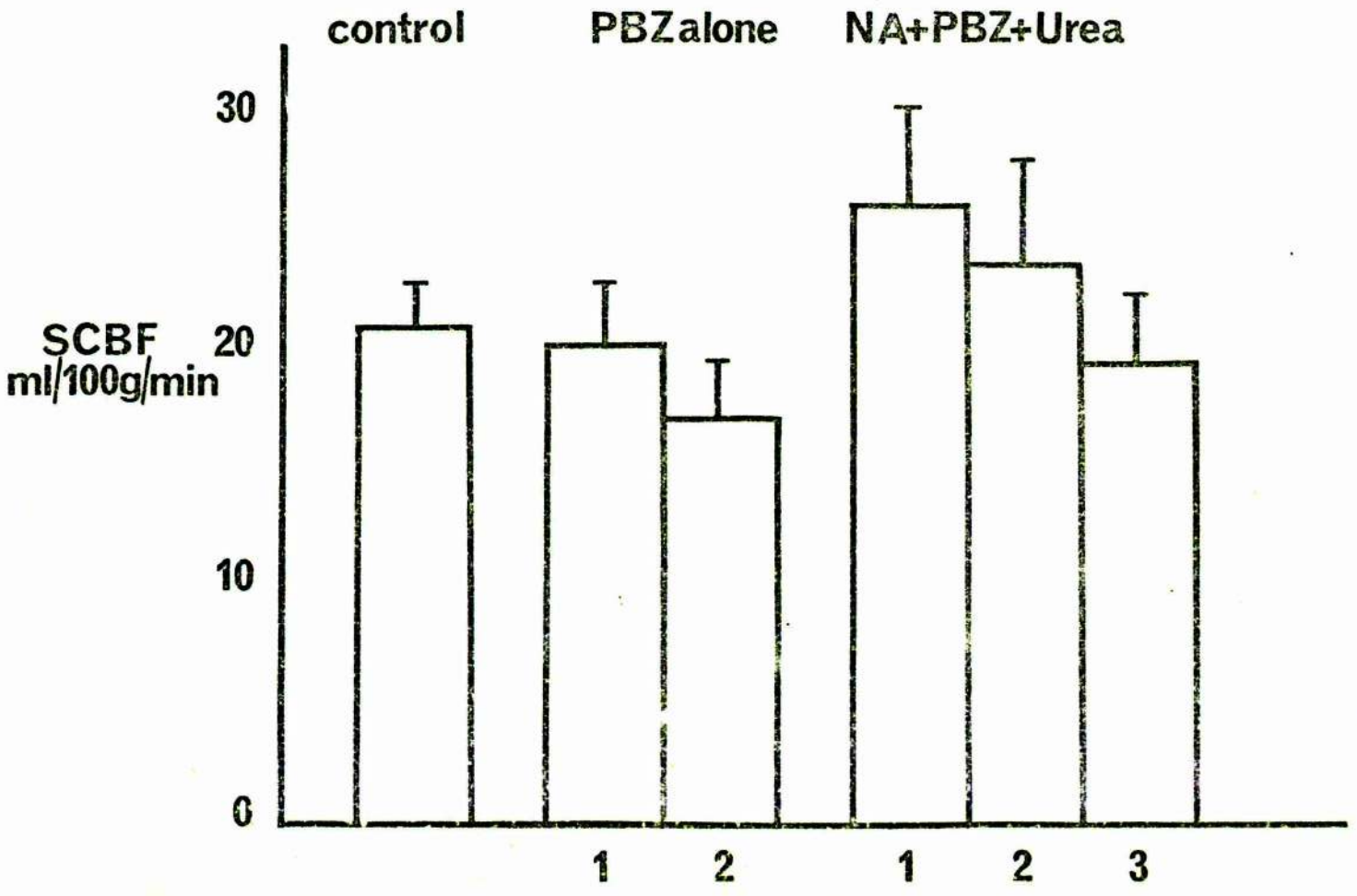

control
PBZalone
NA+PBZ+Urea
30
20
10
0
SCBF
ml/100g/min
1
2
1
2
3

Fig. 13 The effect of topically applied noradrenaline on the vascular diameter of spinal pial arteries and arterioles.

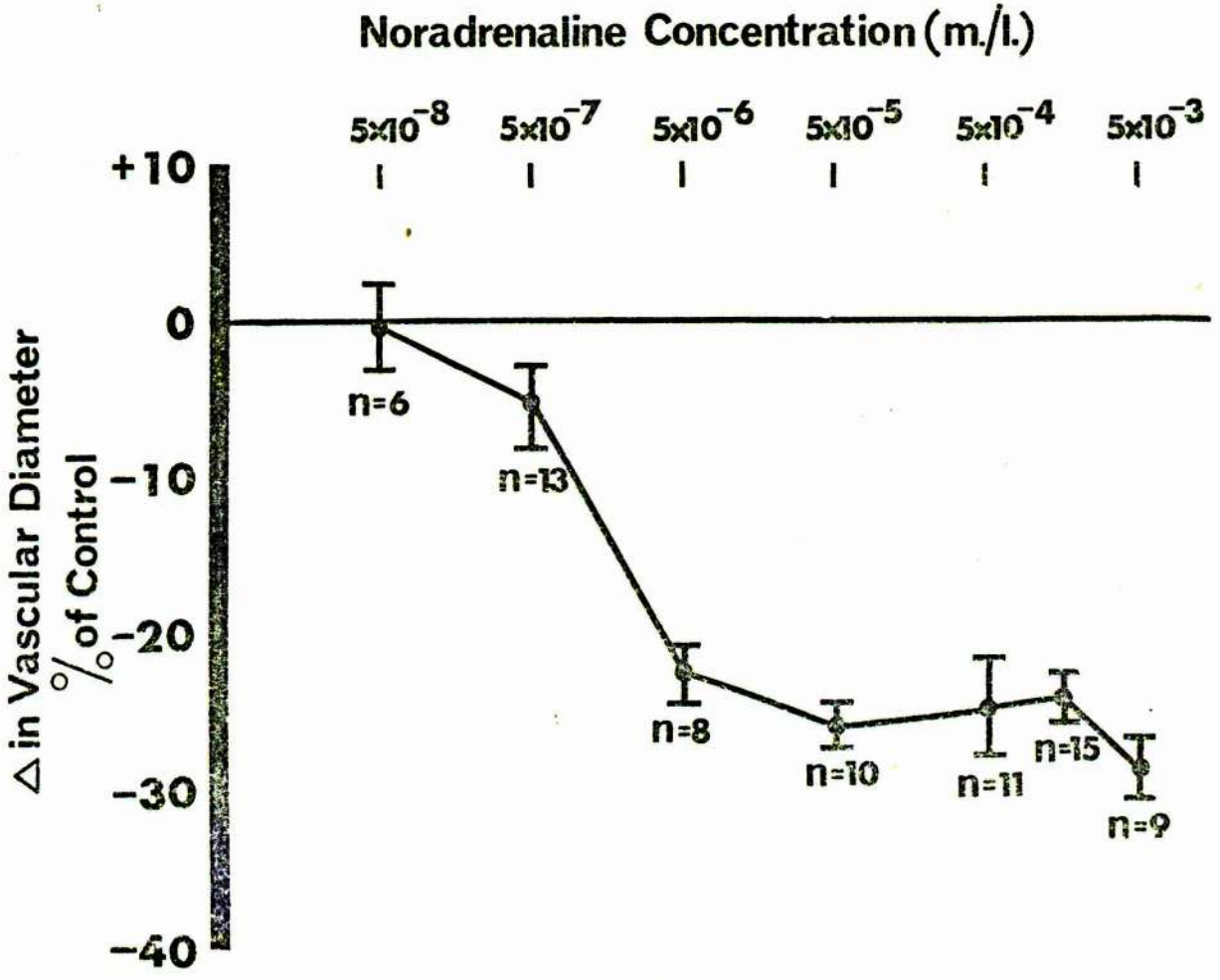
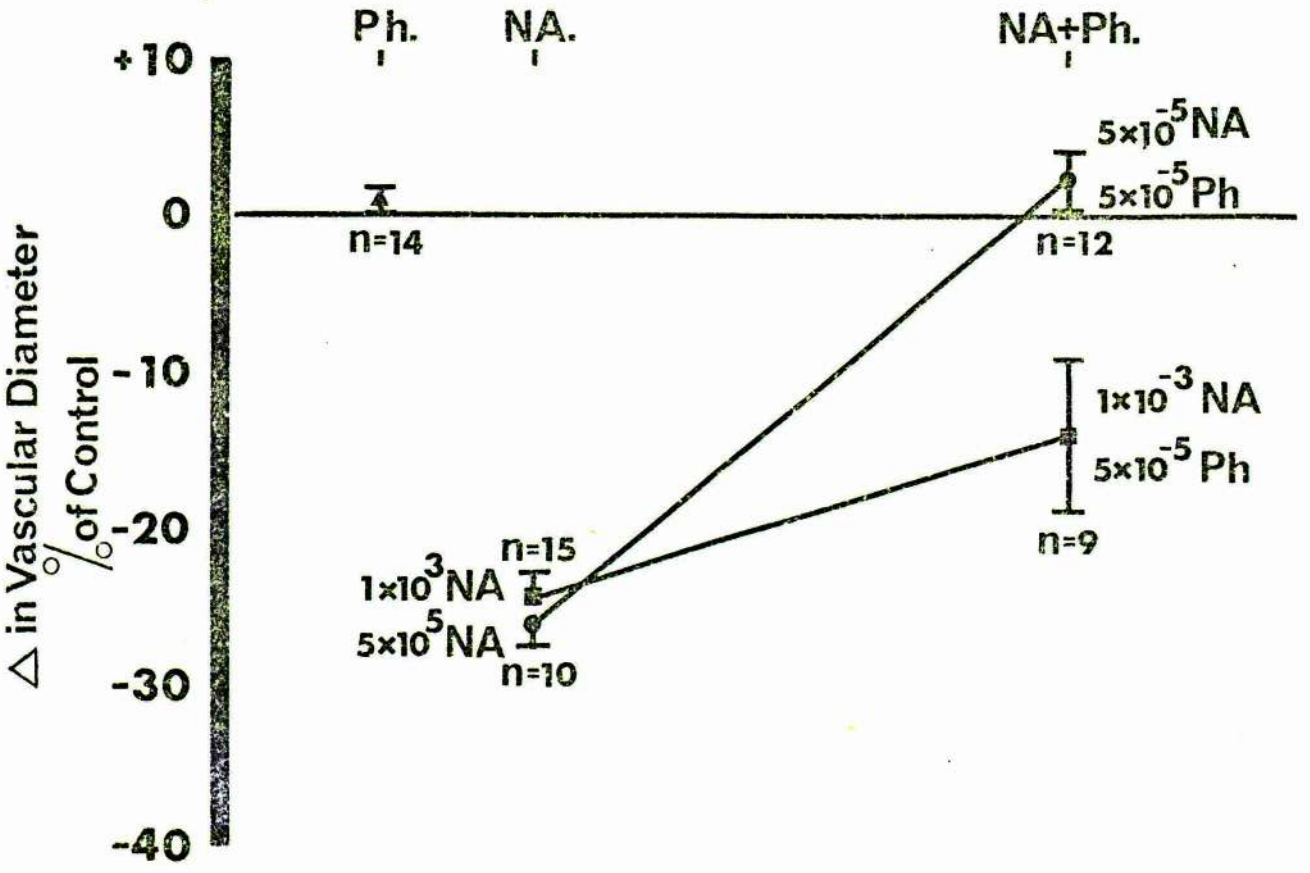

Noradrenaline Concentration (m./l.)
5x10^-8
5x10^-7
5x10^-6
5x10^-5
5x10^-4
5x10^-3
+10
0
-10
-20
-30
-40
Δ in Vascular Diameter % of Control
n=6
n=13
n=8
n=10
n=11
n=15
n=9

Fig. 14 The effect of phentolamine (Ph) on noradrenaline (NA) induced constrictions of spinal pial arteries and arterioles.


Ph.
NA.
NA+Ph.
+10
0
-10
-20
-30
-40
△ in Vascular Diameter
% of Control
5×10⁻⁵NA
5×10⁻⁵Ph
n=14
n=12
1×10⁻³NA
5×10⁻⁵Ph
n=9
n=15
1×10⁻³NA
5×10⁻⁵NA
n=10

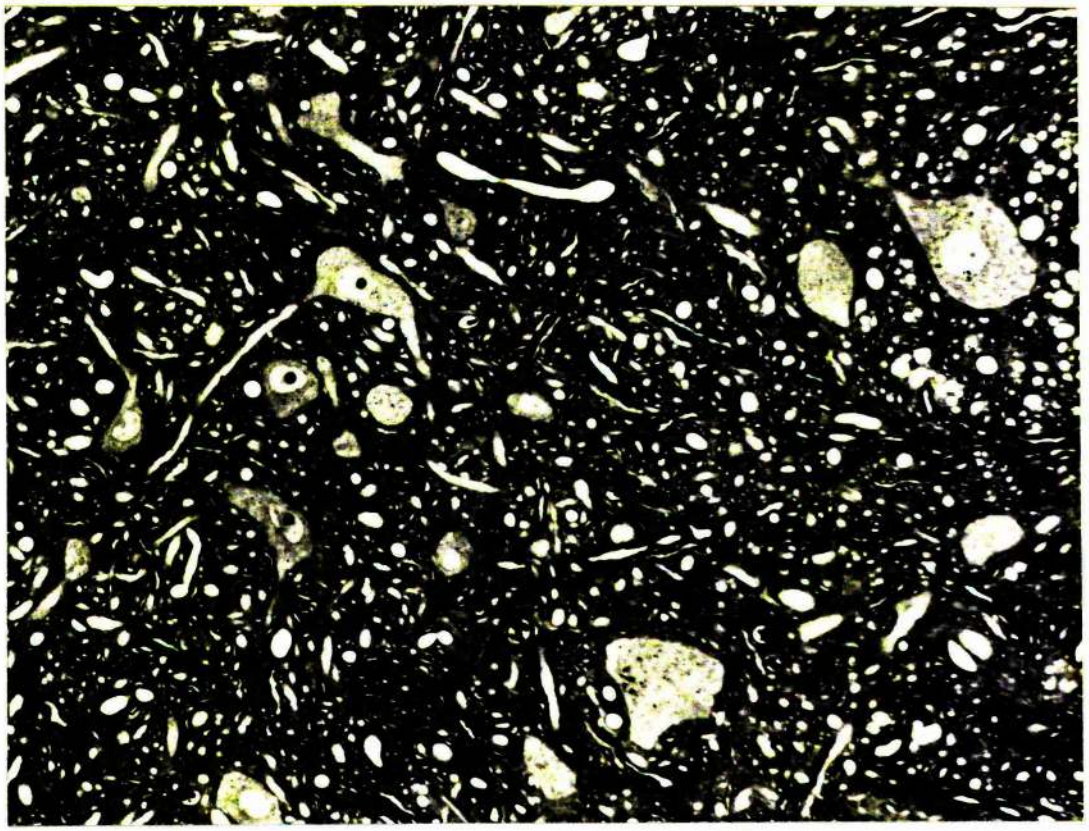


Fig. 15 Area of G.M. from caudal thoracic segment (control). Toluidine blue x 250.

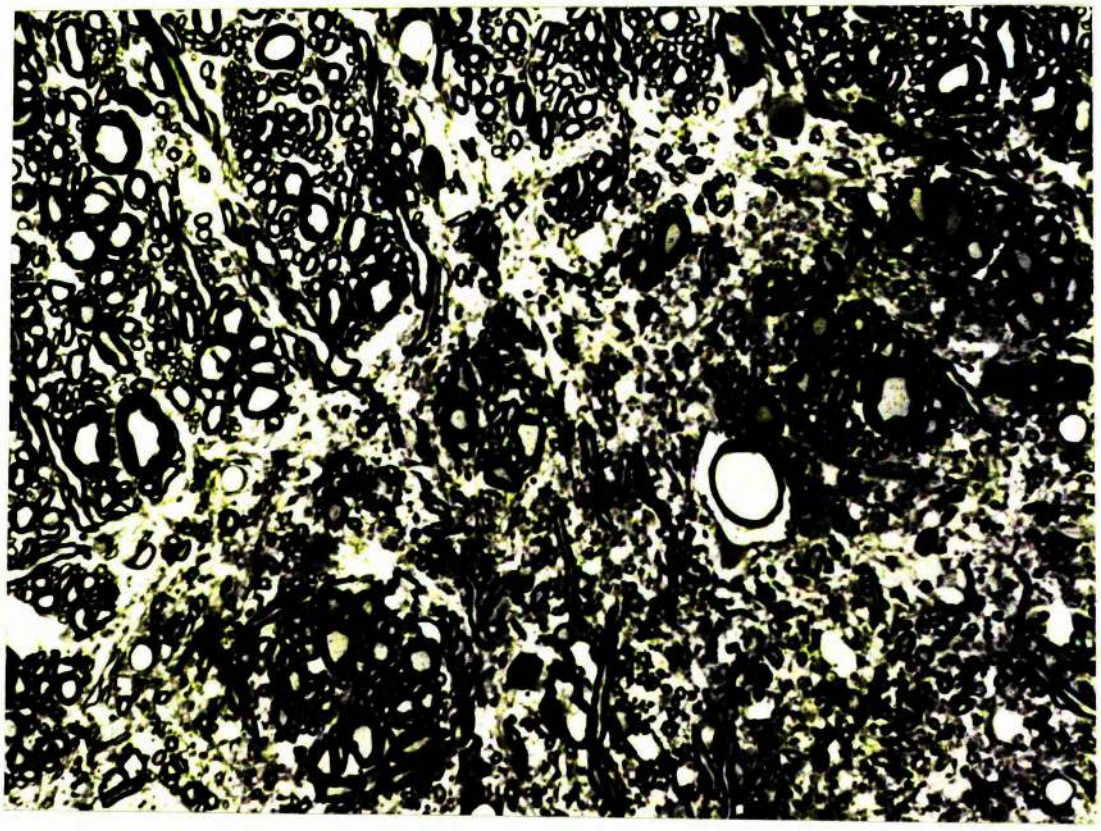


Fig. 16 GM/WM junction which received an injection of 20 µl CSF. There is evidence of oedema in both grey and white matter. Toluidine blue x 400.

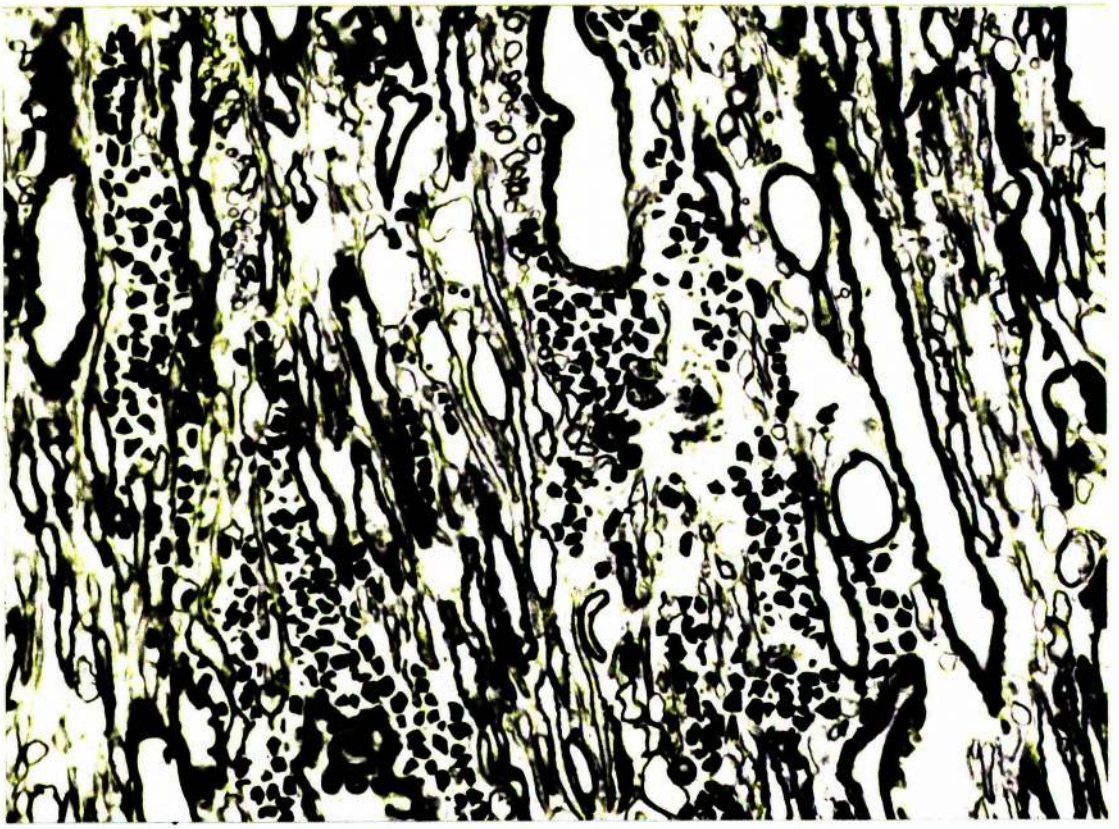


Fig. 17 Area of W.M. which received an injection of 20 µl CSF. Haemorrhage and oedema are evident. Toluidine blue x 400.

Fig. 18 Area of G.M. which received 12 µg NA in 40 µl CSF. Cavitation, with compression of neural tissue, has occurred. Oedema and shrunken neurones are also a feature. Toluidine blue x 250

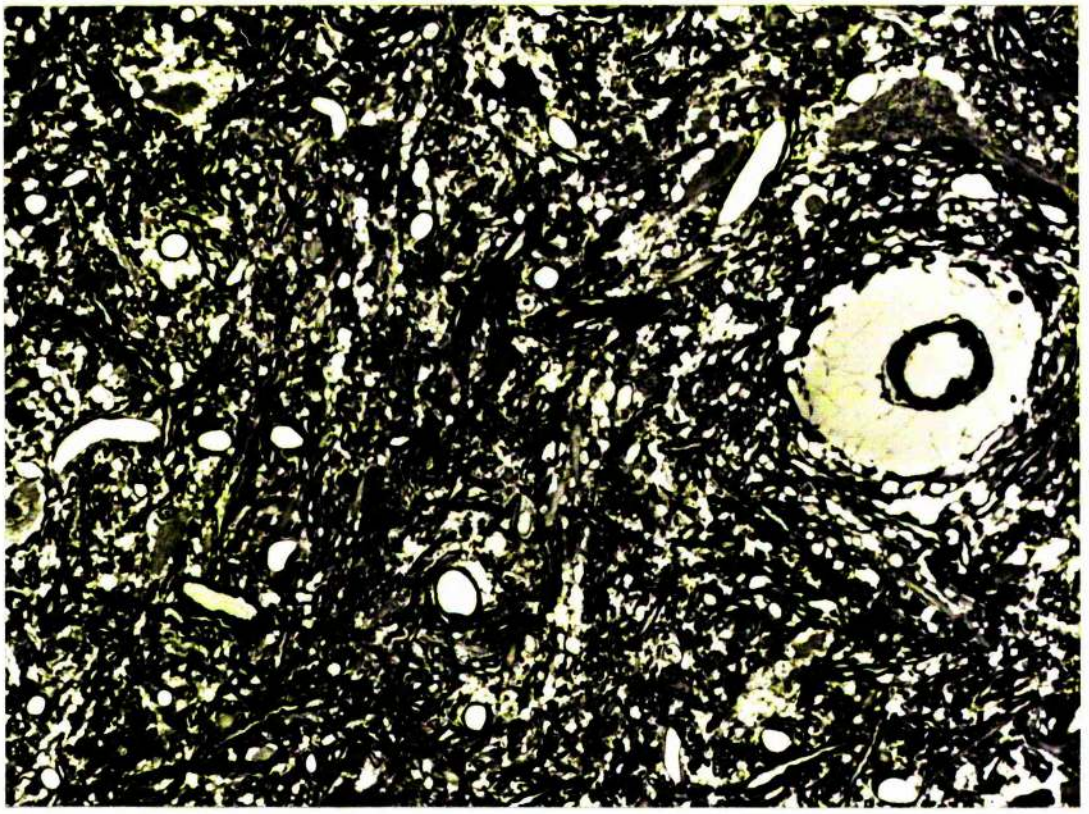


Fig. 19 Area of G.M. receiving 12 µg NA in 20 µl CSF. Generalised oedema with severe perivascular and perineuronal swelling is a prominent feature. Toluidine blue x 250.

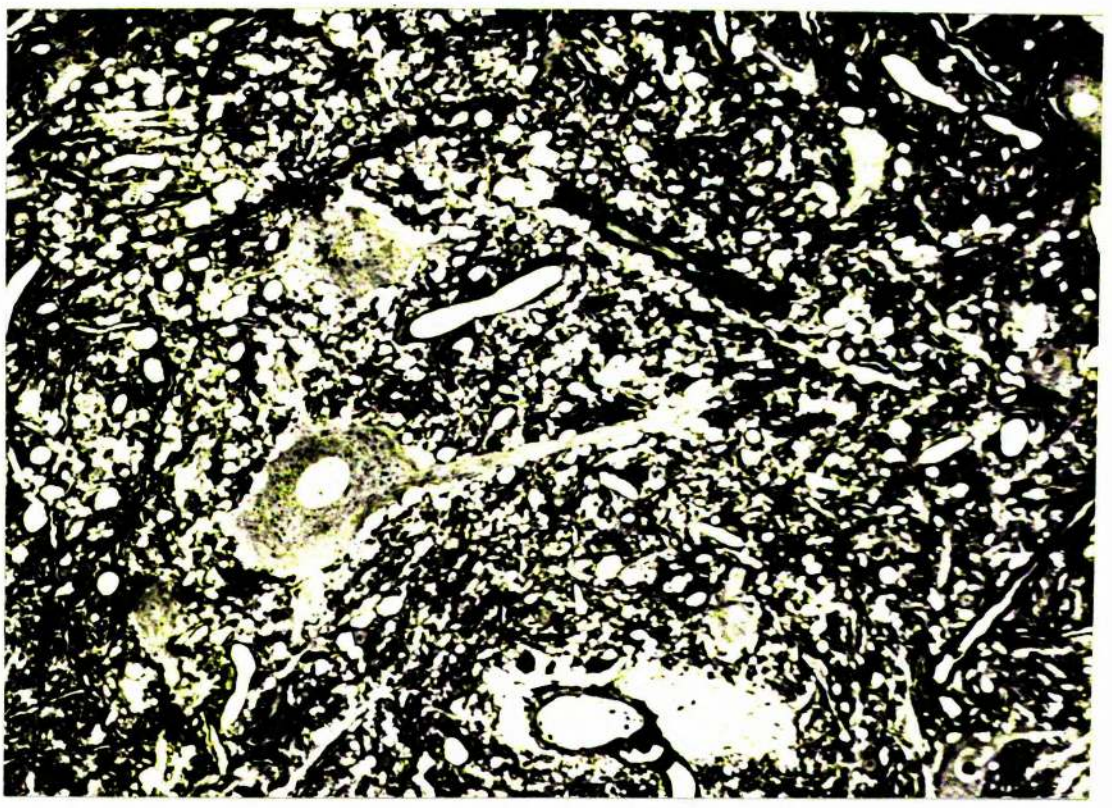


Fig. 20 Area of G.M. receiving 15 µg NA in 20 µl CSF. This shows similar changes to Fig. 19. Toluidine blue x 400.

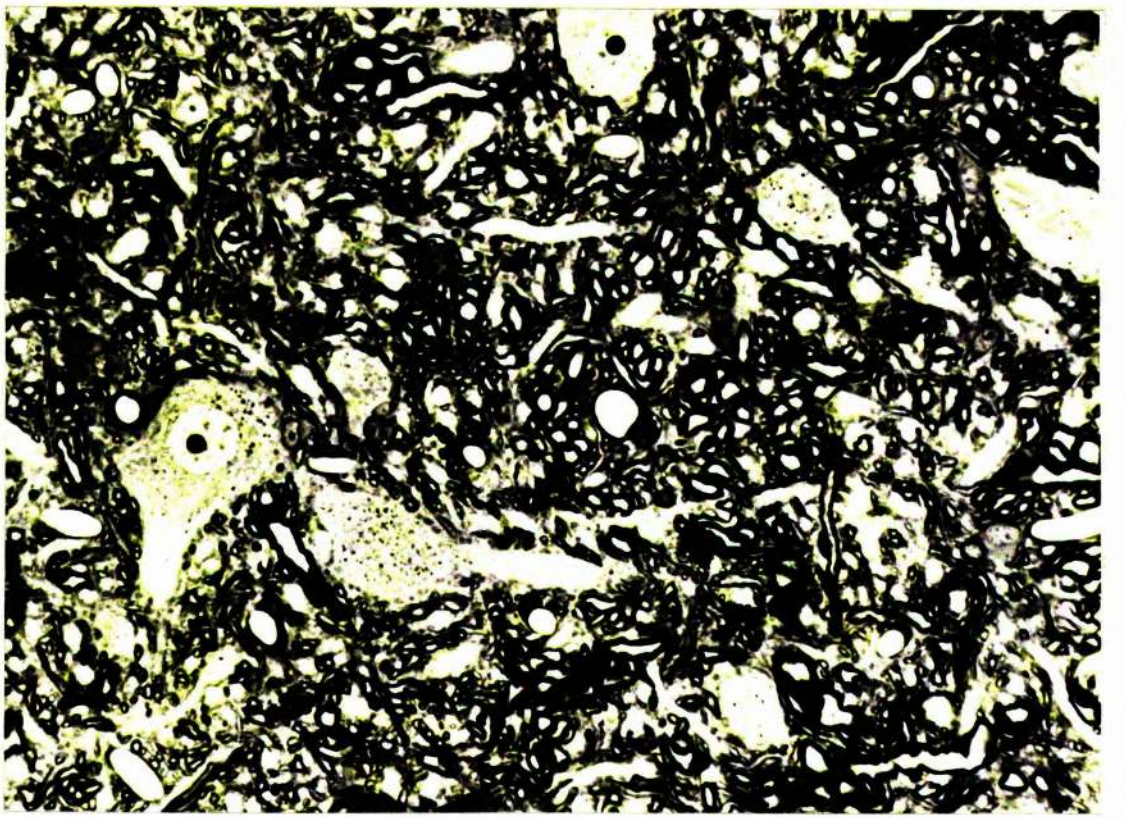


Fig. 21 Area of G.M. which received 30 µg NA in 20 µl CSF. There is no abnormality. Toluidine blue x250.

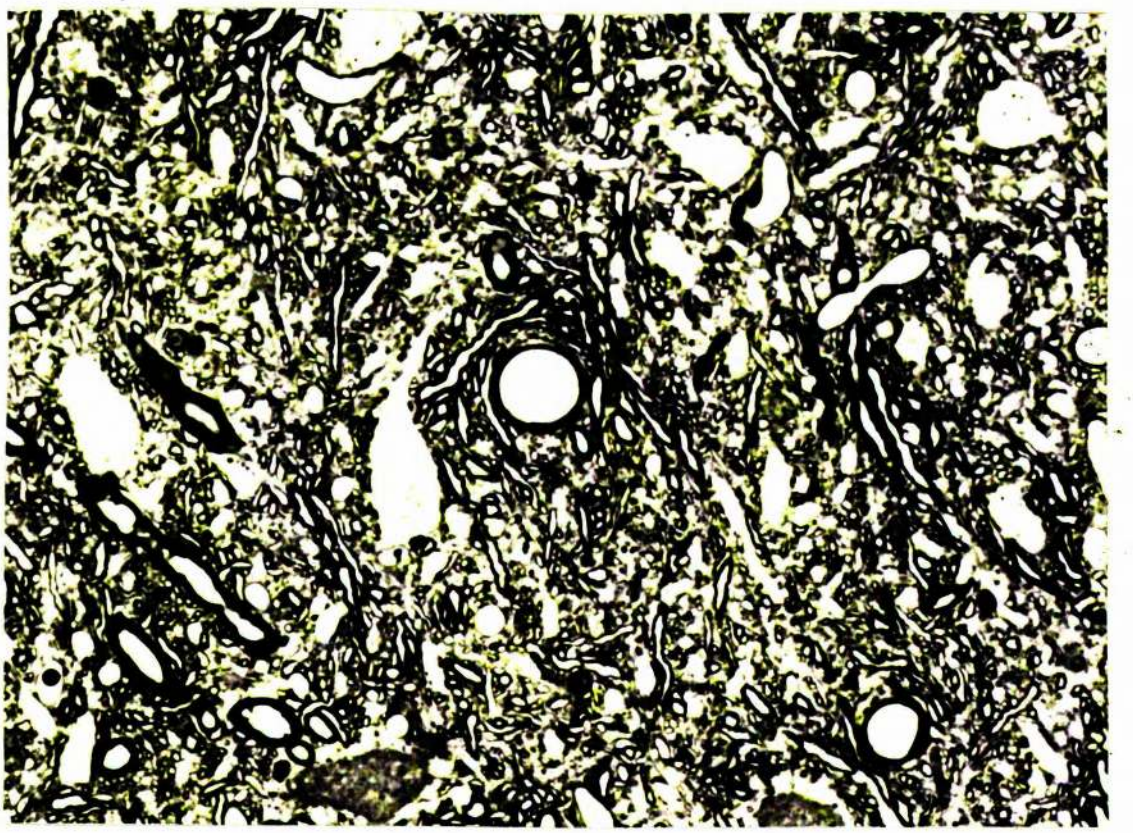


Fig. 22 Area of G.M. which received 30 µg NA in 20 µl CSF. There is moderate oedematous swelling of neural elements. Toluidine blue x 250.

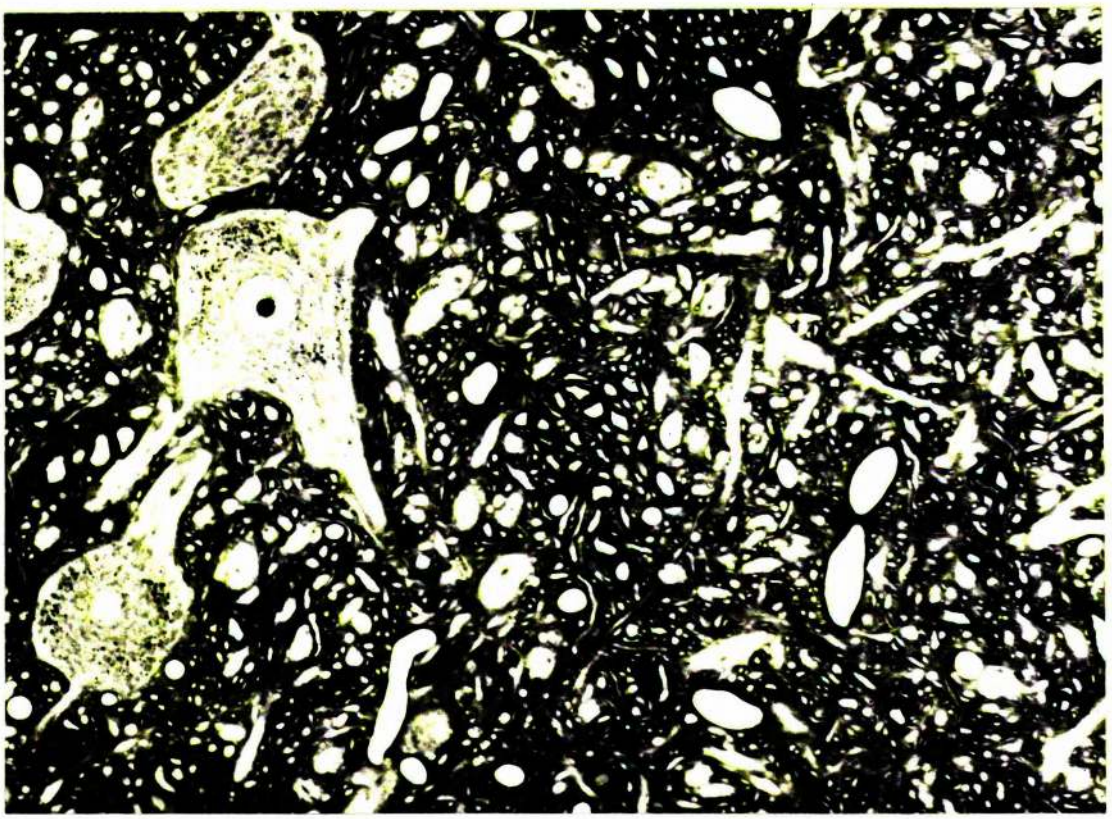


Fig. 23 Area of G.M. receiving 35 µg NA in 20 µl CSF showing normal microarchitecture. Toluidine blue x 250

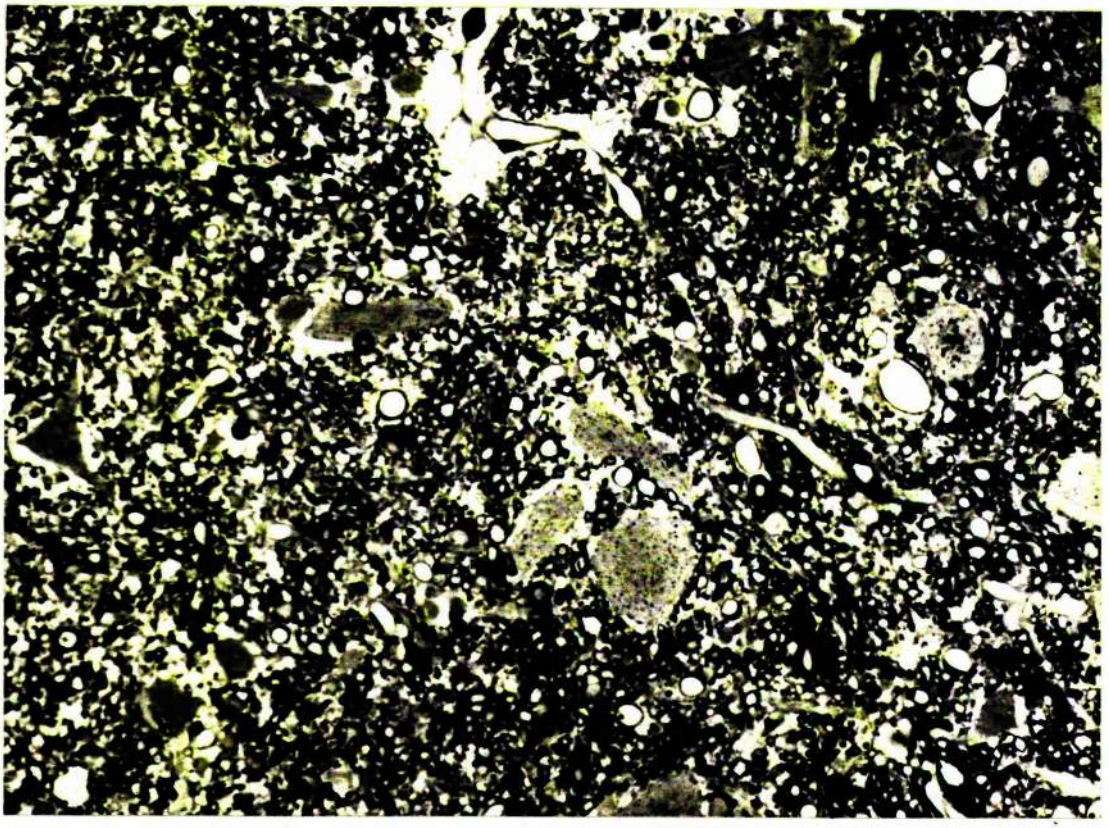


Fig. 24(a) Area of G.M. receiving 35 µg NA in 20 µl CSF. Generalised oedema, with perineuronal and perivascular swelling, is evident. Toluidine blue x 250.

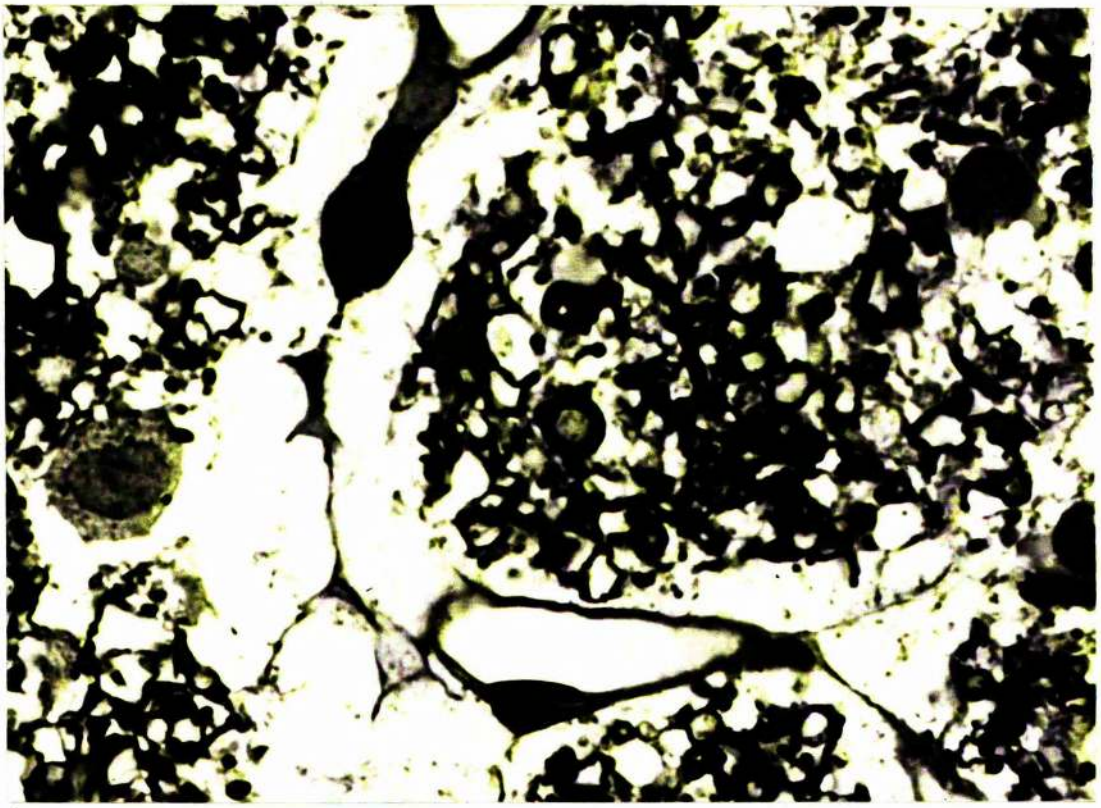


Fig. 24(b) Detail of Fig. 24(a) showing perivascular swelling. Toluidine blue x 1000

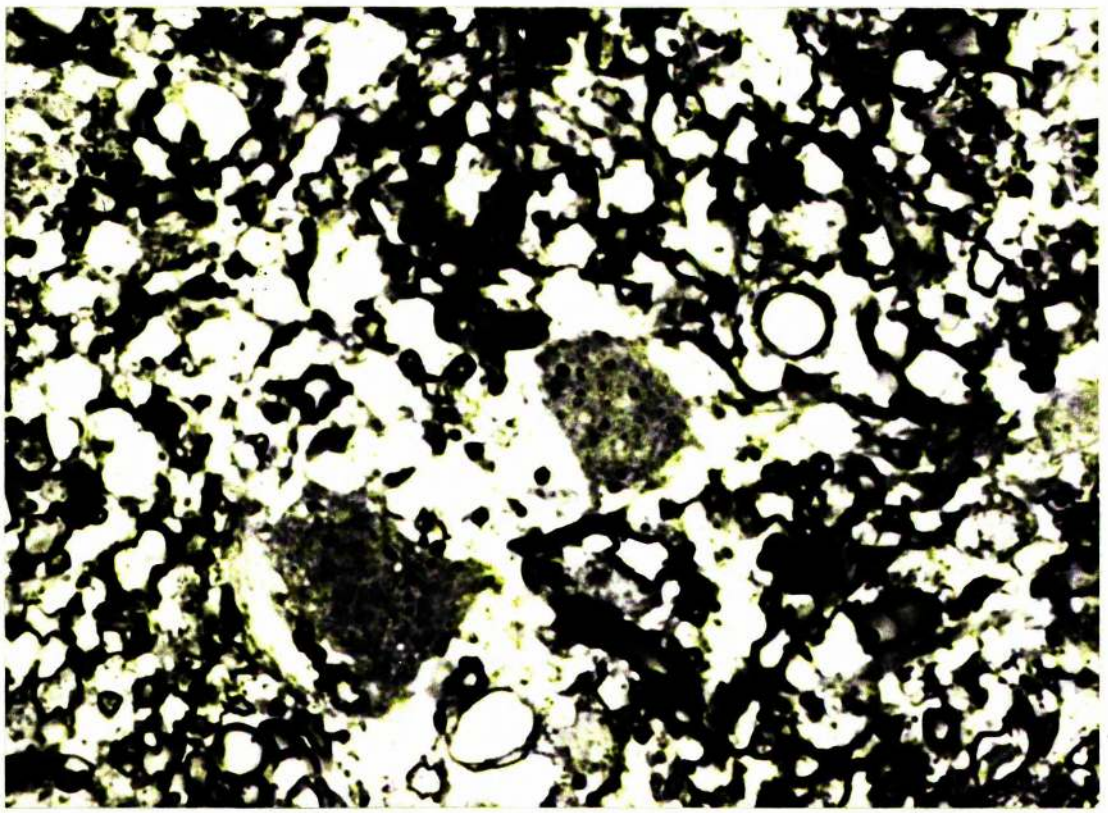


Fig. 24(c) Detail of Fig. 24(a) showing oedema of neuropil. Toluidine blue x 1000.

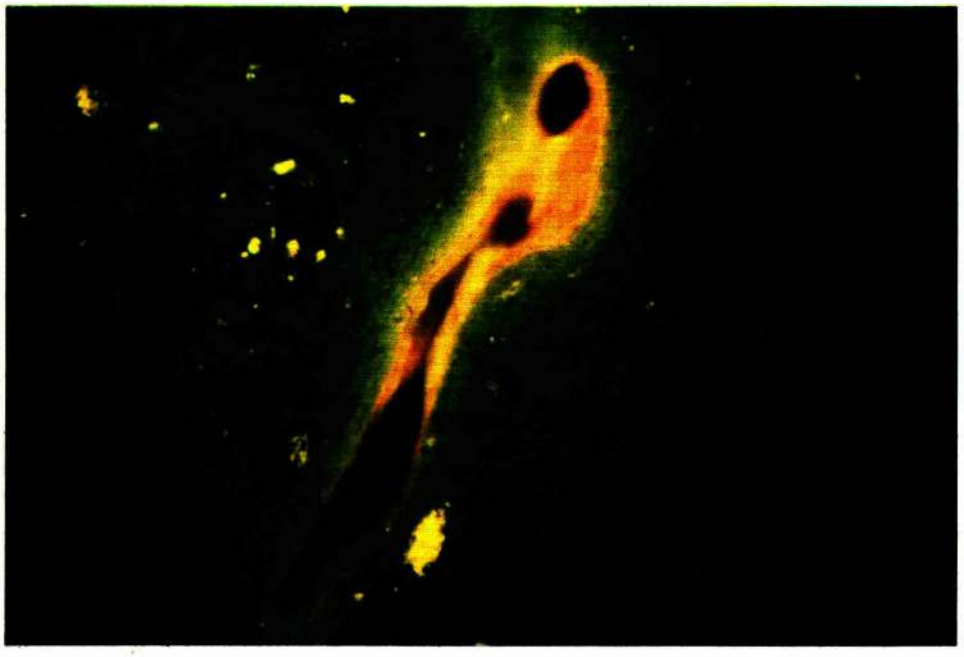


Fig. 25(a) Ventral horn G.M., L_{3-4} junction. Evans blue fluorescence is present in vessel walls and immediately perivascular neuropil. (x 160).

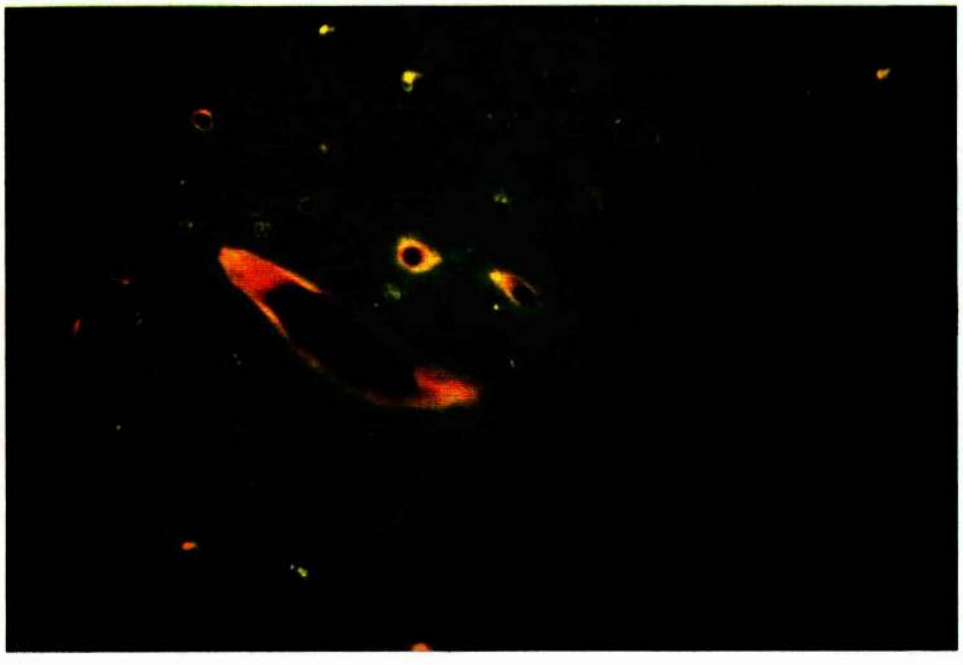


Fig. 25(b) Dorsal horn G.M., L_4. Evans blue fluorescence can be seen in several blood vessels. (x 160).

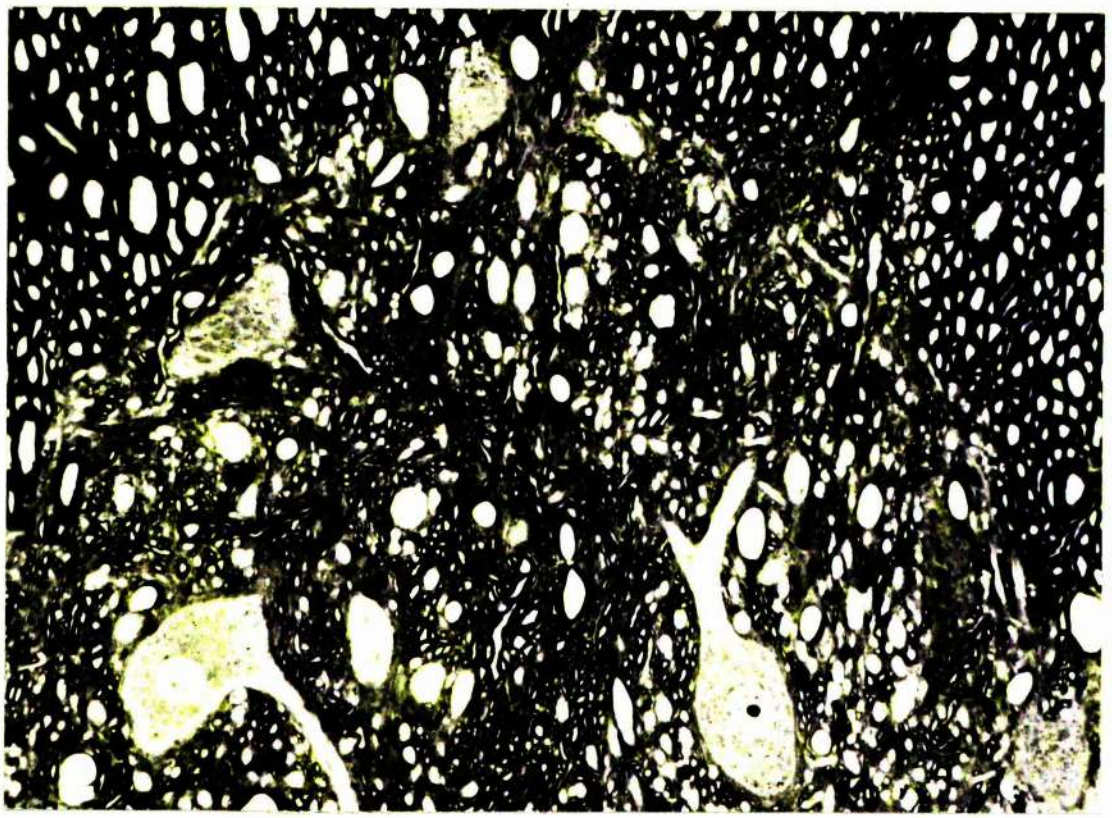


Fig. 26 GM/WM junction from a non-stained area of T_{12} segment. It is normal. Toluidine blue x 250.

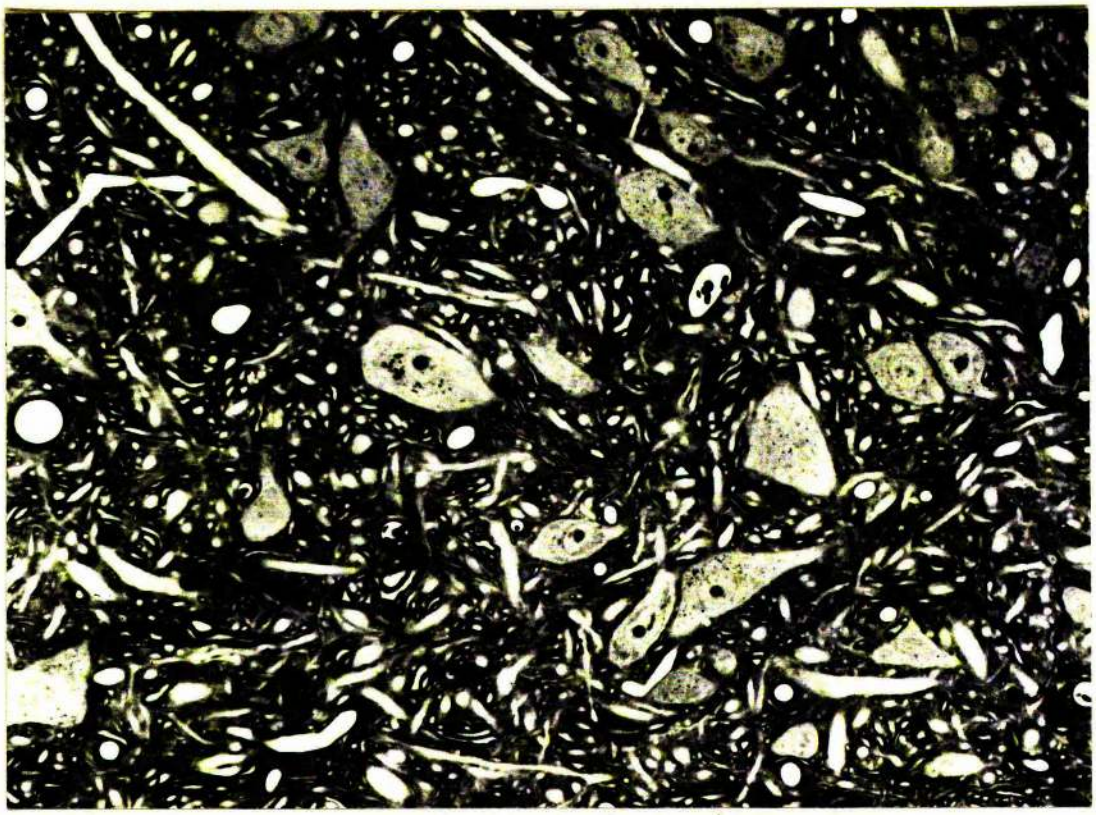


Fig. 27 G.M. from an EBA-stained part of the lumbar cord following NA infusion. There are no abnormalities to be detected. Toluidine blue x 250.

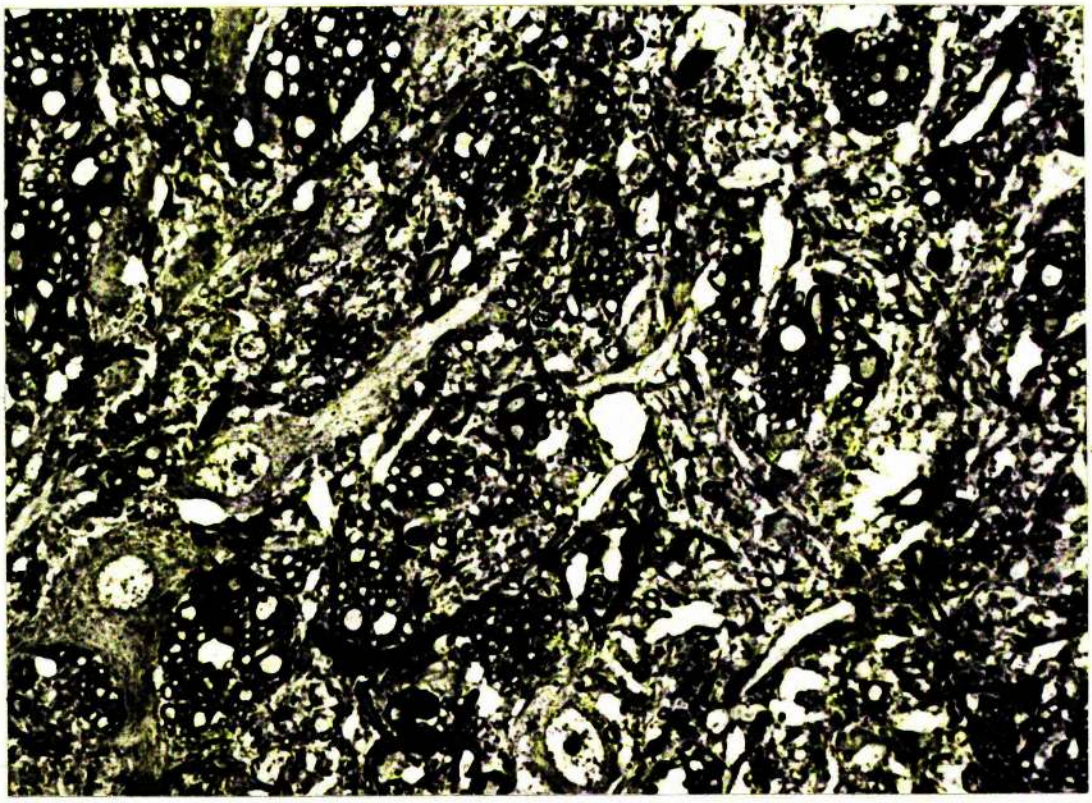


Fig. 28 G.M. from EBA-stained segment of L_4. Oedematous swelling of neuroglial processes are seen. Toluidine blue x 400.

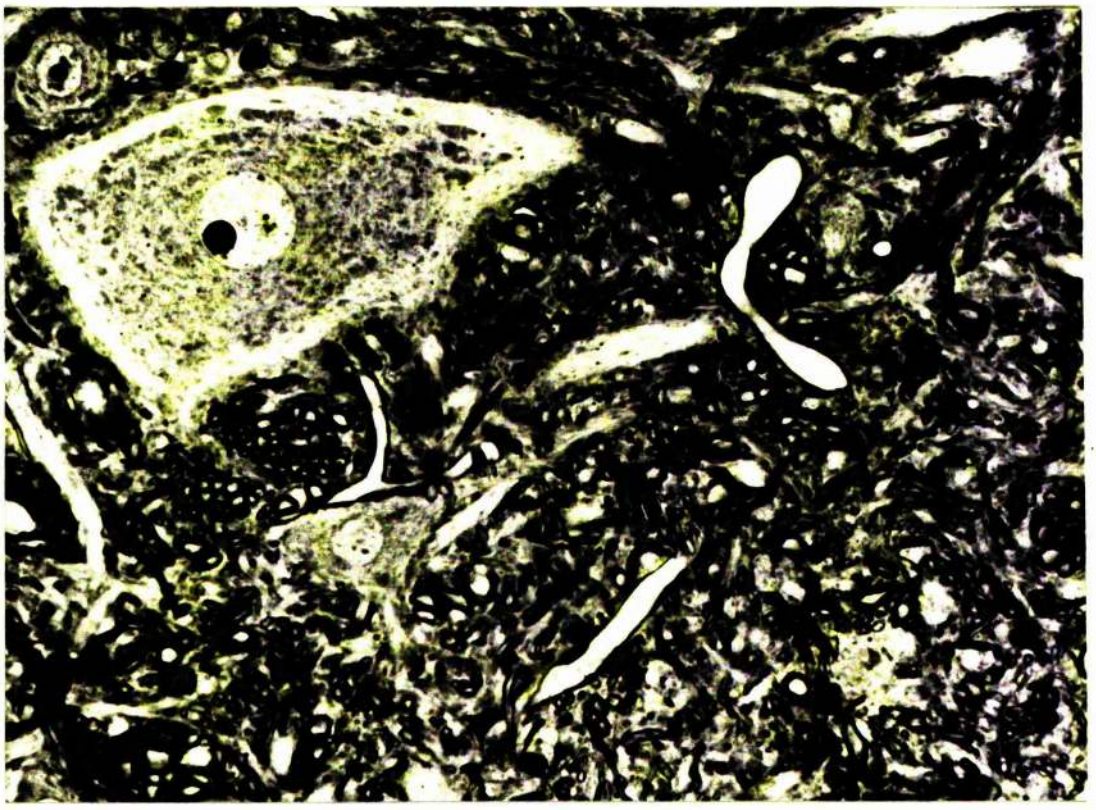


Fig. 29 Area of G.M. from lumbar cord which was EBA-stained. The tissue appears normal. Toluidine blue x 400.

Fig. 30 Lateral radiograph of lumbar spine and abdomen of a cat. Abdominal aortic ligations were carried out as in Fig. 4. The distribution of an intra-arterial injection of contrast media is defined.