AN EXPERIMENTAL INVESTIGATION OF SPINAL CORD BLOOD FLOW, IT'S AUTOREGULATION, AND THE EFFECTS OF CEREBRAL COMPRESSION ON SPINAL CORD BLOOD FLOW

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ABSTRACT

Spinal cord blood flow has usually been considered to be controlled by the same mechanisms that operate throughout the central nervous system. While there is evidence for neurogenic contribution to cerebral blood flow regulation, such evidence is lacking for spinal cord blood flow. We examined the role of intrinsic neurogenic influences upon spinal cord blood flow by inducing a stimulus that consisted of an increase in intracranial pressure. The study was carried out in male Sprague-Dawley rats, anesthetized, paralyzed, and artifically ventilated. The animals were kept throughout the experiments normothermic, normocapneic, and normoxemic. Spinal cord blood flow was measured by the hydrogen clearance technique and C^{14}-iodoantipyrine method. Our findings include: (1) existence of autoregulation in the rat spinal cord, (2) grey matter blood flow is approximately two-fold greater than white matter blood flow, (3) in response to increase in intracranial pressure to the point of eliciting the Cushing Pressor Response, while maintaining blood pressure within autoregulation range, grey matter blood flow is greatly enhanced, while white matter blood flow remains unchanged. We conclude that spinal cord blood flow is to some degree, dependent on suprasegmental surveillance, mediated by as yet unknown descending pathways.

RESUME

Les mécanismes de régulation du débit sanguin médullaire sont habituellement considérés identiques à ceux qui interviennent dans l'ensemble du système nerveux central. Bien qu'il existe des arguments en faveur d'une participation neurogène à la régulation du débit sanguin cérébral de tels arguments n'existent pas pour le débit sanguin médullaire. Nous avons étudié le rôle des influences neurogènes intrinsèques sur le débit sanguin médullaire en utilisant un stimulus non spécifique, l'augmentation de la pression intracrânienne. L'étude fut conduite sur des rats mâles de souche Sprague-Dawley, anesthésiés, paralysés et artificiellement ventilés. Pendant les expériences, les animaux furent maintenus normothermiques, normocapniques et normoxiques. Le débit sanguin médullaire fut mesuré par la technique de clearance de l'hydrogène et la méthode au C^{14}-iodo- antipyrine. Les constatations suivantes ont été faites: 1) il existe une autorégulation au niveau de la moelle épinière du rat, 2) le débit sanguin de la substance grise est approximativement le double de celui de la substance blanche, 3) lorsque la pression intracrânienne est augmentée au point de produire la réponse pressive de Cushing, et alors que la pression artérielle est maintenue dans les chiffres d'autorégulation, le débit sanguin de la substance grise augmente beaucoup, contrairement au débit de la substance blanche qui reste stable. Nous concluons que

le débit sanguin médullaire dépend en partie d'un contrôle suprasegmentaire, médié par des voies descendantes actuellement inconnues.

INTRODUCTION

There have been many reports in the literature concerned with the various aspects of cerebral blood flow in physiological and pathological conditions (Pasztor et al., 1973: Syemon et al.,1973), yet much less is available on the subject of spinal cord blood flow (SCBF). Much of what has been written on SCBF deals with the normal state and the physiological factors affecting it or the changes induced in SCBF with experimental cord trauma (Sandler and Tator, 1976). It is well established that the brain, acting via the sympathetic nervous system can regulate the systemic circulation, and it has been suggested for many years that the brain may neurogenically control its own circulation. However, to the best of our knowledge, it was not yet investigated, quantitatively, whether the brain does participate in regulation of the SCBF. The goal of this study is to clarify whether in the rat, mechanical stimulation of increased intracranial pressure which had been shown to cause vasoactive changes in the brain, will modify also SCBF.

Understanding the physiology of blood circulation in the spinal cord might have great impact on the therapeutic approach to patients with spinal cord injuries, as well as providing important information related to the spinal cord vessels reaction, to pathological events in the brain. In this study, an attempt was made to use an already established method to estimate SCBF – the hydrogen clearance technique. SCBF was

measured under normal physiological state; over a wide range in systemic blood pressure and under induced increased intracranial pressure caused by cerebral compression.

SCBF - REVIEW OF THE LITERATURE

The methods available to measure blood flow (BF) may supply two categories of information; quantitative and qualitative data. The latter can only show a relative increase or decrease in SCBF in contrast to the quantitative studies which provide "actual" values of BF measurements and therefore would appear to be more accurate and reliable.

SCBF - QUALITATIVE METHODS

The most commonly used qualitative method of studying SCBF, has been the heat clearance technique with thermoelectric devices which have been either embedded in the cord (Field et al., 1951) or placed on the cord surface (Palleske and Herman, 1968). Changes in BF produce changes in the temperature which are detected by the thermocouple junctions and in turn lead to variations in electrical current which can be measured. These studies demonstrated a relative increase in the SCBF of the lumbar cord of dwarf pigs when 5-8% CO_2 was added to the inspired gas (Palleske and Herman, 1968), and also an increase in SCBF when blood pressure was elevated (Palleske 1968).

Other studies outline the spinal cord vasculature, thus supplying data concerning patency of vessels in vivo and their capillary density that is higher in the gray than in the white matter, but do not measure flow per se. These methods include the intravenous injection of fluorescent indicators such as fluorescin, or filling vessels with colloidal barium suspension.

and subsequently performing microangiographic studies (Hassler, 1966).

SCBF - QUANTITATIVE METHODS

Quantitative measurements of SCBF require either venous blood collection or the measurement of the quantity of certain tracer in the neural tissue of the spinal cord. The complexity of the anatomy with respect to the venous drainage of the spinal cord precludes use of standard techniques such as venous sampling, and therefore, investigators have used various "tracer techniques". Examples include diffusible indicators, i.e., the uptake of ^{14}C-antipyrine which permit only a single determination of BF (as the animal must be sacrificed post injection) or tracer washout studies including injection to the spinal cord of $^{133}Xenon$, or inhalation of hydrogen by the animal, methods that allow few or unlimited number of flow determinations in the course of an experiment respectively.

The theoretical basis for the use of inert diffuse tracers and for the calculation of measurement SCBF are based on the Fick principle and will be described later (Fick 1870).

Landau et al (1955) performed the first quantitative study of SCBF. They used a radioactive gas, tri-fluoro-iodomethane labelled with ^{131}I - ($CF_3{}^{131}I$)- and autoradiographic techniques to measure regional cerebral and cervical BF in cats. They found the mean cervical cord BF to be

14 ml/100gm/min and 63 ml/100gm/min in the white and gray matter respectively.

Sandler and Tator (1976) measured SCBF in primate thoracic spinal cord using the ^{14}C-antipyrine autoradiography technique, essentially in the same method as Landau et al (1955). The mean value in 22 monkeys for the thoracic white matter BF was 10.3 ml/100 gm/min, and the mean thoracic gray matter flow was 57.6 ml/100gm/min. Whereas white matter flow was much the same in all areas (dorsal columns, ventral and dorsal lateral) ranging from less than 10 to 20 ml/100gm/min, the central gray matter and anterior horn flow values were between 20 and 90 ml/100gm/min. Although these tracer uptake and autoradiographic techniques only measures flow at one particular time, it permit differentiation of white and gray matter SCBF and obviously there is no trauma to the cord.

The indicator fractionation technique was used by Bingham et al (1975) to measure SCBF in monkeys. This method was used originally by Sapirstein (1958). When an indicator such as antipyrine is administered in a single intravenous injection and the killing time is short, the pattern of antipyrine distribution in nervous tissue will be the same as the pattern of the fractional distribution of the cardiac output. The average values obtained in this method for example in T_2 area were 40.3 and 18.3 ml/100gm/min for gray and white matter respectively. There is no trauma to the cord in this technique,

and with microdissection gray and white matter can be measured separately, however with the microdissection there is always possibility of contamination and inaccurate BF readings. The other disadvantage of the method is that it allows only one measurement of flow at one particular time.

Flohr et al (1968) have developed a technique for single measurement of SCBF. They injected to the heart of cats particels of I-131 labelled albumin and after 4 minutes killed the animal. The spinal cord was removed and transected. The amount of indicator found in each segment was taken as an index of the fraction of cadiac output passing to that region. Flow values were calculated from local indicator concentration, the total amount of the tracer injected and cardiac output at the time of injection. Mean SCBF was 19.2 ml/100gm/min. Flow to the thoracic segment (16.8 ml/100gm/min) was lower than to the cervical (19 ml/100gm/min) and lumbar cords (22.1 ml/100gm/min). Once again the method allow only one measurement, and gives values for a whole segment of the cord without differentiation to white and gray matter, however, the method is atraumatic to the cord.

Marcus et al (1977) provided another quantitative date of SCBF by injection of microspheres labeled isotops to the heart. After the animal was sacrificed the segments of the spinal cord were excised and gray and white matter were separated by dissection. Under control conditions gray and white matter

flows to the lumbosacral cord of 5 sheeps were 110 and 25 ml/100gm/min respectively. Mean BF to the cervical and lumbosacral cord segments was 40% higher than flow to the thoracic cord

Another quantitative method was used by Smith et al (1969), by Ducker and Perot (1972), and by Griffiths (1973). They used the inert radioactive gas ^{133}Xenon washout technique, in which the indicator was dissolved in saline and introduced into the spinal cord by direct injection. Diffusion into the peri-injection site tissues occurred, and the partial pressure of ^{133}Xenon at a particular site was dependent on diffusion and solubility. The ^{133}Xenon diffused into the capillaries and was removed by the blood flow, thus allowing further diffusion of the indicator. If follows that the greater the BF, the faster the isotope clearance from the tissue. The recording apparatus consisted of a crystal scintillation counter that was positioned 1-2 mm. above the surface of the cord. The SCBF was calculated from the ^{133}Xenon clearance curves. The mean SCBF for all their measurements at normal PCO_2 levels (for white and gray matter) in the thoraco-lumbar region in goats (Smith et al, 1969) and dogs (Ducker and Perot, 1972) were 16.2 ml/100gm/min and 15.6 ml/100gm/min respectively. With the same method Griffiths (1973) succeeded however to demonstrate in the white matter of the thoracic segment of dogs mean values of 15.7 ml/gm/min, while the mean BF calculated from the fast

component (which represented gray matter) was 48.4 ml/100gm/min.

In contrast to the tracer uptake techniques, i.e ^{14}C-antipyrine, the radioactive isotope clearance of ^{133}Xenon is advantageous in that measurements can be repeated in the same animal. However, the results of Griffiths (1973) demonstrated a marked variation in flows between different dogs, and he had difficulty in obtaining reproducible results in the same dog under similar conditions. Smith et al (1969) also found a marked variation in flows at the same segment between different animals. The trauma to the spinal cord tissue caused by a 29-gauge needle and the difficulty to differentiate between gray and white matter are other disadvantages of this method. However, with respect to the trauma produced by the needle Smith et al (1969) observed no disturbance of normal cell architecture. Although Griffiths (1973) did conclude that he measured flow both in white and gray matter he didn't report if histology was done. Forty percent of his desaturation curves were biexponential and in these instances the slow component was calculate to represent the white matter flow while the fast component was consider to represent the gray matter flow. The other 60% of the washout curves were monoexponential and were considered to represent only the white matter flow. The other investigators employing this technique surmounted this problem by calculating the

average cord blood flow. India ink was injected into a few animals at the same place that the Xenon was injected and was found in both gray and white matter. The SCBF values that were obtained by the various investigators described above is summarized in table 1.

SCBF AND H_2 CLEARANCE METHOD - THEORY

One of the quantitative methods used for measurement of SCBF is the H_2 clearance technique, which we have used in our experiments. Before describing the various groups of investigators who have used this technique, a theoretic background of the method is in order.

Kety (1951) pointed out that hydrogen is metabolic lly inert and not normally present in body tissues. Furthermore, H_2 dissolves readily in lipids and therefore diffuses through the blood brain barrier easily and thereby penetrates the nervous tissue well. Because of its low water-gas partition coefficient of 0.018, (Lawrence et al 1946) the pulmonary circulation should rapidly remove it from arterial blood. These features of hydrogen fulfill the criteria for tracer elements clearance studies of blood flow, developed by Kety (1951). The H_2 clearance method is based upon the detection of hydrogen by a positively polarized platinum electrode implanted in the tissue of interest. Momentarily respired (or injected intra-arterially) hydrogen is transported to the surface of the electrode where it produces a current proportional to the

hydrogen concentration at the electrode tip. As the dissolved gas is cleared from the tissue and removed from the blood by the lungs, tissue hydrogen clearance is reflected by a decreasing current that is related to the rate of blood flow at the site of the electrode. The relationship of the distribution of the hydrogen (according to its partition coefficient between tissues and blood) to the blood flow is described by the Fick principle (1870).

$$J=D\ (\frac{dc}{dx})$$

where J is the flow in moles (the flow of H_2 towards the electrode) and dc is the concentration gradient over distance x from the electrode. The term J, the flow of H_2 can ben converted into a current term, using Faraday's Law. The polarographic technique should reflect H_2 concentration in tissues, and the developed Fick equation tells us that the current is a linear function of the bulk of H_2 concentration. The equations for calculating blood flow from the hydrogen clearance have been recently extensively reviewed by Young (1980a).

Blood flow can be estimated from $t_{1/2}$, the time required by the H_2 concentration to fall to half of its original value. Thus, one of the derivatives of Fick's equation is

$$J=\frac{-\ln(1/2)}{t\ 1/2}=\frac{0.693}{t\ 1/2}$$

Hydrogen clearance technique was introduced as a method to measure blood flow by Aukland et al (1964). They used the polarographic method measuring tissue H_2 concentration in vitro by monitoring the current generated by a platinum electrode. They found it correlated with hydrogen concentration. They also measured blood flow using the same method in animals' kidneys, myocardium and skeletal muscle, and found it to correlate well with flows obtained by venous outflow collection.

Fieschi and Kety (1964) and Fieschi et al (1965) in experimental animals and Gotoh et al (1966) in man were the first to apply the H_2 clearance method to the central nervous system. Good correlation were obtained between the H_2 clearance and the ^{14}C-antipyrine autoradiographic method, measuring BF in cat sub-cortical nuclei and white matter (Fieschi et al., 1969).

SCBF AND H_2 CLEARANCE TECHNIQUE, LITERATURE REVIEW

This technique was first used by Kobrine et al (1974) to measure SCBF in primates. Using a platinum wire 250 m which was placed in the lateral funiculus at T_7-T_{11} segments they were able to measure BF in the white matter which ranged from 14 to 21 ml/100gm/min with a mean of 17.5 ml/100gm/min. They also found the BF in the "center" of the cord to be 14 ml/100gm/min.

Griffiths et al (1975) used the hydrogen clearance method to measure SCBF in dogs and baboons. The platinum electrodes used were 0.2 mm. in diameter, and the results obtained in the white matter of the thoracic cord were 11.5 in dogs and 13.7 ml/100gm/min in the baboons, while gray matter flow was 10.8 and 16.5 ml/100gm/min in the dogs and baboons respectively. The segments of the cord in which these measurements were performed were not given.

Senter et al (1978) using 250 µm diameter platinum electrodes with a sharp 10 m tip, measured SCBF in the white matter of the cats thoracic lateral funiculus to be between 10 to 13.3 ml/100gm/min.

The belief shared by a few authors (Sandler and Tator, 1976, and Marcus et al., 1977) that the H_2 clearance technique cannot differentiate between gray and white matter flow, proved to be incorrect when two groups of investigators independently reported in 1983 their white and gray matter SCBF results.

Hayashi et al (1983) using platinum wire 35 µm in diameter with a tip of 10µm found in rats the mean SCBF in the cervical, thoracic and lumbar segments to be 63 and 20 ml/100gm/min in the gray and white matter respectively. They suggested that the SCBF maintained constant values within the various spinal cord levels despite significant variation in the blood supply to these various levels.

Scremin and Decima (1983) studied the SCBP in cats with the H_2 clearance technique, using a platinum wire 75 m in diameter. The average value of SCBF of the ventral horn gray matter and of the white matter were 43.2 and 16.2 ml/100gm/min respectively. The results obtained by the various investigators using the H_2 clearance technique including the present study are summerized in Table 2.

The apparatus for the H_2 clearance method used by all groups of investigators cited above as well as by us was very similar to that reported by Aukland et al (1964) and had been modified to resemble that used by Pasztor et al (1973).

All of the different authors who studied SCBF using the H_2 clearance method used very similar protocols and methods with a few noteworthy differences; some of which will be described in the discussion session.

SCBF - ^{14}C IODOANTIPYRINE METHOD

A different way to measure SCBF and CBF that was used also in our experiments was by introduction ^{14}C Iodoantipyrine as a tracer to the blood system.

THEORY

This method to quantitatively determine the rate of blood flow to specific component of the brain was first described by Kety (1960) using freely difusable inert gases as tracers. The method is based on the following equation derived by Kety (1951, 1960).

$$Ci(T) = \lambda K \int_0^T Ca\, l^{-K(T-t)} dt$$

Here, where Ci(T) equal the tissue concentration in a homogenous tissue at a given time, T, after introduction of the tracer into the blood, λequals the tissue: blood partition coefficient; Ca is the concentration of tracer in the arterial blood; t, equals the variable time; and K equal a constant that incorporates within it the rate of blood flow in the tissue. The constant K is given as $K=mF/W\lambda$. Where F/λ is the flow per mass of tissue and m is a constant that describes the extent to which diffusion equilibrium between blood and tissue is achieved during passage from the arterial to the venous end of the capillary. Without any diffusion limits or arteriovenous shunt m=1.

VASCULAR ANATOMY OF THE RAT'S SPINAL CORD

Blood flow to the spinal cord, like other organs in the body, is dependent on the cardiac output and on the caliber of the vessels, mainly the arteriols which supply the cord. The intention to use rat for experiments on the SCBF made it necessary to evaluate the vascular arrangement of the spinal cord of the rat. Woollan & Millen (1955) who gave the first account of the vascular anatomy of the spinal cord of the rat, as well as other investigators (Tveten, 1976) were struck by the close similarity to that of man. The latter author used spinal cords of 115 rats, which were cut transversely or in longitudinal plane and were examined by radiography, and stereomicroscopy in different positions, and a summary which cites this study will follow (Teveten, 1976).

Each middle branch of the spinal artery divided outside the dura mater into a ventral and a dorsal root artery (the radicular arteries).

THE VENTRAL RADICULAR ARTERIES

The ventral root arteries provided the main blood supply to the cord. The average number of the ventral radicular arteries in the cervical level was 3, in the thoracic region 4 and in the lumbar region 0 to 1. To the upper thoracic region there was a remarkable poor supply. One artery, larger than the others, the great ventral radicular artery originated between T_{11} to L_2, and in about two thirds of the specimens it arose from

the sub-costal space at T_{13}, slightly more often from the right than from the left side. In the vast majority of specimens, the great ventral radicular artery was the only ventral tributary to the lower thoracic and lumbo-sacral cord. On the ventral surface of the cord each artery divided near the ventral median fissure into an ascending and descending branch. These branches united with those from above and below to form the ventral spinal artery (or anterior spinal artery) which extended the entire length of the cord.

THE DORSAL RADICULAR ARTERIES

These arteries were smaller but more numerous than the ventral radicular arteries, and as for the latters, a profuse supply to the spinal cord enlargements and a relatively poor supply in the upper thoracic cord was evident. These arteries give ascending and descending branches which form the posterior (dorsal) spinal arteries.

THE SURFACE ARTERIES OF THE SPINAL CORD

The surface of the spinal cord possesses three longitudinal arteries, a single ventral (anterior spinal artery) and paired dorsal (posterior spinal arteries). The anterior spinal artery extended the hole length of the cord in front of the ventral median fissure and gave rise to branches transversing the ventral median tissue - the central arteries.

The paired posterior spinal arteries, one on each side, were situated on the dorso-lateral surface, and their size was markedly smaller than that of the anterior spinal artery. The two posterior spinal arteries interconnected by small transverse branches crossing the dorsal surface of the cord. Branches were given off penetrating the tip of the dorsal gray horn to supply the dorsal gray and white matter of the cord.

THE INTRINSIC ARTERIES OF THE SPINAL CORD

The central arteries emerged from the anterior spinal artery and cross the ventral median fissure to reach the base of the ventral gray horn. They terminate in a rich vascular plexus supplying the main parts of the gray and white matter. Their total number is approximately 200. The average number per 1 cm. of the cord was 26, 14 and 32 in the cervical mid-thoracic and lumbar levels respectively. Most of the collaterals of the central arteries coursed to the periphery of the gray matter before they divided into terminal arteriols. The main blood supply to the cord derived from this system.

The penetrating branches from the posterior spinal arteries enter obliquely the tips of the dorsal gray horns. They anastomosed with their pair in the longitudinal and transverse plans to supply the dorsal gray and white matter. At the base of the dorsal gray horn, branches from the central arteries overlapped with penetrating branches of posterior spinal arteries.

As described above, the blood supply of the cord might be divided into two arterial systems, according to the direction of blood flow. a) The anterior blood supply (central artery) in which the direction is centrifugal and b) The posterior spinal arteries and their penetrating branches, with branches of the ventral radicular artery which are entering the cord from the periphery and in which the direction of blood flow is centripetal.

An important extensive capillary network was found in the gray matter, more prominent in the ventral and lateral gray horns than in the dorsal gray horn. The cortico-spinal tract which is located in the ventral part of the dorsal funiculus seem to have much better blood supply than the rest of the white matter.

INNERVATION OF THE BRAIN AND SPINAL CORD BLOOD VESSELS

The cerebral vessels, both extra and intraparenchymal are richly innervated. The cerebral arteries are innervated by both the peripheral aminergic and cholinergic nervous system (Iwayama et al., 1970, Edvinsson et al., 1976), while the cerebral veins have the same aminergic innervatin, however, they have no cholinergic nerve fibers (Nakakita et al., 1983).

By light microscopy Chorobski and Penfield (1932) found a dual sympathetic and parasympathetic innervation of cerebral

arteries. They traced nerve fibers from both the facial nerve and the superior cervical ganglion to the cerebral arteries. They also showed that a large number of these nerve fibers survived after cervical sympathectomy and suggested that the origin of these fibers is in the cranial nerves being parasympathetic fibers. Iwayama et al (1970) studied the anterior cerebral arteries of rats in two techniques; a) fluorescent histochemistry, and b) electron microscopy. a) With the fluorescent method it was shown in the controlled group that fluorescent nerve fibers formed a meshlike plexus over the entire length of the anterior cerebral artery. Two days after removal of superior cerevical ganglia all the fluorescent nerve fibers had disappeared from the anterior cerebral artery. b) After fixation with potassium permanganate, two populations of small vesicles, granular and agranular, could be distinguished in the terminal axons of the nerve fibers, under the electron microscope. Two days after sympathetic denervation, no axons containing small granular vesicles were seen, whereas the fibers containing small agranular vesicles were not affected.

Other studies on organs innervated by adrenergic nerves indicated that the small granular vesciles are sites of storage of noradrenaline. The fact that the fibers containing small granular vescicles and the fluorescent fibers around the cerebral arteries were degenerated after cervical sympathectomy,

suggest that these axons are adrenergic. The fibers that were not affected by sympathetic denervation and which were observed after the degeneration of adrenergic nerves (containing small agranular vesicles) considered by Iwayama et al (1970) to be cholinergic. These nerve fibers seem to have another origin probably non sympathetic, that might be the cranial nerves or more specifically the facial nerve as described by Chorobski and Penfield (1932).

Although much has been learned about the neurogenic innervation of the cerebral blood vessels, there is paucity of morphological information concerning the neurogenic innervation of the spinal cord blood vessels. Nevertheless, few investigators have demonstrated sympathetic innervation of the spinal cord blood vessels in rats and dogs; fibers from paravertebral sympathetic ganglia enter the spinal cord accompanying the blood vessels and synapse on the muscle of these vessels (Ogushi, 1968, McNicholas et al., 1980).

Very recently it was found by histochemical studies that in the spinal cord the arterial system has dual innervation of aminergic and cholinergic nerve fibers, but the venous system has only aminergic innervation, the same as in the brain (Itakura, 1983). This author also demonstrated that the most characteristic morphological difference of the neurogenic innervation between the brain and the spinal cord lie in

intraparenchimal arteriols; those of the spinal cord posses peripheral aminergic nerve fibers, while the intraparenchimal arteriols of the brain do not. The aminergic and cholinergic nerve fibers in the cat spinal cord blood vessels (anterior spinal artery, central artery, and posterior spinal vein) were studied by means of amine histofluorescence and acetylcholinesterase (ACHE) staining (Itakura, 1983).

Concerning the role of the autonomic nervous system in controlling the central nervous system blood flow, Wei et al (1975) suggested that the aminergic system in the blood vessels may play a role in vasoconstriction by performing sympathetic nerve stimulation. D'Alecy and Rose (1977) described the cholinergic system in dogs cerebral arteries to act as a vasodilator. Pharmacological blocking agents were used by Lowe and Gilboe (1971) and by Yordanov and Vlahov (1971) to demonstrate the presence of alpha and beta adrenergic receptors and their different role on cerebral blood flow, in the canine and cat cerebral vessels.

PHYSIOLOGY OF SCBF AND ITS AUTOREGULATION

SCBF has usually been considered to be controlled by the same regulatory mechanisms that operate in the rest of the central nervous system (Marcus et al., 1977).

Several studies have been performed to examine the effect on SCBF of varying physiological parameters such as systemic arterial blood pressure, activation of spinal neurons, arterial carbon dioxide tension ($PaCO_2$), and arterial oxygen tension (PaO_2).

EFFECT OF SYSTEMIC BLOOD PRESSURE ON SCBF (AUTOREGULATION)

Early reports using qualitative methods (thermocouple design) found that SCBF was independent of a wide range of systemic arterial pressure changes (Field 1951). Palleskie (1968) with heat clearance devices and Kindt (1970) using Peltier flow devices studies pigs and monkeys which were under anesthesia. Following injection of vasoconstrictive drugs, and subsequently, elevation of BP they found only initial increase in SCBF that soon returned to normal, although the BP remained high. They also suggested that the cord reacted in a similar way as the brain did, regarding changes in BP.

The quantitative mesurements of SCBF as affected by changes in systemic arterial blood pressure showed clearly that autoregulation in SCBF does exit. Flohr et al (1968) studied anesthetized cats with the particle distribution method,

and concluded, that under normal conditions, no significant correlation was found between mean arterial blood pressure and SCBF when the mean arterial pressure varied between 60-160 mmHg. Griffiths (1973) using the ^{133}Xenon clearance technique demonstrated on anesthetized dogs no significant variation in white matter flow in the BP range of 60-150 mmHg. However, below 60 mmHg flow decreased with reduction of BP. Anesthetized monkeys were shown to have autoregulation to changes in BP as was described by Kobrine et al (1975a) using the H_2 clearance technique. The SCBF in the thoracic white matter remained constant and in the normal range with a mean arterial BP of 50 to 135 mmHg. Below 50 mmHg SCBF fell passively with further decrease in BP, and values above 135 mmHg resulted in increase in SCBF with further increase in BP. In this study, changes in BP were reached by bleeding the animal or by infusion of norepinephrine when BP was lowered or raised respectively. The same tendency of regulation of SCBF in the presence of alteration in BP was emphasized by Marcus et al (1977) using labeled microspheres. Increasing systemic pressure by 40-50 mmHg (from 110 to 152 mmHg in dogs and from 75 to 134 mmHg in sheep) did not alter flow to any region of the spinal cord in the anesthetized dogs or sheep studied. BP was increased in these experiments by infusing phenylephrine. A different range of autoregulation in reaction to elevation or lowering of the BP was observed by Senter et al (1979). Their experiments were

done on anesthetized cats using the H_2 clearance method. Systemic BP was changed using Aramine and Nitropruside for increasing or decreasing the BP respectively. They observed SCBF in the dorsolateral foniculus of the thoracic cord to be increased passively above 90 mmHg and to decrease below 40 mmHg.

EFFECT OF SYMPATHETIC NERVOUS SYSTEM ON SCBF

Few data were published concerning the role of the sympathetic nervous system on control and regulation of SCBF. After the administration of phenoxybenzamime, an alpha adrenergic blocking agent, it was found by Kobrine et al, (1977a) that the SCBF in monkeys white matter was linearly related to mean systemic arterial pressure, which means that the autoregulation phenomena appeared to have been abolished.

The effect of high cervical cord section on the phenomenon of autoregulation was studied in monkeys with the H_2 clearance method (Kobrine et al., 1976). Autoregulation was found to be intact between 50 and 125 mmHg, following a pattern similar to the one observed in the intact animal as described above (Kobrine et al.,1975a). These findings suggested that the sympathetic system can regulate SCBF on a spinal cord reflex basis and not necessarily via the central hypothalamic control of the sympathetic system. The administration of propanolol, a beta adrenergic blocker, emphasized that at high systemic arterial pressure the beta adrenergic component is involved

with initiating a vasodilation and a resultant marked increase in SCBF (Kobrine et al., 1977b). To support part of these observations Young et al (1982) performed paravertebral sympatectomy in the thorax of cats and demonstrated that the blood flow correlated linearly with systemic blood pressure in contrast to the wide range of autoregulation observed in the intact animals. This was a direct proof of the peripheral sympathetic involvement in regulating SCBF.

EFFECT OF METABOLIC STIMULATION ON SCBF

Another factor that affects the SCBF, is increased neural activity which is induced by stimulation of peripheral nerves (i.e. heel pinching or stimulation of the animal sciatic nerve) and has been shown to increase SCBF, either with the qualitative method (Field et al 1951), as well as using quantitative techniques (Scremin and Decima 1983, Marcus et al 1977). The latter group tested the effect of a metabolic stimulus - an electrical stimulation of the femoral and sciatic nerves - on lumbo - sacral blood flow. When these nerves were stimulated in the left side, SCBF to the gray matter on the same side increased by 50%, while simultaneous measurement of flow to the ipsilateral white matter and to the contralateral white and gray matter demonstrated no change. It is generally accepted that blood flow is linked to metabolism in the normal nervous system (Olsen 1970).

EFFECT OF CHEMICAL STIMULATION ON SCBF

Alterations in systemic blood gases, mainly the arterial carbon dioxide tension has been shown to be the most important physiological parameter affecting SCBF.

Kindt et al (1970) studied anesthetized monkeys SCBF by the Peltier flow devices. Recordings were made as the respiratory gas was changed from 100% O_2 to 10% CO_2 in 90% O_2. They reported a prompt rise in SCBF (as well as in cerebral blood flow) when the respiratory gas was changed from 100% O_2 to 10% CO_2 in 90% O_2. When the respiratory gas was again changed to 100% O_2, there was a reduction in SCBF. Another SCBF qualitative study on anesthetized pigs has been performed using heat clearance probes (Palleske and Herman 1968). They also obtained consistently, increases in SCBF (and in brain) with increases in arterial pCO_2. Following hypoxia they observed a reactive hyperaemia of the cord.

The majority of the evidence elaborated by the investigators who used the quantitative methods indicates that extreme hypercarbia causes a marked increase in SCBF and hypocarbia causes a marked decrease in SCBF. Flohr et al (1968) using the particle distribution method in anesthetized cats found that SCBF in whole segments was linearly related to pCO_2. Smith et al (1969) in anestetized goats using the ^{133}Xenon clearance method, reported mean SCBF to fall from

17.4 ml/100gm/min when pCO_2 was 39.3 torr, to 8.5 ml/100gm/min when pCO_2 was reduced to 16.8 torr. Conversely, elevation of pCO_2 from 37.4 to 59.4 torr increased mean SCBF from 13.0 to 19.6 ml/100gm/min. Using the same method in anesthetized dogs, Griffiths (1973) showed a high linear correlation between the change in flow and pCO_2, and raising the pCO_2 from 40 mmHg to approximately 85 mmHg doubles the blood flow in the white matter. Hypocarbia was consistent with vasoconstriction and this effect was absent in coexistent hypoxia. No change in flow occurred until pO_2 had reached 60 mmHg when a sharp increase in flow occurred reaching maximal levels at pO_2 of 30-40 mmHg. Very similar conclusions were obtained by Marcus et al (1977). They measured SCBF in anesthetized dogs and sheep. For example, their total SCBF (in dogs) with pCO_2 of 39 mmHg and pO_2 of 123 was 32 ml/100gm/min. With a pCO_2 of 24 and 55 mmHg the total SCBF was 18 and 55 ml/100gm/min respectively. Thus, hypercapnia increased flow and hypocapnia decreased flow. Hypoxia in sheep also markedly increased flow, i.e. under pO_2 of 32 mmHg lumbosacral blood flow was 53 ml/100g/min while the control measurement under pO_2 of 100 mmHg SCBF in the same segment was 32 ml/100gm/min.

The normal value for the pCO_2 in the thoracic white matter of an awake unanesthetized monkey was 31 mmHg based on the observations of Kobrine et al (1975a) using the H_2

clearance technique. However, relatively large changes in pCO_2 in either direction did not result in significant change in SCBF, and unlike the cerebral blood flow they found that between 10 and 50 mmHg, SCBF remained constant at 17 ml/100gm/min. On the other hand they demonstrated a linear increase in SCBF from 50 to 110 mmHg of pCO_2.

Thus, it can be concluded that SCBF in experimental animals increases markedly when pCO_2 is above 50-55 mmHg and decreases in pCO_2 values below approximately 25 mmHg. Hypoxia (pO_2 below 60 mmHg) also increases SCBF reaching maximal levels at pO_2 of 30-40 mmHg. In addition, SCBF demonstrates autoregulation in reaction to systemic arterial blood pressure changes, and increases significantly in the presence of metabolic stimulation. SCBF is regulated by the sympathetic nervous system on the level of the spinal cord and is not necessarily under central sympathetic control.

EFFECT OF DRUGS ON SCBF

The effects of drugs on SCBF are dependent upon their capability to cross the blood cord barrier (BCB) and to reach the extracellular fluid. Anesthetic agents however in addition to having direct vasoactive effects on the blood vessels (i.e. vasoconstriction or vasodilation) may have secondary effects on SCBF; e.g. by affecting blood pressure, metabolism, or respiration (e.g. changing the pCO_2).

Concerning cerebral blood flow (CBF), the anesthetic drugs that are given intravenously (i.e: sodium pentobarbital) cause vasoconstriction and reduction in CBF, secondary to reduction in cerebral metabolism. In contrast, the inhalational anesthetics tend to dilate cerebral vessels (Smith and Wollman, 1972). For example, Fieschi et al (1969), (the investigators who introduced first the H_2 clearance method to the brain in experimental animals) demonstrated that caudate nucleus blood flow tends to decrease significantly (37%) during deep barbiturate anesthesia in cats (Pentobarbital 50 mg/kg/i.p.) as compared to awake cats. There is no up to date detailed study, yet to the best of our knowledge, that investigates the role of anesthetic compounds on SCBF. Nevertheless, using the heat clearance method, a non-quantitative technique , it was shown by Field et al (1951) that intravenous injection of barbiturates (sodium pentobarbital) or D-tubocurarine caused a decrease in the rabbits SCBF. However, Landau et al (1955), using a short

acting barbiturate, thiopentone, reported no difference in the cervical white matter SCBF from values obtained in unanesthetized cats. Sokoloff (1958), in a quantitative measurement using radioactive inert gas in the feline, found also that light thiopental anesthesia had no significant effect on the SCBF. A further support to these studies was emphasized by Griffiths (1973) using the ^{133}Xenon clearance method on dogs. Although he didn't test unanesthetized dogs, the comparison of the effects of trichlorethylene, halothane, and pentobarbitone anesthesia on the flow in the white matter showed no significant difference in SCBF with any of the three drugs.

Practically all the various investigators in SCBF are using either light anesthesia with barbiturate (i.e. 25/mg/kg of pentobarbital, Young et al., 1982) or N_2O and O_2 in approximately a 2:1 mixture (Kobrine et al., 1977a).

Not much is known either on the effects of non-anesthetic vasoactive agents on the normal SCBF. Propranolol (a beta adrenergic blocker) was given via slow intravenous infusion at a dosage of 1mg/kg and it failed to affect the SCBF (which was measured at the normal resting blood pressure) which remained constant (Kobrine et al., 1977b, H_2 clearance method). Thus, despite the adrenergic innervation of the spinal arteries, at least the beta receptors in "normal resting flow pressure" are not activated, perhaps due to the relatively small number of beta receptors in the spinal cord, compared to the rest of the

central nervous system (Alexsander et al, 1975).

Crawford et al (1977) studied on dogs the effect of norepinephrine (NE) on SCBF using the H_2 clearance method. Multiple ligations of branches of the posterior aorta was done (except those supplying the lumbar and sacral segments), and further pharmacological disruption of the blood cord barrier (BCB) using hyperosmotic compounds (2.5 M urea) was performed. Then, they injected NE directly into the aorta, and concluded that SCBF decreased in direct correlation with an increase of the NE dosage. However, no change of SCBF was observed while the BCB remained intact. This decrease in SCBF was prevented by treating the dogs (before the administration of NE) with phenoxybenzamine (an alpha adrenergic blocker). The last observation suggests that the decrease in SCBF (after the NE treatment) is via an alpha adrenergic mechanism. It is worth noting however that injection of NE after BBB disruption caused increase of cerebral blood flow and cerebral metabolism (MacKenzie et al., 1976). The reason it didn't happen in the spinal cord might be, as we noted above, due to the relatively small number of beta receptors in the spinal cord as compared to the brain. In the same study (Crawford et al., 1977), they also measured SCBF between 0 to 30 minutes after urea administration (intrarterially), and found that urea had no significant effect on SCBF.

It was recommended by Young et al (1980b, 1982) to use carefully, acetylcholine blockers (i.e. tubocurare, gallamine, and succinylcholine) to paralyze animals in spinal injury experiments because of the varying degrees of sympathetic or parasympathetic blockade they produce, that subsequently might mask the pressor response to the trauma, resulting in higher post-traumatic flows.

In conclusion, the experimental evidence reviewed here tend to agree that light barbiturate anesthesia has no significant effect on normal SCBF. Beta adrenergic blockade agents do not change normal SCBF, and do not abolish autoregulation phenomenon in SCBF. Alpha adrenergic blockade agents, do have a role in modulating SCBF. Treatment with phenoxybenzamine abolishes both, the decrease in SCBF following NE injection and the autoregulation to changes in blood pressure in the SCBF, as well. NE administration only after disruption of blood cord barrier decrease significantly SCBF (via an alpha adrenergic receptors) in contrast to its effect on cerebral blood flow.

The Effect of Intracranial Pressure on Cerebral and Spinal Blood Flow

Two experimental methods to simulate clinical circumstances of increased intracranial pressure (ICP) were summarized by Cushing (1902). The first is a local compression of the brain (e.g. inflation of an extradural balloon) leading to a resultant unequal distribution of ICP, in contrast to the other technique (e.g. perfusion of the sub-arachnoid space with saline) which brings about a generalized compression.

One important factor that influences CBF is the pressure difference between its arteries and veins, and this is defined as the cerebral perfusion pressure. The pressure inside the cerebral veins is practically the same as the ICP in almost all situations, so that cerebral perfusion pressure (CPP) is commonly regarded as being the difference between the systemic arterial pressure (SAP) and intracranial pressure (ICP).

Thus, CPP=SAP-ICP (Jennett and Teasdale, 1982)..

The other factor considered to define CBF is the resistance of the cerebral vessels. Thus cerebral blood flow (CBF) varies with changes in perfusion pressure and the diameter of the cerebral vasculature.

CBF=Perfusion pressure/vascular resistance

This assumption is a derivative of the quation which was given for flow through rigid tubes:

$$\text{Flow} = \frac{(\text{Inflow pressure} - \text{outflow pressure})\,\text{radius}^4}{\text{length} \times \text{viscosity}}$$

Since the length of the cerebral vessels changes very little, and the viscosity can be assumed to be constant the denominator of the equation can be ignored. The inflow pressure minus outflow pressure and the radius^4 represent the perfusion pressure and vascular resistance respectively. Despite the fact that the interactions between ICP and CBF are complex, there is general agreement that a) the cerebral circulation is able to compensate, within limits, for rising intracranial pressure and b) a critical point is reached when the ICP is within 40 to 50 mmHg of the mean arterial pressure, after which CBF falls as perfusion pressure further decreases (Symon et al., 1973, Jennett and Teasdale, 1982).

Interestingly, two recent studies demonstrated that such reduction in CBF during intracranial hypertension was heterogeneous and flow to the medulla was preserved much more efficiently than flow to the cerebrum. In these studies, increases in intracranial pressure were similar in the cerebrum and medulla (Sadoshima et al, 1981) and higher supratentorially than infratentorially (Malik et al., 1977).

While many investigators demonstrated that increased intracranial pressure (the so called Cushing response) caused a reduction in CBF, in contrast, electrical stimulation of centers of the brain stem (the Fastigial nucleus of the cerebellum and the Dorsal Medullary Reticular Formation) which had caused also the Cushing reflex, (this time, electrically with no pressure effect), the blood flow in the brain (cerebrum, cerebellum and brain stem) was found to increase markedly (Nakai et al., 1982, Iadecola et al, 1983). These centers appear to function as tonic vasomotor centers and mediate the reflex cardiovascular responses to the increased intracranial pressure (the Cushing reflex). Whether or not the changes in blood flow resulting from stimulation of these nuclei, are restricted to the cerebral hemisphere, basal nuclei and brain stem or are widespread and affecting also the spinal cord, is unknown. While we failed due to technical reasons to perform electrical stimulation and blood flow measurement of SCBF with H_2 clearance simultaneously, we decided to investigate the SCBF during acute increased intracranial pressure. The same idea was tested by Palleske et al (1970) using the heat-clearance technique. With this qualitative method they were able to demonstrate in dwarf pigs that an increase of ICP by means of an epidural balloon, caused an increase in SCBF, although the blood pressure did not change. A stronger compression resulted in cerebral circulatory arrest, while the circulation of the spinal cord rose.

AIM OF THESIS

The hydrogen clearance technique has been used for almost 20 years to measure cerebral blood flow (Fieschi and Ketti, 1964) and for approximately 10 years for quantitative measurement of SCBF (Kobrine et al, 1974). This method is one of the most widely used and accepted approaches for studying spinal cord injury. We have chosen the H_2 clearance technique mainly because of the fact that during an H_2 clearance study, one can take many measurements, using different stimuli and the same animal might serve as the control as well as the induced stimulus group.

The main purpose of this work is: 1) to study a method to measure normal SCBF in the rat as well as to test the aspect of autoregulation in rats SCBF, with the devices and techniques of H_2 clearance, 2) in an attempt to evaluate the validity of the SCBF measurements obtained by the polarographic technique, a comparison study of blood flow between the latter method and iodoantipyrine as a tracer done. 3) the role of central nervous system on SCBF regulation is not fully understood.

Kobrine et al (1976) tested the autoregulation phenomenon in monkeys SCBF. After high cervical cord section, autoregulation was still observed, following a pattern similar to the one observed in the intact animal. This, therefore, indicates a local regulatory mechanism of the spinal cord. The same notion was expressed by Scremin and Decima (1983) who

failed to provide evidence for a role of supraspinal connections in the maintenance of resting SCBF. Thus, during the period immediately following an acute cord transection, SCBF did not change several segments below, in spite of a profound depression of reflex activity. Furthermore, the same investigators demonstrated that supraspinal connections are not necessary for the effect of CO_2 on SCBF, since SCBF varied according to pCO_2, similar to what had been described in the intact animal. It is well established that the brain, acting via the sympathetic nervous system, can regulate the systemic circulation; it has also been suggested for many years that the brain may neurogenically control its own circulation (Reis et al, 1982). The spinal cord and the brain have quite the same histology, embryology, and similar physiological control, yet the role of the brain on the SCBF is not yet known. An attempt was made to study the effects of an acute increase in intracranial pressure on SCBF. The elevation of the intracranial pressure will cause increae of systemic blood pressure (via sympathetic discharge, circulating catecholamines and vasoconstriction) and a raising in the pressure in the spinal canal.

Since the pressure effect is known to cause a decrease in cerebral blood flow (Symon et al., 1973), and norepinephrine after disruption of the blood-brain-barrier produces an increase in cerebral blood flow (Mackenzie et al., 1976) and a decrease

in SCBF (Crawford et al., 1977), it will be interesting to determine the effect of these factors on SCBF. Moveover, since, a significant (2.4 fold) increase in CBF can be elicited by electrical stimulation of the Dorsal Medullary Reticular Formation (Iadecola et al, 1983), the anatomical subtrate for the Cushing Response, we intend to study the effects on SCBF associated with the Cushing Response elicited by raising intracranial pressure. Thus the role of supraspinal influences on SCBF are to be examined.

MATERIAL AND METHODS

H_2 CLEARANCE TECHNIQUE-IN VITRO STUDY

In order to test our polarographic technique, to assess how accurately it reflects H_2 concentration, the same H_2 clearance methodology was used in an in vitro model.

Saline saturated with hydrogen gas was injected into a closed 100 cc. reservoir which was agitated by magnetic stirrer. Normal saline was than run through the reservoir to an outlet which contained a platinum electrode 130 μm in diameter and a gold reference electrode (Fig.1). The rate of flow was first measured with a stopwatch and a graduated cylinder and then calculated from the washout curve on the recorder, both measured values were then compared. The theory behind the method will be stressed again. H_2 polarography is based on the principle that the oxidation of H_2 generates electrons.

$$H_2 \rightarrow 2H^+ + 2e^-$$

If provided an acceptor surface, such as a platinum electrode, the reaction will donate electrons to the electrode, causing a current flow. In vitro - when the polarized platinum electrode is inserted into a solution containing H_2, the H_2 molecules closest to the electrode surface oxidizes to form H^+ ions. As the H_2 adjacent to electrode surface becomes depleted, a concentration gradient is established between the bulk solution and the immediate electrode vicinity. This concentration gradient causes a migration of H_2 molecules,

controlled by the diffusion coefficient of H_2 in the solution. When the H_2 depletion is sufficiently rapid, the electrode is diffusion limited, and this is described by Fick's Law (1870), as explained above.

ANIMAL PREPARATION, MONITORING AND OPERATIVE PROCEDURES

Male Sprague Dawly rat, weighing 300-400 gm. were anesthetized with sodium pentobarbital, 30 mg/kg intraperitoneally.* Polythylene catheters, filled with 12 IU sodium heparin and 0.009g NaCL per ml of water, were tied into the right femoral artery for blood pressure and gases monitoring as well to the right femoral vein for the purpose of drug administration. The trachea was cannulated and the skin was sutured. The Ag/Cl gold reference electrodes were implanted subcutaneously in the abdomen. The animal's head was placed in a stereotaxic frame and the temperature was maintained at 36-37°C with a heat lamp, and monitored by rectal probe connected to a YSI Tele-Thermometer. (Anesthesia was maintained if needed by supplemental intravenous injections of sodium pentobarbitol). The animal was then paralyzed with (Tubarine) Tubocurarine chloride (3 mg/kg i.p.) and mechanically ventilated with a small volume respirator. The respiratory volume and rate were adjusted to maintain arterial blood pH at 7.38-7.42, pO_2 at 100 to 150 mmHg, and PCO_2 between 30 to 35 mmHg. Blood pressure (BP) was monitored directly from the femoral artery

with a pressure transducer connected to a Hewlett Packard 8 channel recorder.

One level laminectomy was performed between L2 to L5 or T10 to T12 or C_3 and C_4 as needed, using a micro rongeur, taking care not to damage the spinal cord. Two small dural incisions (1 mm) were made with a No. 11 surgical blade, at the dorso-lateral aspect of the cord (0.5 mm. medially to the posterior spinal artery) in both sides, when the gray matter blood flow was to be measured. When the white matter was to be measured the dural incision was situated 1 mm. lateral to the midline which was marked by the posterior central vein. The animal was allowed to recover from the operation for 30 minutes because of the depression of SCBF immediately following laminectomy (Anderson et al., 1978). The polarographic electorodes were inserted through the dural incisions with micromanipulator. The connecting wires were fixed to the animal's skin, allowing the electrodes to float freely within the spinal cord, in synchrony with the respiratory movement. The electrodes were advanced to a depth of 0.5-1.0 mm without visible trauma to the cord. No methods of immobilization of the vertebral column were performed except for the head which was fixed by the frame. The surgical procedurs described above were done under surgical microscopic illumination and magnification. The recording electrodes were connected to the amplifiere and recorder, (Fig.2) then polarized with +350 MV

(positive to the gold reference electrode) and left for 15 minutes to stabilize. Once a steady baseline has been achieved and monitored in the ampmeter and on the recorder, the local SCBF measurement was performed using the hydrogen clearance technique. Ten to 15 percent hydrogen mixed with air (Fig.3) was administered to the rats through the mechanical ventilator, for 3-4 minutes, until the tissue was saturated and a plateau was reached on the chart recorder. The hydrogen was turned off and the decreasing current was recorded (Fig.4). The local SCBF was calculated from the exponential clearance rate of tissue hydrogen. An artist drawn of the experiment model is plotted in Fig. 5.

A computer was used to sample the current (arbitrary numbers) versus the time every 30 seconds during the initial 2.5 minutes of the clearance curve, excluding the first 45 seconds that were disregarded because of arterial recirculation (Pasztor et al., 1973). By the programed computer, the logarithm of the H_2 signal was taken and a linear regression over time was performed and the correlation coefficient of the line was calculated and the exponential slope was obtained. The slopes were multiplied by 100 to expres the flow rates in units of ml/100gm/min. Most clerance curves were recorded from the gray matter but blood flow was also measured in the white matter. Clearance curves were recorded with a polygraph (Hewlett

Packard recorder), and by the same instrument, the blood pressure and the heart rate were recorded.

Arterial blood samples (0.2ml) were drawn during the experiment to detemine pH, pCO_2 and pO_2.

The duration of the experiments was between 5 to 7 hours; 2 hours for preparing the animal for the measurements and 30 min. between the consecutive clearance observations.

In the end of the experiment the animal was killed with saturated KCL and after staining the place of the electrods with Fuxin, the spinal column segment was removed and embedded in 10% buffered formalin. After 3 days the cord segment was removed and serially sectioned and stained with Hematoxilin Eosin, and the position of the electrods was microscopically verified. A comparison between blood flow values obtained by the observations in the experimental groups was done by statistical methods relying on the student T-test for paired and unpaired data.

An attempt was done to evaluate the functional neurological damage caused by insertion of the electrodes to the spinal cord. A low thoracic one level laminectomy was performed in three rats and the two platinum electrodes were put into the thoracic spinal cord, in the same technique as it had been performed in the other experimental groups of animals. The electrodes were left in place for 4 hours and then were removed and the incision was sutured. The animals were allowed to recover from

anesthesia and were examined daily for motor activity regarding the posterior limbs.

ELECTRODES AND MEASUREMENT CIRCUIT

ELECTRODES

The microelectrodes were made from Teflon coated platinum were (10% iridium, 90% platinum) 180 μm in diameter. Both of the electrodes had the ends scraped off of Tephlon insulation and a plantinum tip was exposed (130 m bare diameter) 0.5 mm in length and tapered to a point, while the other end is connected to a thin nylon-insulated copper wire.

CIRCUIT DESCRIPTION (FIG.6)

Amplifer A1 buffers the source of polarization voltage and connects it to the Pt electrode, +350 polarization voltage was used.

Amplifier A2 senses the algebraic sum of the polarization voltage e_p and the potential e_H (generated by the oxidation of molecular hydrogen to hydrogen ions at the Pt electrode). Amplifier A2 is connected for unity gain, and was selected because of the following characteristics (OP-07 Precision Monolithic):

1.	Offset voltage	10uv
2.	Voltage drift/temp	0.2 v/^{o}C
3.	Viktage drift/time	0.2 v/month
4.	Noise	0.35 v p-p
5.	Input current	0.7 nA

Parameters 1 to 4 of the above show the negligible error that the device itself contributes to the measurement in terms of changes of temperature and duration of the experiment. Parameter 5 means that the measuring circuitry presents an impendance of approximately 100 M to the potential source, errors due to circuit loading are thus negligible.

Amplifier A3 is connected as a difference amplifier and subtracts out the polarization voltage from the output of A2, thus the output of A3 is the hydrogen potential e_H. Amplifier A3 was also chosen to be the OP-07 as the preceding circuit characteristics were also desirable in this section.

Amplifier stage A4 was configured to provide maximum noise rejection particularly at baseline frequencies.

Amplifier stage A5 interfaces to the Pen recorder (Hewlett Packard). It provides for various stages of gain selection and the facility for setting the recorder trace to zero for zero input at the electrodes.

In the various experiments four groups of animals were tested. In group No. 1, normal SCBF was measured in the cervical cord. In group No. 2, SCBF was measured in the lumbar region under normocapnic conditions, over a wide range of

systemic blood pressure. In group No. 3, thoracic SCBF was measured after induced increased intracranial pressure. In group No. 4, SCBF was measured in the same animal using both methods, H_2 clearance and ^{14}C-iodoantipyrine.

GROUP NO. 1

Animals were prepared and SCBF was measured in the cervical spinal cord as described above under normocapnic and normotensive conditions. In few experiments while one Pt electrode was in the cord, the second electrode was inserted into the caudate nucleus using stereotactic methods. A fronto-parietal skin incision was done, and the periost was retracted. Using the point where the sagital and coronal sutures cross each other as the O, 2.5 mm anteriorly, and 3.5 mm laterally, a small hole was done in the cranium. A platinum electrode 0.25 mm was then advanced to a depth of 5 mm in 90^0 angle to the caudate nucleus.

GROUP NO.2

Using the same surgical conditions as described above lumbar SCBF was measured. After few control readings were done, changes in perfusion pressure (autoregulation phenomenom) was tested. Systolic blood pressure was increased or decreased by infusion of metaraminol (a peripheral vasoconstrictor) or bleeding the animal respectively. While manipulating the systemic blood pressure, simultaneous measurement of SCBF was done (Fig.7).

GROUP NO. 3

After the animals were prepared as described above, 3 to 5 blood flow determinations in the lower thoracic segments were done in every animal of this group to serve as control.

A craniectomy was then performed in the left posterior parietal region, an area of dura about 3 mm. was exposed for the insertion of the epidural balloon.

A deflated Fogarty biliary catheter balloon (0.4 ml maximal balloon inflation capacity, Edwards Laboratories, California) was then applied into the epidural space in the fronto-parietal region of the left hemisphere. The small craniectomy defect in the skull was filled completely by the polyethylen tube that was connected to the balloon. At this stage (with the balloon deflated intracranially) another 2 flow measurements were performed. The polyethylene tube which was connected to the balloon from one side, was connected (in its other end) to a syringe filled with saline and attached to a Harvard perfusion pump for gradual inflation of the balloon (Fig.8). Hydrogen gas was given by inhalation to the animal and at the time while the saturation was almost completed and just before the hydrogen turned off, the intracranial balloon started to fill causing an acute increase in intracranial pressure. Each measurement consisted of the stepwise inflation of the extradural balloon by increments of 0.2 ml fluid, delivered over a 5 min period. When blood pressure was elevated about 50 to 100 mmHg above its

baseline value the infusion of the balloon was stopped, and simultaneously the washout clearance curves from the spinal cord were recorded (Fig.9). The balloon was then partially deflated in purpose to lower the blood pressure back into the autoregulation limits while the washout clearance took place. The animal was allowed to rest for 30 min. and then the observation was repeated.

GROUP NO. 4

CERVICAL SCBF DETERMINED BY H_2 CLEARANCE TECHNIQUE AND IODO-ANTIPYRINE METHOD

Twenty male Sprague Dawly rats were prepared, anesthetized and monitored as described above with few exceptions; both femoral arteries and veins were cannulated and a cervical laminectomy of C_3 and C_4 were performed. After the dura was incised and the platinum electrodes were inserted into the spinal cord, the paralyzed artifically ventilated animal was given hydrogen to inhale and a few washout curves were recorded in each animal to determine the cervical SCBF with the H_2 clearance technique.

In this stage, 500 IU of sodium heparine was injected intravenously, ^{14}C iodoantipyrine (New England Nuclear) 40-60 MCi/mmol specific activity was infused (5 μCi/100gm) for approximately 30 seconds into one of the venous lines (with the Harvard pump). Immediately after it, the animal was sacrificed

with a bolus of 2-3 ml of saturated KCL which was injected to the other venous line.

During the infusion period, blood samples of 40 liters were taken from one of the arterial lines (while the second monitor blood pressure and gases) during 0 to approximately 30 seconds, for every 3 to 5 seconds. A cassette tape recorder was on, to record times during which the samples were taken. Later these time periods were measured with a stopwatch. Immediately after the animal was killed, the skull and vertebral column were opened and the brain and the segments of spinal cord (cervical and lumbar) were removed as soon as possible and were placed on cool watch glasses. The dural and sub-arachnoidal vessels were carefully removed. The brain was hemisected at the mid-line and dissected into anatomical regions that were placed in preweighed scintillation vials. The spinal cord segments were bisected under the operative microscope and then white and gray matter were dissected and separated and were placed also in preweighed scintillation vials.

MEASUREMENTS OF ^{14}C CONCENTRATIONS IN LOCAL SPINAL AND CEREBRAL TISSUES

The brain and spinal cord tissues (placed in scintillation vials) were reweighed. One ml of tissue solobilizer ETOH Protosol (New England Nuclear) were added and were shaken in a water bath at 60°C overnight, 10 ml of Econoflour (New England Nuclear) was added and vials were counted for 10 minutes.

Twenty μ liters C^{14} taluene (New England Nuclear) $4.0x10^5$ dpm/ml was added as an internal standard and recount for 10 minutes.

MEASUREMENT OF ^{14}C CONCENTRATION IN BLOOD

Forty micro liters blood samples were placed in vials containing 1 ml of tissue solobilizer ETOH Protosol (New England Nuclear) and placed in an incubator for 30 minutes at 60°C. 0.2 mls of hydrogen peroxide was then added, and five minutes later, in order to let the gases escape, the vials were shaken in a water bath for 15 minutes. 15 mls Bioflour (New England Nuclear) was added and vials counted for 10 minutes. 20 μliters of ^{14}C taluen was added as an internal standard and vials recounted for 10 minutes. The concentration of tracer in the blood samples were calculated from the measured amount of radioactivity and the volume of the sample of blood.

RESULTS

H_2 CLEARANCE TECHNIQUE - IN VITRO STUDY

In fourteen obervations, the rate of flow of the saline saturated with hydrogen gas was calculated by both methods (as described above, page 46). The flow of saline that was accumulated in the graduated cylinder during 1 minute, (see Fig.1) and was measured simultaneously from the washout clearance curves are shown in table 3.

The data obtained was analysed with the linear regression method to find out the correlation between these two techniques of calculation, in order to evaluate the accuracy of SCBF values which have been measured from the washout clearance curves using the same technique. These flow data were analyzed by calculating the regression of one measurements (Y) on the other ones (X) in the pairs. The regression equation obtained was $Y=0.98X+6.4$ The correlation coefficient (r) was 0.98 (Fig. 10).

GROUP No. 1 - Normal SCBF was measured in eight animals. In only three rats were the two platinum electrodes recording simultaneously the cervical SCBF, while in five rats one electrode traced the washout clearance curve from the cervical cord while the other electrode recorded data from the caudate nucleus. For 8 animals the range of systolic arterial pressure (SAP) was 80 to 150 mmHg and the means $\pm$ SEM's were as follows:

pH = 7.36 ± 0.02 units, $PaCO_2$ = 29.6 ± 1.32 mmHg, PaO_2 = 126.9 ± 16.1 mmHg. For all 45 consecutive blood flow data in the gray matter of C_3-$_4$ the mean value was 53.6 ± 2.5 ml/100gr/min. The data obtained in this experimental group is shown in table 4. Under the same physiological conditions for all 21 flow data in the caudate nucleus the mean local blood flow was 41.0 ± 2.6 ml/100gm/min. (table 5).

GROUP No. 2 - Lumbar SCBF was measured in nine animals under normocapnic and normoxemic conditions (means ± SEM's were as follows: pH = 7.40 ± 0.02 units, $PaCO_2$ = 36.8 ± 2.8 mmHg, and PaO_2 = 111.5 ± 21.7 mmHg) and over a wide range of systolic arterial blood pressure (SAP)-220 to 25 mmHg, to find out the autoregulation curve in the rat's SCBF. The SAP was either lowered by bleeding the animal or raised by the intravenous administration of metaraminol (Aramin). Both electrodes recorded simultaneous washout clearance curves from the gray matter of the lumbar spinal cord. The SCBF remained relatively constant with a mean of 52.3 ± 1.9 ml/100gr/min between quite a wide range of SAP of 45-50 to 160-165 mmHg. When SAP was between 50 to 15 mmHg SCBF fell to 24.9 ± 1.8 ml/100gm/min, while below 15 mmHg the spinal circulation fell down to zero value. SCBF rose to 88.2 ± 4.0 ml/100gm/min. when SAP reached 165 to 220 mmHg. The data obtained in this experimental group is demonstrated in table 6 and 7 and is plotted in Fig.11.

GROUP No. 3 - In this group of eleven animals the effects of increased intracranial pressure on SCBF was tested. Under normocapnic and normotensive conditions ($PaCO_2$ = 31.4 $\pm$ 1.8 mmHg; SAP = 127.6 $\pm$ 22,7 mmHg) the control SCBF in the gray matter of the lower thoracic cord was 41.2 $\pm$ 12.6 ml/100gr/min. In some animals after the control washout clearance were done, additional readings were made after the balloon was placed (deflated) intracranially (still without increasing the intracranial pressure). At this stage SCBF was 45.1 $\pm$ 11.4 ml/100gm/min. This was to ensure that application of the balloon itself did not alter the SCBF. This was indeed true $p < 0.05$, student's unpaired T test (Table 8).

Few changes progressed in association with the increasing balloon volume and increased intracranial pressure. Once the balloon reached the "critical volume" for eliciting the Cushing response (approximately 0.2 ml) a few observations were made. The systolic and diastolic arterial pressure increased, in each of the animals, to a mean value of 200 mmHg. The heart rate increased in most of the animals and decreased in few. Immediately before the appearance of the arterial hypertensive response pupillary changes were evident. The left pupil (ipsilateral) to the balloon was maximally dilated and unresponsive to light. At the peak of the systemic arterial pressure in one animal both pupils were fully dilated. The balloon at this stage was partially deflated so the pupillary

changes and the cardiovascular parameters were immediately reversed and SCBF measurement took place.

After induced increased intracranial pressure (ICP) in the same animals (with SAP of 132.5 ± 20 mmHg = within autoregulation) the thoracic gray matter SCBF rose to 53.2 ± 19.3 ml/100gr/min. The raising of SCBF after the elevation of ICP when compared to the control was statistically significant ($P<0.005$). In few animals, after repeated observations of increased ICP, although the SAP was only 29.3±5.3 mmHg (quite below autoregulation) the SCBF remained close to the control value 38.2±10.7 ml/100gm/min (Table 8).

When the electrodes were inserted in the white matter of the lower thoracic cord, with SAP of 118 ± 12 mmHg the control SCBF was 22.2 ± 2.4 ml/100gm/min, and almost did not change after the induced increased ICP, to be 21.0 ± 1.8 ml/100 gm/min. during SAP of 128 ± 5 mmHg (Table 9). The data obtained in this experimental group is demonstrated in tables 8 and 9 and is plotted in Fig. 12 and 13.

GROUP No. 4 - In this group a comparison of blood flow measured with H_2 clearance and ^{14}C iodoantipyrine methods was done independently, within few minutes in the same animal. In nine animals the blood flow was measured by both methods, while in three animals only with ^{14}C iodoantipyrine technique. The arterial blood pressure and blood gases were

stable during both measurements. The range of SAP was 100 to 140 mmHg, and the mean PH, $PaCO_2$ and PaO_2 was 7.41 ± 0.01 units, 33.2 ± 0.9 mmHg and 152.8 ± 11.7 mmHg respectively. The average value of total cervical SCBF (Gray and White Matter) in 90 consecutive flow data with the H_2 method was 47.8 ± 1.5 ml/100gm/min. The cervical SCBF measured separately with the same technique, in white matter and gray matter was 26.8 ± 1.0 and 51.9 ± 1.2 ml/100gm/min respectively (Table 10).

By the ^{14}C iodoantipyrine technique the total (entire) cervical SCBF (Gray and White Matter) in 23 observations was 70.9 ± 6.6 ml/100gm/min. With the same method the cervical blood flow measured separately in white and gray matter was 50.4 ± 5.3 and 93.2 ± 8.5 ml/100gm/min respectively.

The values of blood flow obtained with the ^{14}C iodoantipyrine method, in the cervical and low thoracic spinal cord, and in the caudate nucleus, pons, cerebellum and cortex are demonstrated in tables 11 and 12. The data of this group concerning SCBF and blood flow in the caudate nucleus obtained from this group by both methods (^{14}C iodoantipyrine and H_2 clearance) and from group no 1 (caudate nucleus by H_2 method) is plotted in Fig. 14.

MOTOR FUNCTION AFTER INSERTION OF THE ELECTRODES INTO THE CORD

In this experimental group of 3 animals in whom insertion of the platinum electrodes to the thoracic spinal cord had been

performed and the animals were allowed to recover from anesthesia, only one animal had mild paresis of the left lower limb which had been resolved after four days. The other two rats were neurologically intact immediately after recovering the operation and during the following week. The histological preparations of these animals were not different from the other preparations obtained from the various experimental groups and showed small hemorrhages along the electrode tip and tract.

HISTOLOGY

The tips of the electrodes were identified after staining with Hematoxilin Eosin to stay in the gray or the white matter of the spinal cord or in the caudate nucleus. In some preparations diffuse small hemorrhages were seen around the electrode tracts.

CLAIM TO ORIGINAL WORK

SCBF under physiological conditions as well as under different states of systemic blood pressure was described already with the hydrogen clearance technique and by other methods in various species. To the best of our knowledge autoregulation of SCBF concerning changes in perfusion pressure by hydrogen clearance technique in the rat's spinal cord has not yet been described. In addition, we presume that this is the first experiment aimed at determining quantitatively SCBF values in the presence of induced increased intracranial pressure. The idea that SCBF rose in association with acute increase of

intracranial pressure was described, though qualitatively, by others (Palleski and Herman, 1968).

DISCUSSION

One of the most devasting diseases of this century is the spinal cord trauma and unfortunately there is no treatment available once the damage has been done. Alteration in SCBF has been suggested to be one of the main factors responsible for the neural dysfunction following spinal cord injury. Therefore it is of paramount importance from the general physiological and the clinical points of view to acquire continous information and knowledge concerning the circulation of the spinal cord in normal and pathological conditions.

The study of SCBF before and after experimental trauma, is best performed with the H_2 clearance technique, because of the unique capacity of this method to provide serial measurement in the same animal over time (Young 1980a). Thus, if it is desirable to observe changes in SCBF produced by physiological or pathological events over a period of time, the H_2 clearance technique is an excellent method to choose, since in our experiments, the blood flow values obtained were quite constant with respect to time for each animal as well as constant for series of animals. It should be stressed that this is not the case with the radioisotope tracer techniques and autoradiography because the animal has to be sacrificed at the time of measurement of the SCBF, thus blood flow can be measured only once in any experiment. The conclusions in such experiment could be obtained on statistical basis only, using large series

of animals, and it is impossible that the same animal will serve as the control and as the induced stimulus.

H_2 CLEARANCE MEASUREMENT OF SCBF - AN ANALYSIS OF TECHNIQUE

Traditionally, there are two major disadvantages of the H_2 clearance technique, namely, that this technique traumatizes the cord with the electrodes, and cannot differentiate between gray and white matter blood flow. However, it seems in our and other hands to present much less difficulties than with previous studies using H_2 clearance techniques.

Concerning the aspect of tissue damage, a few considerations should be noted: 1) in order to minimize tissue injury from the electrode implantation, the various investigators in this technique showed a tendency to use smaller and smaller electrodes. Thus, Aukland et al (1964) used 2 mm. diameter electrodes, Fieschi et al (1969) used 0.4 mm. tissue electrodes, Kobrine et al (1974) used 250 micra, and the diameter of electrodes used in the present experiments was 130 micra. Two recent studies used even smaller diameter such as 75 and 35 micra (Scremin and Decima 1983, and Hayashi et al., 1983) respectively. 2) The post-mortem microscopic preparats revealed minimal tissue disruption along the electrode tract only, with few red blood cells along the tract. This was

demonstrated in the present experiments as well as in others (Kobrine et al., 1974, Senter et al., 1979). 3) Although the H_2 clearance considered to be an invasive technique, it was shown in the present experiments and in others (Kobrine et al., 1974, Hayashi et al., 1983) not to interfere with neurological function following electrode implantation. 4) It was suggested by Pasztor et al (1973) that the areas from which the hydrogen electrodes record, although small, are themselves beyond the immediate area of tissue damage, and therefore, reflect perfusion through relatively normal tissue. 5) Perhaps the most important scientific proof for this issue was manifested by Aukland et al (1964) who had pointed out that a small zone of devitalized cells must surround the implanted electrode. He showed that there is a delay in clearance curves resulting from a diffusion layer of varying thickness between the actual clearing tissue and the electrode. He also calculated that for theoretical membrane of 0.2 and 0.4 mm., which covers the electrode, the electrode response does not approach the H_2 concentration until the first second of clearance or 16 seconds later, respectively. Thus, it is clear from this calculation that the clearance curve may be somewhat delayed if there is an area of necrotic tissue around the electrode, however, the electrode response should relfect H_2 concentration during most of the clearance curve. It has been the experience in the present study that no serious tissue

damage results from the implantation of the electrodes. In few experiments, however, a delay in the desaturation curve of more than 30 seconds after the hydrogen gas was turned off, suggested that tissue damage has occurred. Indeed, a small hematoma was found around the electrode in the post-mortem dissection and these clearance curves were discarded.

SCBF - GRAY AND WHITE MATTER RESOLUTION

With earlier studies using H_2 method, there have been difficulties with the measurement of local SCBF within white and gray matter areas. Thus, Kobrine et al (1974, 1975b) obtained in monkey values of 17.5 and 14.0 ml/100gm/min in the white and gray matter respectively. Griffiths et al (1975) reported 11.5 and 10.8 ml/100gm/min in dogs and 13.7 and 16.5 ml/100gm/min in baboons white and gray matter respectively. It is clear, however, that these flow rates for gray matter would be more appropriate for white matter. It has been suggested that low blood flow values for gray matter were obtained due to rapid diffusion of hydrogen from white to gray matter (Halsey et al., 1977). The same authors were impressed by their inability to demonstrate appropriate gray matter clearance rates in the 2 mm thickness of rabbit cortex and by the same phenomena in the 1 mm thickness of monkey's spinal cord dorsal horn gray matter (unlike the 5 mm. thickness of baboon cortex in which gray matter flow was separately demonstrated) and concluded that the spatial resolution of the hydrogen method would be about 2 mm,

and when the electrodes are within 2 mm. of another tissue compartment the errors are greatest. On the other hand, Kobrine et al (1974) claimed that the technique measure focal flow in a volume of tissue less than 0.5 mm, and Young (1980a) in his extensive review of the hydrogen technique doubted the ability of the H_2 clearance to localize blood flow to tissue volumes of less than 5 mm.

Interestingly, while it was established and generally accepted that the H_2 method cannot differentiate gray and white flow, it was demonstrated that values of 64 and 20 in rat and 43 and 16 ml/100gm/ min. in cat for gray and white matter respectively can be obtained with the H_2 method (Hayashi et al., 1983, Scremin and Decima, 1983, respectively). In the present study, as well, flow rates of 54 and 23 ml/100gm/min were achieved in the rat spinal cord gray and white matter respectively.

It is beyond the scope of this presentation to find out what have been changed in the methodology of the technique in this decade that brought to this new area of resolution of gray and white matter. However, the hydrogen method has differed in its technique of application in the hands of various authors, and an attempt to analyze it will follow.

As mentioned above; 1) the diameter of the platinum electrode became smaller, and 2) the length of the exposed tip became shorter, i.e. 1) 250 micra-diameter, 2) 1000 micra-tip

(Kobrine et al., 1974) 1) 250 micra-diameter, 2) 700 micra-tip (Senter et al., 1978) 1) 150 micra-diameter (Young et al., 1982),1) 130 micra-diameter, 2) 300 micra-tip (present experiment, 1984) 1) 75 micra-diameter, 2) 75 micra-tip (Scremin and Decima, 1983) 1) 35 micra-diameter, 2) 50-100 micra-tip (Hayashi et al., 1983).

The time of inhalation of hydrogen was stressed as an important precaution in use of the method, to obtain homogeneous tissue saturation by prolonged inhalation (Halsey et al., 1977). In the present experiment the impression was that very short inhalation time (obviously due to a very high H_2 concentration that caused systemic changes in the animal like hypotension) tended to give flows on the high side. The H_2 concentration and time of inhalation used by the various investigators was between 5 to 15% and between 3 to 5 minutes respectively.

The reference electrode used by most authors was a standard silver/silver chloride or a stainless steel electrode. We found the reference electrode of gold (used in the ElectroMyograph) to be important in maintaining a constant baseline over the course of the experiment.

The external polarizing voltage applied to the electrode (to reduce contributions from O_2 changes in ischemic tissue) had been varied between 650 mV (Kobrine et al., 1974) and 350 mV (Young et al., 1982 and present experiments) to 110 mV (Senter et al., 1978). It was clear from the present experiment that

the greater the polarization voltage used, the longer the time required by the electrode to equilibrate.

Few investigators (e.g. Kobrine et al., 1974) stressed the importance that the tip of the electrode should be coated electrolytically with platinum black to increase the surface of the area of the electrode tip. Others (e.g. Scremin and Decima 1983) covered the spinal cord after the laminectomy was done with mineral oil. In the present experiments it was not necessary to use the platinum black nor the mineral oil and it doesn't seem to change the stability and reproductability of the technique.

In purpose to overcome movements of the spinal cord with respiration the animals were immobilized in a metal spinal frame (Scremin and Decima, 1983) or by passing sutures through the supraspinous ligaments and paraspinal muscles (Hayashi et al., 1983) or by introduction of a negative intrathoracic pressure (Senter et al., 1978). None of these immobilization methods were used in the present experiment without detected disturbance from the respiratory movements.

Anesthesia with sodium pentobarbital was maintained throughout the whole experiment, with spontaneous respiration (Young et al., 1982, Hayashi et al., 1983) while the others paralyzed the animals with pancuranium and ventilated them with oxygen-nitrous oxide gas (Kobrine et al., 1974, Senter et al., 1978, Scremin and Decima, 1983).

From analysing the differences in the methods, it seems that the biggest critical contribution done recently towards the satisfactory resolution and differentiation of gray and white matter is the tendency to use smaller electrodes and short exposed tip. (Although originally this tendency to use smaller electrodes was directed to prevent tissue damage and perhaps indeed, the less the damage the less is the diffusion of the gas between the gray and white matter). It is almost obvious, from looking at the data, that only electrodes with diameter of less than 150 micra and with an exposed tip of less than 300 micra will be able to give satisfactory gray and white flow rates in such a tiny structure as the rat's spinal cord with a diameter of approximately 2 mm. The problem with these micro electrodes rest with the small amplitude of the current produced by oxidation of the hydrogen in the level of the exposed tip (Young, 1980a). Therefore the electrical circuit that can resolve nanoampers or even picoamperes should be available.

As was mentioned above, one of the drawbacks of the hydrogen clearance method was its inability to differentiate between gray and white matter in the spinal cord. The values of the blood flow measured with this technique, especially concerning the gray matter, were in conflict with data obtained by other methods (Table 2). We have been able to define quantitatively the blood flow of gray and white matter

separately in the spinal cord in rats, and our results are close to those previously reported (Table 2).

It is known that the gray matter of the spinal cord has a richer capillary network than the white matter (Tveten, 1976). It was also showed that the use of glucose and oxygen is higher in the gray than in the white matter (Hayashi et al., 1983). These observations are in consistent with our results as well as others (Bingham et al., 1975, Scremin & Decima, 1983) namely that the SCBF in the gray matter was found to be twice as high in comparison to flow in the white matter. Other investigators found the same tendency, though with different ratio; threefold (Hayashi et al., 1983) and fivefold (Marcus et al., 1977) high values in the gray as oppose to white matter.

Only a few groups of investigators measured the SCBF in different segments of the cord. The present experiments support the results obtained by Flohr et al (1968), by Bingham et al (1975), and by Marcus et al (1977), that SCBF's at the thoracic level were lower than flows measured at the cervical and lumbar segments. This was true for the regional SCBF as well as for the more specific flow measurement of the gray and white matter (Table 1&2). These findings might reflect the diminished blood supply to the thoracic cord and its lower metabolic demand. In contrast, Hayashi et al (1983) concluded that there is no significant difference between SCBF values for cervical, thoracic, and lumbar levels. Interestingly, they also found the

ratio of white to gray matter area within each spinal cord level to be 3:1, 1,8:1 and 1:1 in the thoracic cervical and lumbar regions respectively. A decrease in the gray matter area of the thoracic cord in respect to white matter, demands for a lower vascular density and neuronal metabolism in the same segment and subsequently manifests a decrease in blood flow requirements. This assumption fits well with our and others findings, namely, that the thoracic cord has a lower blood flow than the cervical and lumbar regions.

AUTOREGULATION PHENOMENA IN SCBF

Fog (1938) first described the phenomen of autoregulation in the brain. He noted changes in the caliber of pial vessels in response to fluctuations in systemic arterial blood pressure. The idea of autoregulation tells us that the blood vessels maintain the capability of changing their caliber (to constrict or dilate) in order to keep a constant blood flow during variations in their intraluminal pressure, or accordingly during changes in perfusion pressure. Many investigators confirmed this concept in the cerebral circulation.

In the present study, an excellent autoregulation to changes in perfusion pressure was found in the gray matter of the lumbar spinal cord. As can be seen from Figure 11, SCBF remained relatively constant and within the control values when systolic arterial pressure (SAP) was 50 to 165 mmHg. Below 50 mmHg and above 165 mmHg, SCBF was a function of SAP; decreased

with further lowering of blood pressure below 50 mmHg, and increased when raised above 165 mmHg.

In the introduction to this study we described the results of various workers that contributed their experiments to demonstrate how changes in systemic arterial blood pressure affects SCBF. Our data is in general agreement with these previous reports. With the exception of Marcus et al (1977) who defined in sheep's lumbo-sacral gray and white matter the existence of autoregulation to systemic blood-pressure, the other authors in the last decade summarized their findings concerning autoregulation and SCBF, only in the white matter of dogs, monkeys and cats (Griffiths, 1973, Kobrine et al, 1975, and Senter et al., 1979) respectively. In this respect we were able to report autoregulation to changes in SAP in the gray matter of the rat's spinal cord. The present investigation makes it appear also that the regulation of the blood flow of the spinal cord, in relation to perfusion pressure, is similar to that of the brain.

INCREASED INTRACRANIAL PRESSURE AND SCBF

The quantitative methods of studying SCBF which had been performed in experimental animals, supported essentially the earlier qualitative evidence on changes in SCBF mainly due to chemically active agents and perfusion pressure. We had the opportunity in the present study to confirm and validate the

experimental investigation that had been carried out by Palleske et al (1970), using the heat clearance technique. Thus, compression of the left cerebral hemisphere by means of an epidural balloon, led to an increased blood flow in the spinal cord.

Our observations were also in consistent with those described by others (Rodbard and Stone, 1955, Zidan, 1978), concerning the Pressor Response. It is true that the rate of inflating the intracranial balloon is important, and the faster it is done the shorter will be the latency to the response, however the crucial factor in acute increased ICP, is the volume of the expanding mass rather than its rate of growing. We could define a constant critical volume to cause cerebral disfunction and consequently the Cushing Response in the rat, to be approximately 0.2 ml. At that point the intensity of the Pressor Response was dependent on the degree of the compression, which means the volume of the blown up intracranial balloon. In other words, the raising of the blood pressure (BP) was dependent on the grade of cerebral compression; the higher the ICP the higher the BP. Once the critical volume was achieved, the elevation of BP was immediate (within a second) and related to the ICP. A decrease in the ICP (by partially deflating the balloon) brought to immediate drop of the BP.

An interesting finding in this study was that in association with increase in ICP to the point of eliciting the

Cushing Pressure Response - white matter blood flow remained unchanged (Figure 13) when compared to the control. By contrast, gray matter blood flow consistently increased although BP elevations associated with the ICP increase were maintained within the range of autoregulation (Figure 12). Furthermore, in few animals in whom during repeated induced Cushing Response, BP was below autoregulation and therefore one would expect SCBF to be impaired (according to the above described autoregulation phenomen and to our results of decreased SCBF in the present of BP below 50 mmHg). This was not the case and relative hyperemia was observed in the gray matter still within the limits of the control values. This hyperemia cannot be related to CO_2 accumulation (caused by apnea or respiratory difficulties - known as features of the Cushing Response) since the animals were paralized, artifically ventilated and under controlled CO_2 levels. Other putative mechanisms as well can't explain these results.

One of the disadvantages of the present study is that we failed to get direct measurements of intracranial and intraspinal pressures. However, during the expansion of the supratentorial balloon it was clear that both intracranial and spinal pressures were elevated. The response to the inflation of the balloon caused as described above the clinical signs of increased ICP; tentorial herniation with unilateral pupil dilation that was reversed immediately while decreasing the

ICP (by deflating the balloon). Moreover with the first animals in this group we tried to measure SCBF in the cervical region, and cervical laminectomies (C3-5) were performed. Surprisingly, when the ICP was elevated during the induced Cushing Response the cervical spinal cord was severely herniated out of the spinal canal. It didn't occur however in the thoracic spine, thus we believe the ICP was transmitted to the spinal canal, though not in the same intensity to the lower thoracic canal.

The cerebral circulation is able to compensate, within limits, for rising ICP. However, there are numerous studies to point out that acute increased ICP during expansion of an extracerebral balloon, in ventilated animals, causes cerebral blood flow to fall as soon as the ICP raised (Langfit, 1969, Malik et al., 1977). If the spinal circulation in pathological condition reacts as the brain does, we would expect the SCBF to decrease during the increased intraspinal pressure.

Furthermore after the Cushing Response was elicited, the acute rise in blood pressure might cause a disruption of the blood-cord-barrier and consequently the elevated nor-epinephrine will cause a further decrease in the SCBF (Crawford et al., 1977).

Since the above described suggestions do not fit to the findings obtained in this study, we might argue the following.

The Cushing Pressor Response can be evoked by raised ICP (Cushing, 1902) as well as by spinal cord compression (Alexander and Kerr, 1964). This pressor reflex elicited with both mechanisms can be prevented by adrenergic blockade and is believed therefore to be mediated by the sympathetic nervous system.

That the peripheral sympathetic system regulate SCBF on a spinal cord reflex basis and not through central control was showed by various authors. Cervical cord transection does not interfere with SCBF autoregulation (Kobrine et al, 1976), and thoracic sympathectomy did abolish SCBF autoregulation (Young et al., 1982), thus, the spinal sympathetic system has its own control on the spinal circulation in the case of changes in perfusion pressure or to CO_2 reactivity. However, there is evidence from this study to postulate that SCBF is still dependent at least partially upon suprasegmental neurogenic input and further experiments like cervical cord transection in association with the Cushing Response should be carried out.

We hypothetize that mechanical stimulus (e.g. increase ICP) sensitize central vasomotor nuclei that via descending pathways stimulated a chain of pressor or depressor reactions into motion, abolishing autoregulation. As a result SCBF is reacting independently from the sympathetic ganglia, mediate vasodilation with concomitant increase of flow in spinal gray matter. The

exact locus and pathways that mediates this vascular response remains to be identified. However, nor-adrenergic pathways originating in the pons that reach the spinal gray laminae are candidates for the putative pathways.

SCBF- RAPPORTS BETWEEN ^{14}C IAP and H_2 CLEARANCE METHODS

Further convalidation of the hydrogen clearance technique was based on a comparison with blood flow values in the same structures measured at short interval of time by means of using the Kety principle and ^{14}C iodoantipyrine (IAP) as a tracer. We choose the latter agent because it had been emphasized recently that the IAP appears to be satisfactory non-gaseous agent for exact measurement of local cerebral blood flow (CBF), mainly for its higher solobility in oil and hence its better diffusional penetration of the blood-brain-barrier (Sakurada et al., 1978, Ohno et al., 1979).

Our results with both methods in the rat's cervical cord gray and white matter gave a ratio of approximately 2:1; the IAP values being almost twice these of the hydrogen clerance technique. These findings confirmed, although indirectly, the results and conclusions obtained by others. Sakurada et al (1978) and Ohno et al (1979) demonstrated that IAP provides higher (almost twofold) values of CBF than does ^{14}C-antipyrine in the conscious rat. The latter author also concluded that IAP provides higher CBF values than does the

hydrogen clearance technique. Furthermore, in a comparative study of hydrogen clearance method and ^{14}C-antipyrine as a tracer, done by Fieschi et al (1969), no significant difference has been found in CBF values recorded by the two techniques. We might conclude from these data that a) the IAP furnishes BF values that are nearly twice those obtained with hydrogen clearance technique, and this consumation is in consistent with our results. b) our gray matter values in the spinal cord obtained with IAP method, were quite similar to those elicited by Sakurada et al (1978) with the same tracer and autoradiography.

Although quantification of perfusion is an important issue in physiology and pathology of spinal cord circulation, the flow values obtained by various techniques are often different and sometimes even discrepant. However, the method to be employed should be chosen according to the individual experimental problem. When SCBF is to be measured before and after an induced stimulus, the hydrogen clearance technique which permit repeated measurement should be elected, rather than methods based on the determination of the concentration of tracer material during tissue saturation (e.g. IAP), which yield a single flow measurement only.

TABLE 1

SCBF MEASUREMENTS IN REPORTED SERIES

AUTHORS	METHOD	SPECIES	AREA MEASURED	SCBF* GRAY	SCBF* WHITE
LANDAU ET AL (1955)	I(CF I)+ AUTORADIOGRAPHY	CAT	CERVICAL	63	14
SANDLER & TATOR (1976)	^{14}C-ANTIPYRINE+ AUTORADIOGRAPHY	MONKEY	THORACIC	57.6	10.3
BINGHAM ET AL (1975)	^{14}C-ANTIPYRINE	MONKEY	C6 CERVICAL T12 THORACIC L3 LUMBAR	48.4 37.1 43.7	19.7 16.2 21.7
FLOHR ET AL (1968)	^{131}I-ALBUMIN PARTICLES	CAT	CERVICAL THORACIC LUMBAR	+19.9 +16.8 22.1	
MARCUS ET AL (1977)	MICROSPHERE LABELLED ISOTOPS	SHEEP	LUMBO-SACRAL CERVICAL THORACIC LUMBO-SACRAL	110 +32 +23 30	25
SMITH ET AL (1969)	(INTRASPINAL) ^{133}XENON CLEARANCE	GOATS	THORACO LUMBAR	+16.2	
DUCKER & PEROT (1972)	" "	DOGS	THORACO LUMBAR	+15.6	
GRIFFITHS (1973)	" "	DOGS	THORACIC	48.4	15.7

*ml/100gm/min
+TOTAL SCBF (Grey and White)

TABLE 2

SCBF - H^2 CLEARANCE TECHNIQUE

AUTHORS	METHOD	SPECIES	AREA MEASURED	SCBF* GRAY	SCBF* WHITE
KOBRINE ET AL (1974)	H_2 CLEARANCE	MONKEY	THORACIC		17.5
GRIFFITHS ET AL (1975)	" "	DOGS	THORACIC	10.8	11.5
GRIFFITHS ET AL (1975)	" "	MONKEYS	"	16.5	13.7
SENTER ET AL (1978)	H_2 CLEARANCE	CAT	THORACIC		10.9
YOUNG (1982)	" "	CAT	THORACIC		13.6
HAYASHI ET AL (1983)	" "	RAT	CERVICAL THORACIC LUMBAR	63 62 64	20 19 20
SCREMIN & DECIMA (1983)	" "	CAT	LUMBAR	43.2	16.2
PRESENT STUDY (1986)	" "	RAT	CERVICAL THORACIC LUMBAR	52 41 52	27 22

*ml/100gm/min.

TABLE 3

SALINE FLOW IN VITRO, MEASURED SIMULTANEOUSLY WITH H_2 METHOD AND A DIRECT TECHNIQUE

NO	FLOW/MIN-DIRECT METHOD	FLOW/MIN-CLEARANCE CURVES
1.	50	58
2.	29	39
3.	21	30
4.	38	43
5.	9	16
6.	33	41
7.	48	59
8.	118	122
9.	24	26
10.	54	54
11.	57	55
12.	96	110
13.	116	129
14.	98	90

TABLE 4

CERVICAL GRAY MATTER SCFB CALCULATED WITH H_2 CLEARANCE TECHNIQUE

RAT NO.	n	$\bar{X}$	SEM	n	$\bar{X}$	SEM
		ELECTRODE A			ELECTRODE B	
1.	(4)	49.5	6.5	(4)	42.5	5.8
2.	(4)	33.0	3.0			
-	(4)	50.0	7.0			
4.	(4)	45.2	5.2			
5.	(5)	63.2	12.2	(5)	55.6	8.5
6.	(4)	74.5	5.9			
7.	(4)	52.2	3.7	(3)	58.0	11.3
8.	(4)	58.2	7.1			
TOTAL	(45)	53.6	2.5			

n = CONSECUTIVE FLOWS $\bar{x}$ = MEAN BLOOD FLOW ml/100gm/min:

SEM = STANDARD ERROR OF MEAN

TABLE 5

CAUDATE NUCLEUS BLOOD FLOW CALCULATED WITH H_2 CLEARANCE TECHNIQUE

RAT NO.	n	$\bar{X}$	SEM
		ELECTRODE B	
2	(4)	29.2	1.2
3	(3)	25.6	2.9
4	(6)	49.1	2.3
6	(4)	38.7	2.1
8	(4)	54.3	5.0
TOTAL	(21)	41.0	2.6

n = CONSECUTIVE FLOWS. $\bar{X}$ = MEAN BLOOD FLOW ml/100gm/min

SEM = STANDARD ERROR OF MEAN

TABLE 6

SCBF AT DIFFERENT SYSTOLIC ARTERIAL PRESSURE

SYSTOLIC B.P.	SCBF*	n
10-19	6±0	2
20-29	21.1±1.2	6
30-39	27.4±1.6	14
40-49	38.1±3.5	2
50-59	51.6±12	2
60-69	45.0±1.7	6
80-89	51.6±4.0	4
90-99	40.3±2.3	2
100-109	55.6±5.7	12
110-119	46.7±3.9	10
120-129	52.3±4.4	8
130-139	52.8±6.6	2
140-149	55.4±1.5	8
150-159	41.8±2.9	2
160-169	78.1±7.3	4
170-179	83.0±6.0	6
180-189	87.7±9.7	4
190-199	90.3±8.0	6
200-225	118.9±3.4	2

*MEAN ± SEM ml/100gm/min

n= NUMBER OF OBSERVATIONS

TABLE 7

SCBF AT DIFFERENT SYSTOLIC ARTERIAL PRESSURE

EXPERIMENTAL GROUP	BLEEDING	CONTROL	ARAMINE
B.P. RANGE mmHg	50	50-160	>160
SCBF ml/100gm/min	+24.9±1.8 n=24	+52.3±1.9 n=34	+88.2±4.0 n=22

+MEAN ± SEM, n = NUMBER OF OBSERVATIONS

TABLE 8

GRAY MATTER THORACIC SCBF BEFORE AND AFTER INDUCED INCREASED INTRACRANIAL PRESSURE

EXPERIMENTAL GROUP	CONTROL	
	NO BALLOON	BALLOON IN
+SCBF ml/100gm/min	41.2±12.6 n=63	45.1±11.4 n=16
SAP mmHg	127.6±22.7 n=35 (60-150)*	

INCREASED ICP

EXPERIMENTAL GROUP	WITHIN AUTOREGULATION	BELOW AUTOREGULATION
+SCBF ml/100gr/min	53.2±19.3 n=56	38.2±10.7 n=7
SAP mmHg	132.5±20 n=30 (75-150)*	29.3±5.3 n=7 (25-40)*

*RANGE OF SYSTOLIC BLOOD PRESSURE

n=NUMBER OF OBSERVATIONS

+MEAN ± SEM

TABLE 9

WHITE MATTER THORACIC SCBF BEFORE AND AFTER INDUCED INCREASED ICP

EXPERIMENTAL GROUP	CONTROL	INCREASED ICP
+SCBF ml/100gm/min	22.2±2.4 n=10	21.0±1.8 n=9
SAP mmHg	118±12 n=10	128±5 n=9

n=NUMBER OF OBSERVATIONS

+MEAN ± SEM

TABLE 10

NORMAL CERVICAL SCBF CALCULATED WITH

H_2 CLEARANCE TECHNIQUE

RAT NO.	n	$\bar{X}$	SEM	n	$\bar{X}$	SEM
	ELECTRODE A			ELECTRODE B		
1	(4)	*34.7	6.0	(3)	*26.0	1.0
2	(7)	60.4	6.7	(8)	51.4	3.1
3	(6)	38.0	1.1	(9)	47.3	3.7
4	(4)	58.2	5.8	(4)	53.0	4.8
5	(6)	50.5	5.2	(4)	58.2	5.8
6	(4)	46.3	4.6	(5)	*34.5	3.5
7	(5)	55.6	4.1			
8	(5)	*24.8	1.1	(5)	60.0	5.4
9	(6)	47.8	4.7	(5)	55.2	7.8
TOTAL (A&B)	(90)	47.8	1.5			
CERVICAL WHITE	(17)	26.8	1.0			
CERVICAL GRAY	(73)	51.9	1.2			

*WHITE MATTER, n=CONSECUTIVE FLOWS. $\bar{X}$=MEAN BLOOD FLOW

ml/100gm/min.

SEM=STANDARD ERROR OF MEAN

TABLE 11

NORMAL CEREBRAL AND SPINAL CORD BLOOD FLOW CALCULATED WITH ^{14}C IODOANTIPYRINE METHOD

RAT NO.	1	2	3	4	5	6
L FRONTAL CORTEX	81.0	56.9	63.1	63.3	51.8	57.7
R " "	54.3	57.1	61.1	60.9	53.7	56.8
L PARIETAL CORTEX	61.2	51.6	91.3	73.7	56.2	70.2
R " "	62.9	61.1	80.0	63.8	64.0	71.8
L CEREBELLUM	60.5	59.2	71.1	63.9	55.4	68.1
R "	62.8	60.6	74.8	65.2	65.6	70.7
L CAUDATE NUCLEUS	74.9	48.9	93.7	59.6	48.2	64.1
R " "	73.4	68.4	87.5	63.5	46.7	64.6
L PONS	61.9	79.4	127.3	60.5	50.8	66.9
R "	67.0	73.7	86.9	63.6	50.3	---
W CERVICAL	49.3	61.2	22.8	45.3	35.8	42.6
G "	108.2	68.0	72.3	93.0	79.1	63.2
W THORACIC	---	56.6	---	53.7	42.5	31.4
G "	---	87.1	86.2	84.8	61.6	54.8

RAT NO.	7	8	9	10	11	12
L FRONTAL CORTEX	69.5	109.7	78.5	44.2	97.5	83.4
R " "	63.2	113.0	76.6	65.6	74.6	86.4
L PARIETAL CORTEX	75.1	115.0	77.8	---	108.2	108.7
R " "	82.4	116.1	80.4	73.0	87.2	102.2
L CEREBELLUM	84.0	103.2	87.8	57.7	87.8	95.5
R "	59.3	104.3	90.5	90.5	88.2	95.5
L CAUDATE NUCLEUS	91.3	115.6	80.4	68.0	131.4	108.7
R " "	79.0	124.3	79.0	81.8	122.5	111.1
L PONS	73.5	105.4	110.5	68.4	79.9	99.9
R "	65.8	91.3	90.7	59.6	100.8	103.6
W CERVICAL	25.5	69.6	60.3	40.1	75.5	76.6
G "	155.8	124.3	76.2	79.8	105.9	---
W THORACIC	56.6	65.1	56.9	33.4	55.1	81.0
G "	138.2	95.8	78.6	58.3	79.5	111.9

L = LEFT, R = RIGHT, W = WHITE, G = GRAY

BLOOD FLOW VALUES IN ml/100gm/min

TABLE 12

NORMAL CEREBRAL AND SPINAL CORD BLOOD FLOW CALCULATED WITH ^{14}C IODOANTIPYRINE METHOD

(TOTAL OF 12 EXPERIMENTS)

	n - LEFT	n - RIGHT	n - ENTIRE
FRONTAL CORTEX	(12) 72.6±5.5	(12) 68.6±5.0	(24) 70.0±3.6
PARIETAL CORTEX	(11) 80.8±6.6	(12) 78.7±4.9	(23) 79.7±4.0
CEREBELLUM	(12) 74.5±4.7	(12) 74.8±4.5	(24) 74.7±3.3
CAUDATE NUCLEUS	(12) 84.6±7.1	(12) 83.5±7.0	(24) 84.0±4.9
PONS	(12) 83.5±6.9	(11) 77.6±5.4	(23) 78.1±5.5
	n WHITE MATTER	n GRAY MATTER	n TOTAL
CERVICAL SPINE	(12) 50.4±5.3	(11) 93.2±8.5	(23) 70.9±6.6
THORACIC SPINE	(10) 53.2±4.6	(11) 85.2±7.3	(21) 69.9

n = NUMBER OF EXPERIMENTS

VALUES OF BLOOD FLOW = MEAN ± SEM in ml/100gm/min.

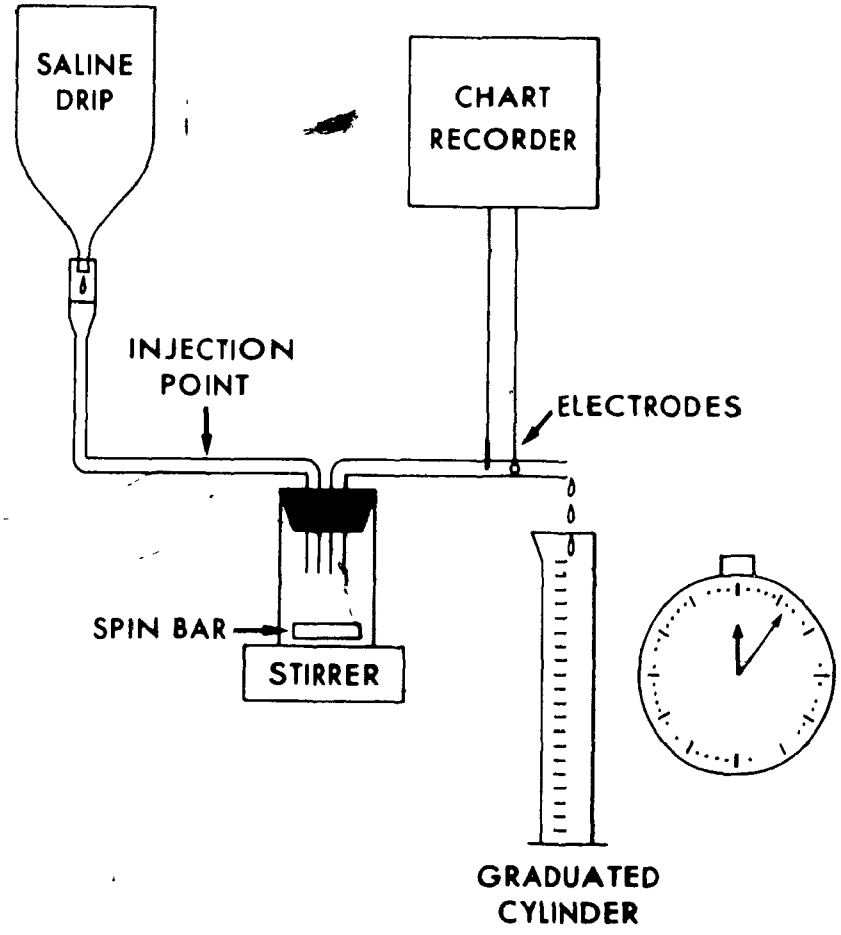

Figure 1

An in vitro model used to verify the blood flow results obtained by the clearance method. The flow of saline measured simultaneously by the washout clearance curves (record) was compared to the flow of saline in the graduated cylinder.

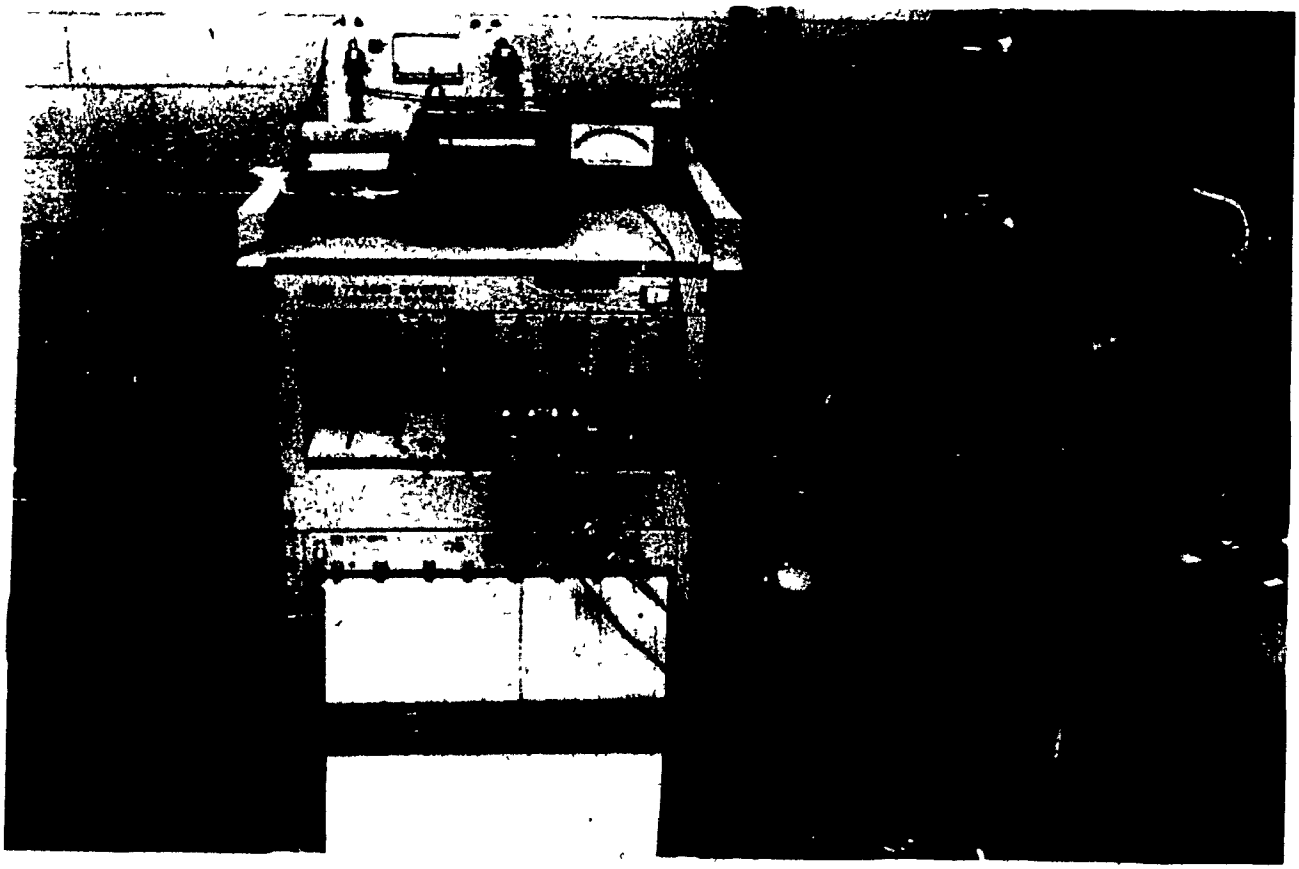

Figure 2

The experimental set-up demonstrates the rhodent respirator (right), the amplifier and the polygraph (left) for recording the clearance curves, the blood pressure and heart rate.

Figure 3

The experimental set-up showing the rhodent respirator, the hydrogen container (left), the animal within the stereotoxic frame and microscope (right).

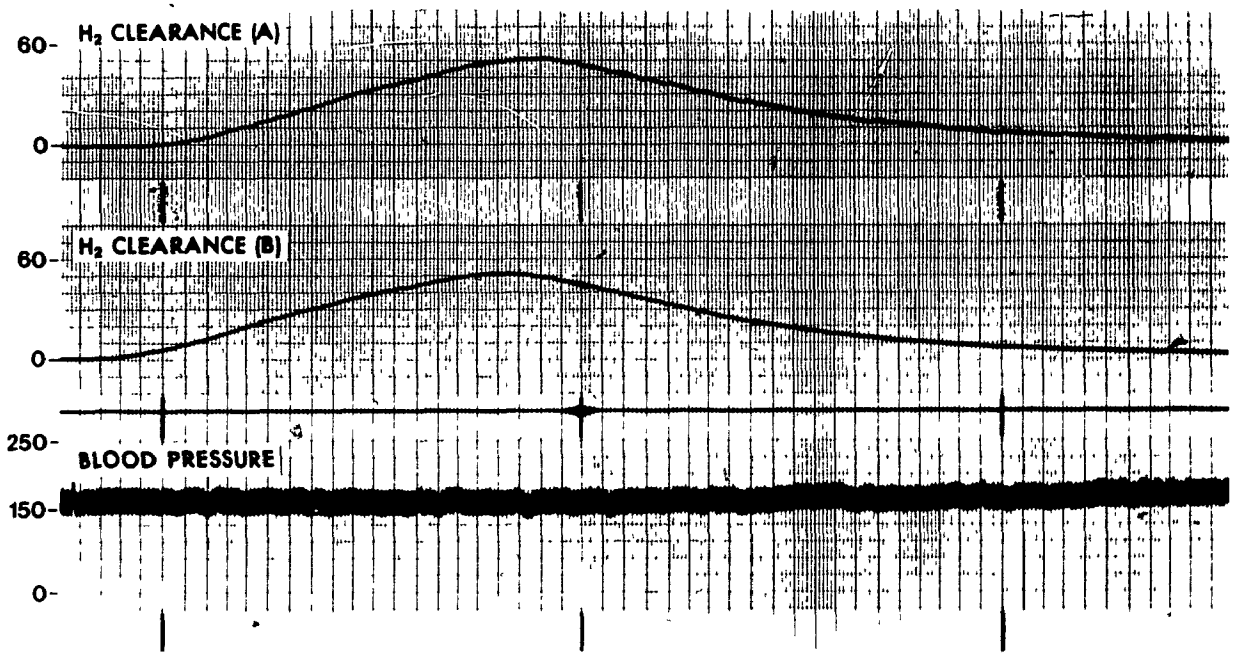

Figure 4

The chart recorder shows recorded curves of the hydrogen clearance technique, and blood pressure. After the baseline is stabilized the curves climb until the tissue is saturated with hydrogen, and a plateau (saturation) is reached. Then the H_2 hydrogen inflow is discontinued and the decreasing current (washout) is recorded. Note the curve returns to baseline.

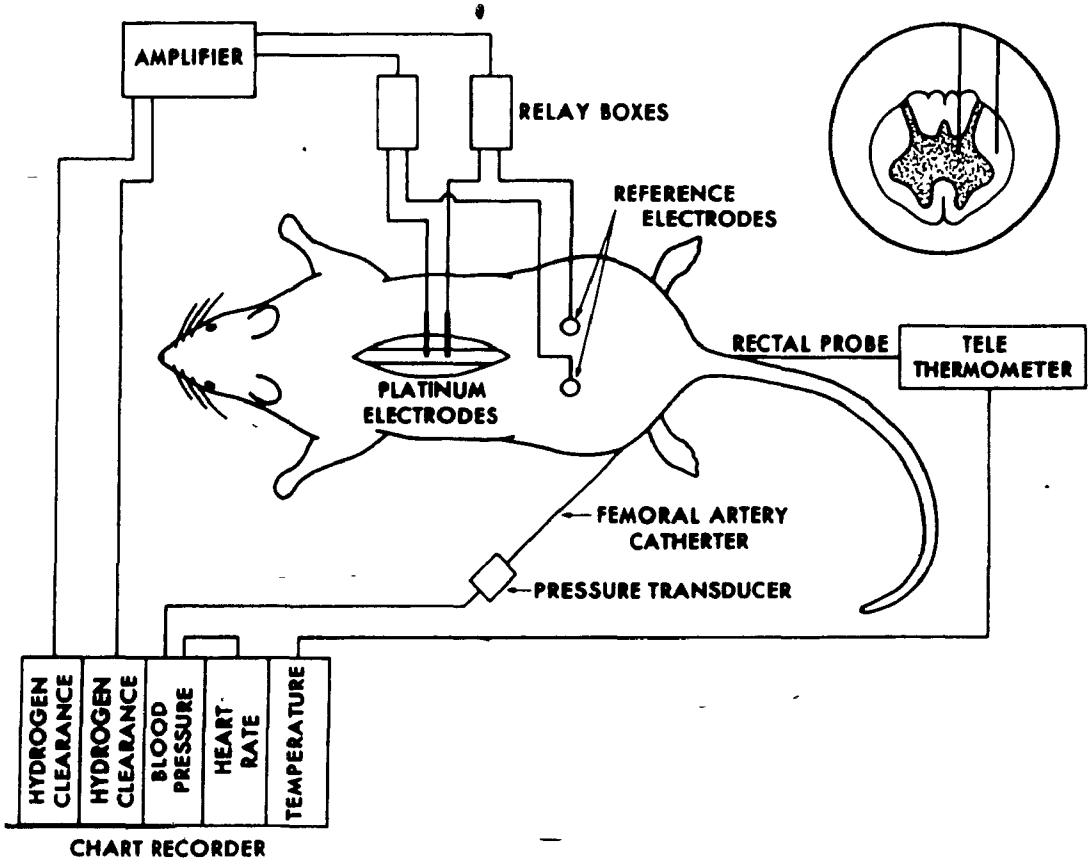

Figure 5

An artist drawing of the experimental set-up. The platinum electrodes are inserted in the cord and are connected with the reference electrodes to the relay box, through the amplifier to the chart recorder. The rectal probe and the femoral catheter (for temperature and blood pressure recording respectively) are connected to the chart recorder. Insert shows a section of the cord with the electrodes placed in the grey and white matter.

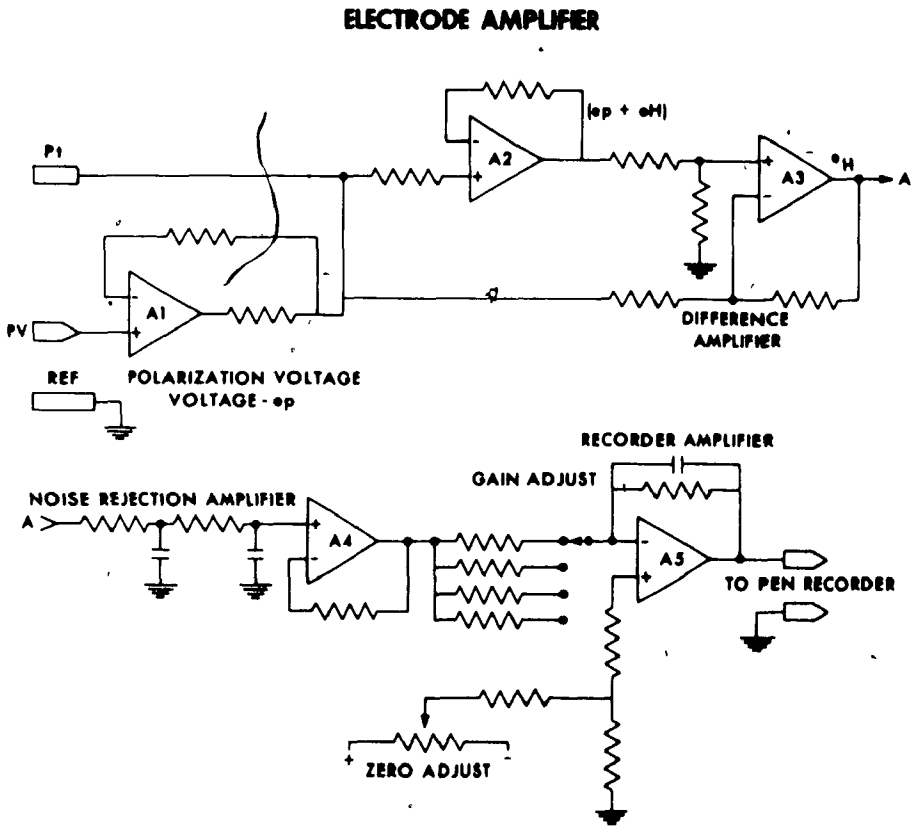

Figure 6

The electronic circuitory (description see text)

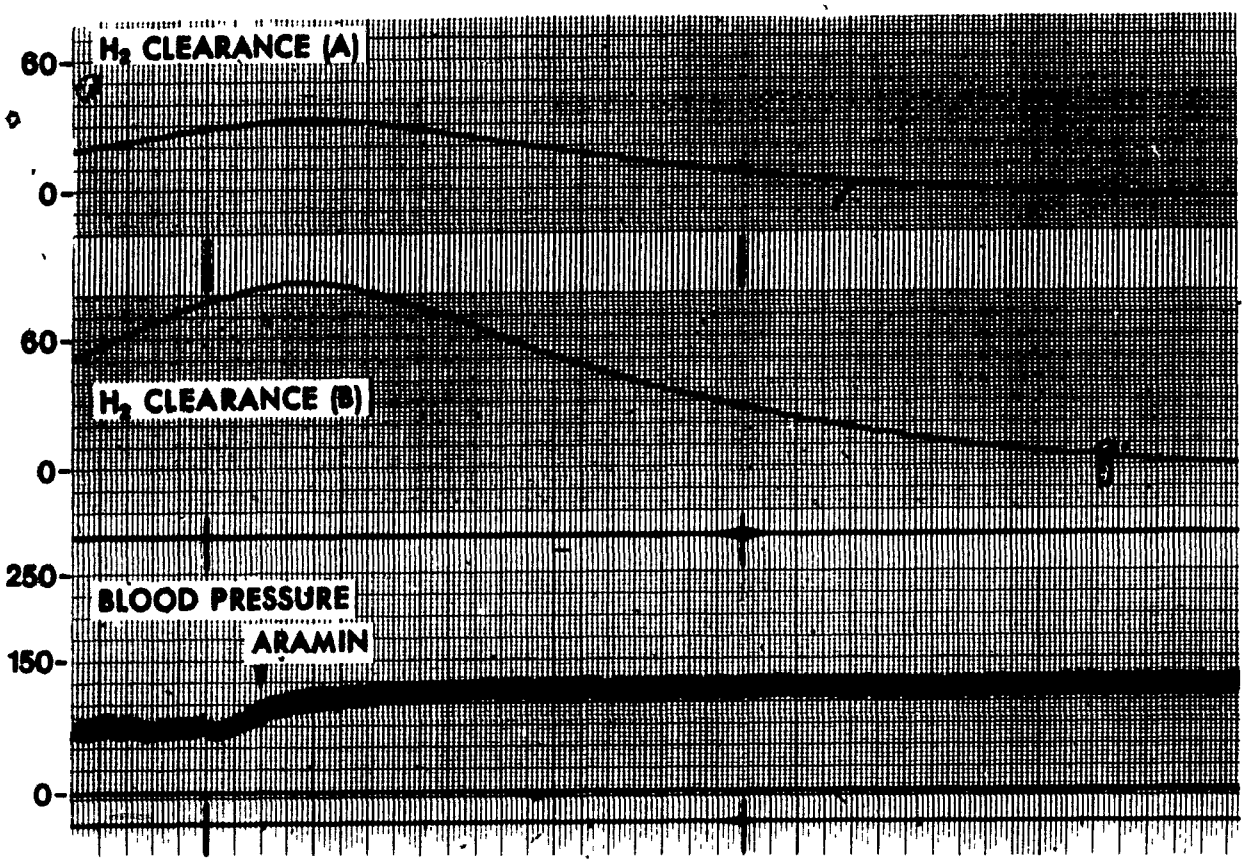

Figure 7

A washout curves from two electrodes inserted in the cord, while raising blood pressure by administration of metaraminol. Blood pressure in this example was raised from 75 to 125 mmHg and the mean measured SCBF was 49±2.4 ml/100gr/min. Blood pressure was raised while the tissue was saturated with hydrogen and a plateau was reached.

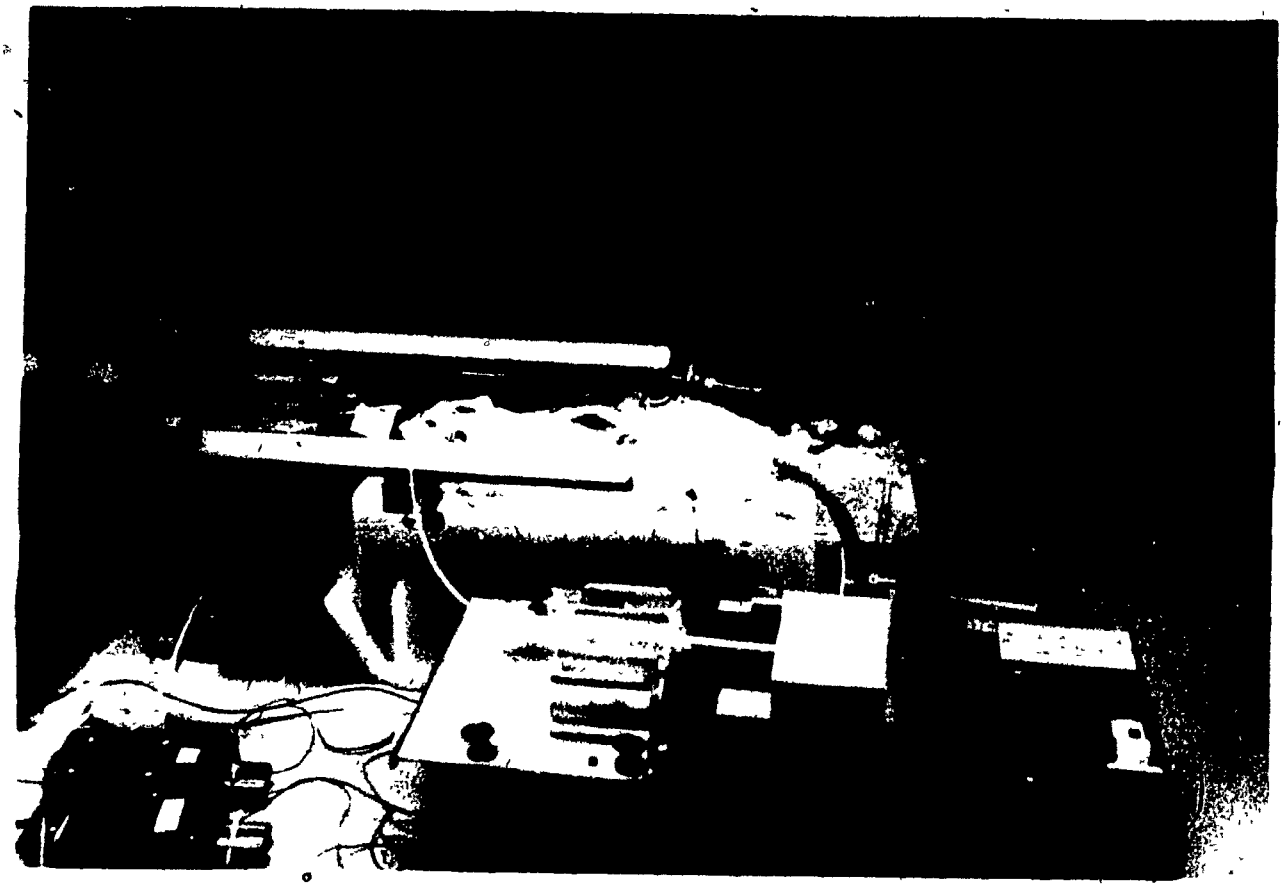

Figure 8

The experimental set-up of group number 3, demonstrating the polyethylen tube connected to the epidural balloon from one side and to a syringe (on its other end) filled with saline (attached to a Harvard perfusion pump) for gradual inflation of the balloon.

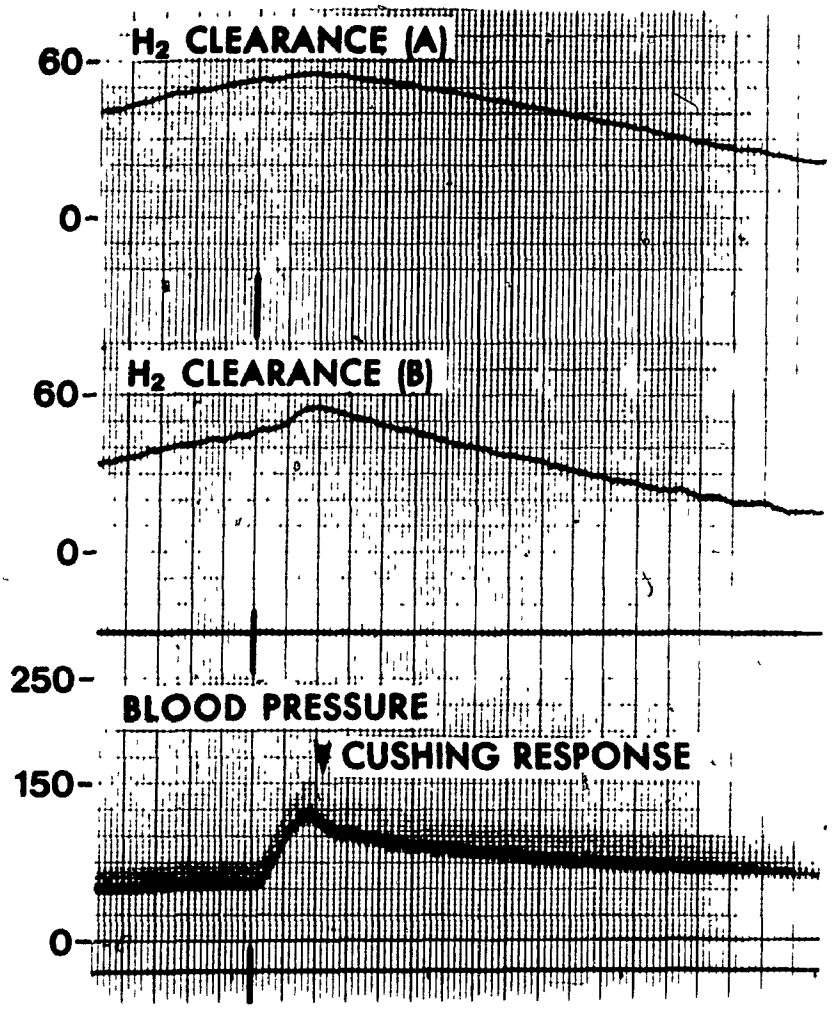

Figure 9

A washout curves from two electrodes inserted in the cord to demonstrate the Cushing Response. After the saturation of hydrogen was completed and the gas turned off, the intracranial balloon was inflated to induce an acute increase in intracranial pressure. Once blood pressure was elevated about 25 to 50 mmHg above its baseline value, the inflation of the balloon was stopped, and SCBF was measured.

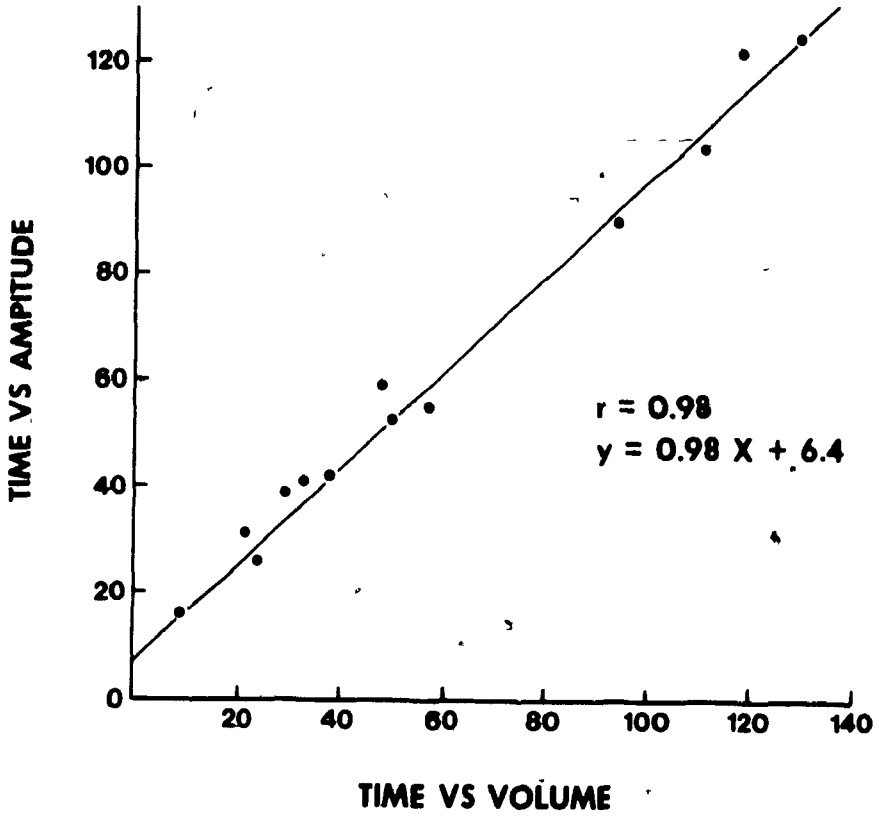

Figure 10

With the linear regression method it can be seen that there is good correlation (correlation coefficient (r) is 0.98) between the two techniques of calculating the flow; the flow of saline that was accumulated in the cylinder during 1 minute and was measured simultaneously from the washout clearance curve. The regression equation of these two measurements is Y=0.98Y+6.4

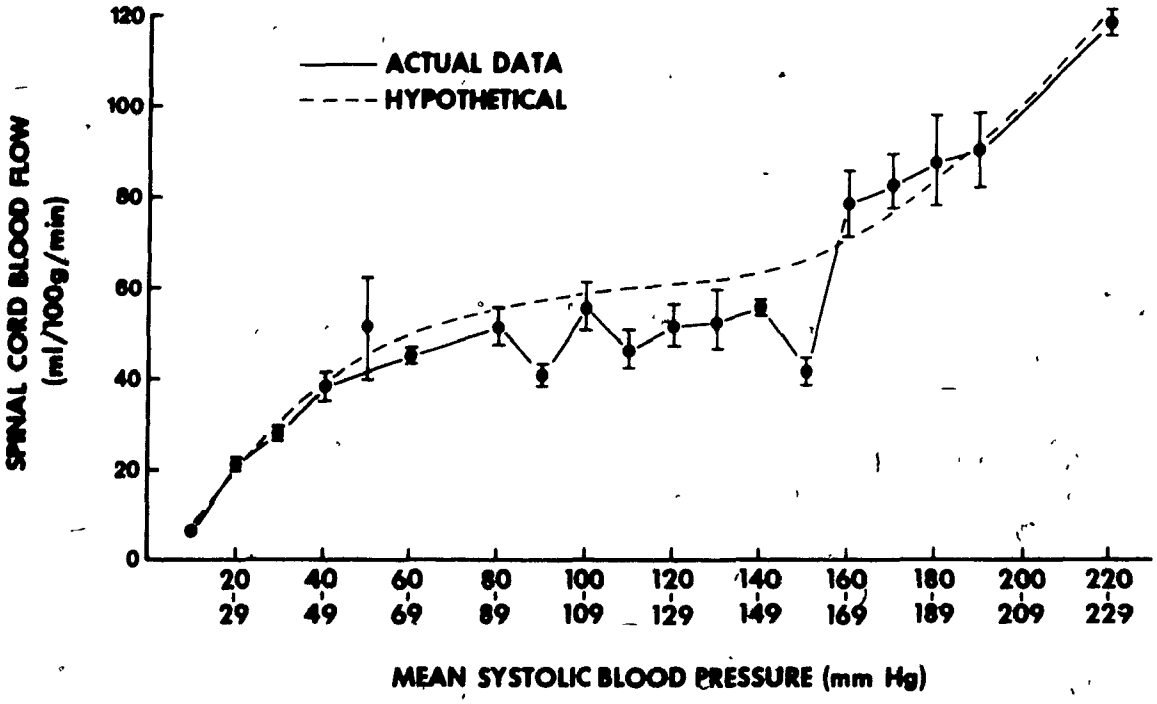

Figure 11

Autoregulation in SCBF in relation to changes in blood pressure. Over a wide range of systolic arterial blood pressure (eg. 50-165 mmHg), SCBF remained relatively constant. Below 50 mmHg and above 165 mmHg, SCBF was a function of systolic arterial pressure.

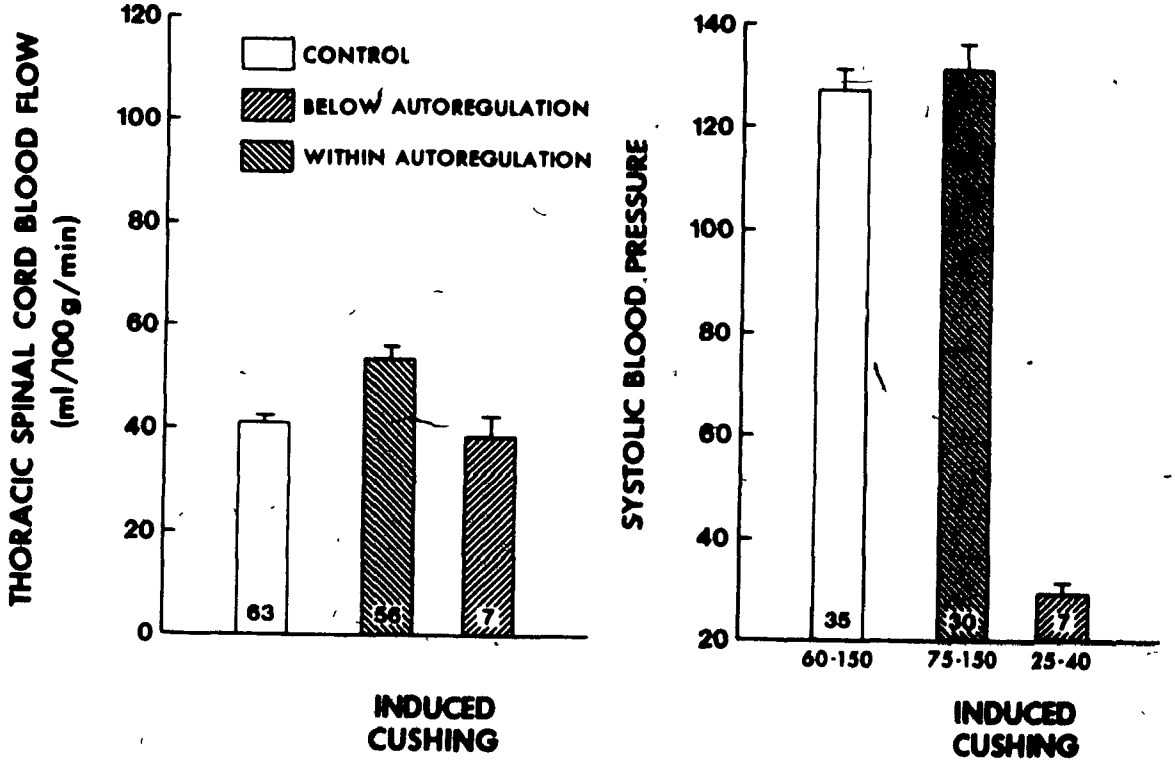

Figure 12

This diagram demonstrates clearly elevation of the thoracic grey matter SCBF after induced increase in intracranial pressure (ICP). Under this stimulus and while the blood pressure was maintained within autoregulation (132±20 mmHg) SCBF rose to 53.2±2.6 ml/100gr/min. The control SCBF was 41.2±1.6 ml/100gr/min with systolic pressure of 127.6±22.7 mmHg. Note that when blood pressure was far below autoregulation (29.3±5.3 mmHg) under the same induced increase in ICP, relative hyperemia was observed close to the control values.

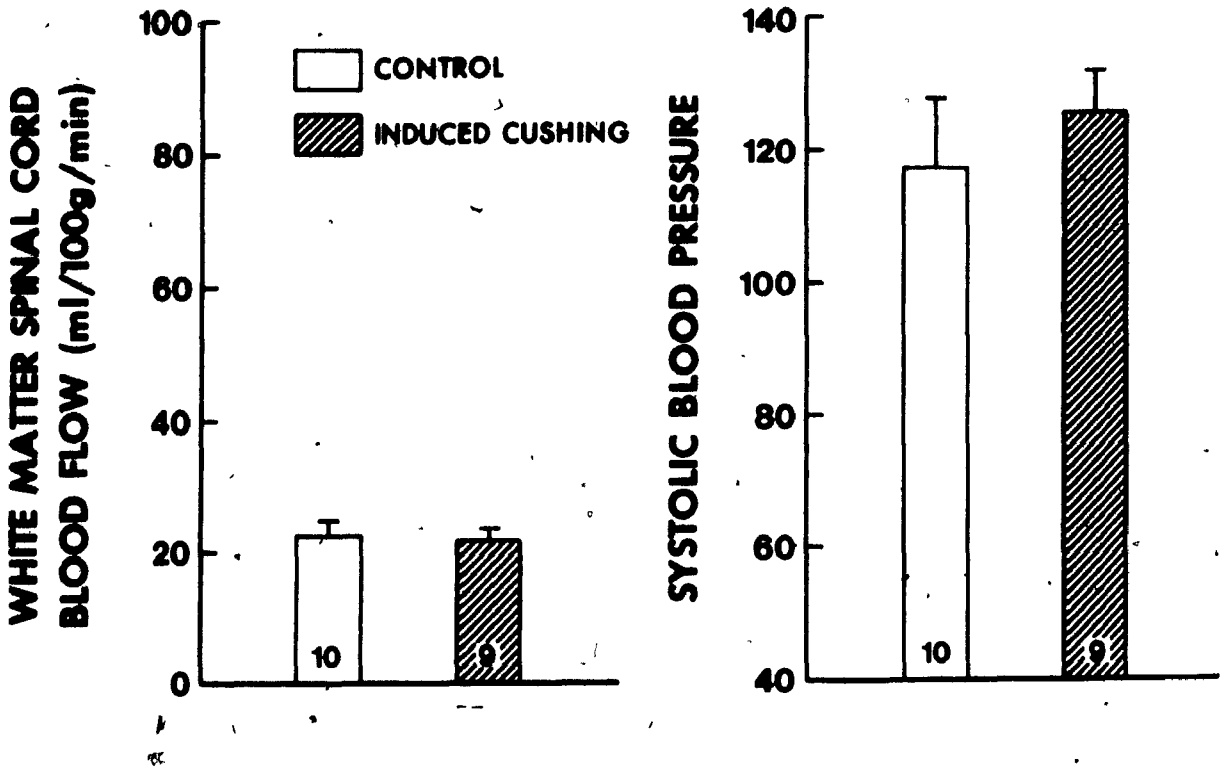

Figure 13

SCBF in the white matter of the thoracic cord did not change after the induced increase in intracranial pressure. The blood pressure values shown on the right are within autoregulation limits.

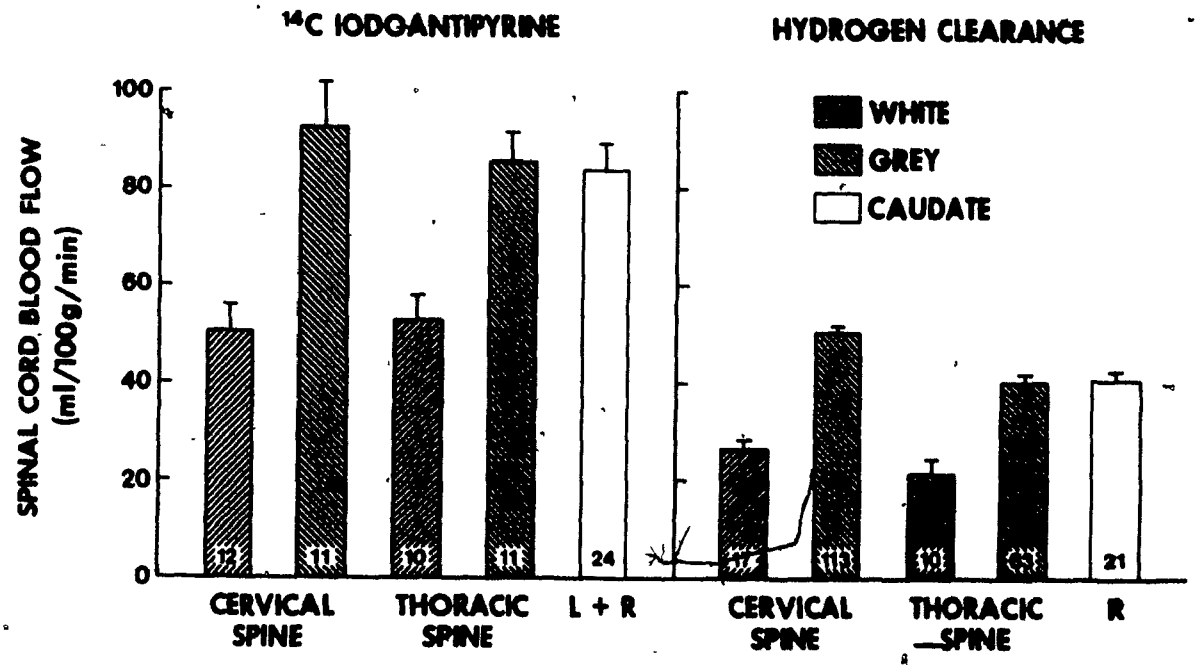

Figure 14

A comparison of blood flow measured with H_2 clearance and ^{14}C-iodoantipyrine methods. The IAP values are almost twice as large as these of the H_2 clearance.

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