X-ray Fluorescence Measurements of Regional Cerebral Blood Volume in Rabbits: Effect of Functional Load, Epinephrine and Papaverine

by

Charles J. Hannan, Jr.

Submitted to the Faculty of the School of Graduate Studies

of the Medical College of Georgia in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

June

1976

X-ray Fluorescence Measurements of Regional Cerebral Blood Volume in Rabbits: Effect of Functional Load, Epinephrine and Papaverine

This dissertation submitted by Charles J. Hannan, Jr. has been examined and approved by an appointed committee of the faculty of the School of Graduate Studies of the Medical College of Georgia.

The signatures which appear below verify the fact that all required changes have been incorporated and that the dissertation has received final approval with reference to content, form and accuracy of presentation.

This dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 3, 1976
(Date)

[redacted]
Advisor

[redacted]
Chairman, Department of Pharmacology

[redacted]
Dean, School of Graduate Studies

ACKNOWLEDGMENTS

The author is indebted to Dr. Raymond P. Ahlquist for affording him the opportunity to pursue a course of graduate study in pharmacology, and for his encouragement and interest in the author's progress.

The supervision of the present work by Dr. Louis P. Gangarosa is regarded with deep appreciation to him for his guidance and patience.

In thanks for a productive laboratory rapport, and stimulating exchange of ideas; the author is grateful to Mrs. Ching Hung, Dr. James Matheny and Dr. Walter Hubbard.

To the members of his committee, Drs. Raymond P. Ahlquist, Louis P. Gangarosa, Gene Howard, David C. Jerram, S. David Stoney and Dean Sam Singal, the author extends his thanks for their support and many helpful suggestions.

The author is extremely grateful to Dr. Jon Trueblood for his joyful extension of time and energy in the solution of many technical problems.

The author expresses thanks to Drs. James Matheny, Tom Swift and Jon Trueblood for their willing efforts as readers of this dissertation.

The author is deeply grateful to Mrs. Brenda Griffin for her talents in the preparation of this manuscript.

Finally, the author wishes to record his gratitude to Drs. Bobby Moore and Herb Swartz whose introduction to the science of pharmacology

decided the author on his present course of study.

Financial support for this research came from the U.S. Public Health Service, National Institutes of Health, Grant Number ROl DE 03022, and a Sigma Xi Grant-in-Aid of research.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

Ach	acetylcholine
BBB	blood brain barrier
CBF	cerebral blood flow
CBV	cerebral blood volume
CSF	cerebrospinal fluid
EEG	electroencephalogram
Epi	epinephrine (adrenaline)
FET	field effect transistor
FWHM	full width at half maximum
HSA	human serum albumin
HZ	hertz (cycles per second)
IA	intraarterial
IV	intravenous
kVp	peak kilovoltage
MAP	mean arterial pressure
mAs	produce of milliamperes and seconds
MCA	multichannel analyzer
mm Hg	millimeters of mercury (units of pressure)
NE	norepinephrine (noradrenaline)
pCO_2	partial pressure of carbon dioxide
pH	negative logarithm of the hydrogen ion concentration
pO_2	partial pressure of oxygen
rCBF	regional cerebral blood flow
rCBV	regional cerebral blood volume
ROM	read only memory
SCA	single channel analyzer
Si(Li)	lithium drifted silicon; as used for semiconductors
Tc-99m	technetium-99m ("m" symbolizes the metastable nature of the isotope)

LIST OF TABLES

INTRODUCTION

A. Statement of problem

The general aim of this research is to determine the effect of selected vasoactive drugs on regional cerebral blood volume (rCBV) in the presence and absence of functional loading. The drugs to be included are the adrenal medullary hormone epinephrine and the vasodilator papaverine. The nature, or even existence of an effect by epinephrine on the cerebral vasculature has been debated ever since epinephrine was recognized as an agent with sympathomimetic properties. Controversy also surrounds the distinct disparity between *in vivo* and *in vitro* vasodilatory responses to papaverine. Used together and in conjunction with functional loading, we hoped to gain insight into the role these agents play in altering cerebral hemodynamics.

To achieve this goal a non-invasive technique for measuring rCBV was developed and evaluated using modifications of methods described by Hoffer and co-workers (1969). For comparative purposes selected drugs and similar techniques were evaluated in the hindlimb, where it was possible to measure the blood flow (BF) or blood volume (BV) by alternative techniques with and without a metabolic load present.

B. Review of related literature

1. Cerebral vasculature reactivity to drugs

a. Functional vasodilation

During and immediately after a period of neural activity it seems probable that there is metabolic activation of the synaptic region. Since there is constant bombardment of the post-synaptic membrane, from impulse transmission, an efficient mechanism by which to achieve dynamic maintenance of neural synapses and cells would appear to be required. Such a mechanism might require alteration of a rate limiting event which holds in check a reaction for supplying energy for the redistribution of ions across membranes and resynthesis of transmitters. Metabolites, such as glucose and oxygen are essential to maintain the biosynthetic pathways required for maintenance (and growth) of brain cells (Meyers and Ericsson, 1971). These metabolites must be available for the critical time when they are needed during bursts of cellular activity. In a like manner noxious waste products must be removed from sensitive nerve sites. Not only is increased metabolic activity necessary for renewal of transmitters and maintenance of the sodium pump, but also continuous stimulation in the absence of proper substrate supply might lead to atrophy or cell death (Roberts and Matthysse, 1970). Although synaptic events are short-term, they may be linked to a longer termed biochemical event which replenishes used metabolites in at least two ways involving the action of agents released into the extracellular fluid: 1) by feedback control on the active cell, or 2) by establishing a neurogenically

produced vascular event. This latter alternative may operate through a population of nerve cells that is uniquely sensitive to metabolic state having far reaching effects on the hemodynamics in extensive parts of the brain where the neurons project. Such mechanisms are well-known for the peripheral systems where special cells detect pCO_2, pO_2 or pH of the blood and alter circulatory or respiratory functions accordingly.

Although frequently demonstrated the effect of a functional load or metabolic activation in the CNS on regional blood flow (rCBF) has not been generally appreciated. Sokaloff (1961) made a key observation in qualitative terms using a radioactive inert tracer gas clearance rate to demonstrate rCBF. The concentration of tracer in brain regions was determined by producing autoradiograms of brain sections and comparing the density of exposed film over regions which were under increased functional load with those having normal activity. There was evidence of increased blood flow in the lateral gyrus, lateral geniculate ganglion, and superior colliculus in response to photic stimulation to the eye, these studies refined previous observations (Serota and Gerard, 1938). However, the areas affected in Sokaloff's experiments were so small compared to the entire brain that total blood flow was not significantly changed from control values. To attempt to relate cerebral functions to total CBF and metabolic rate would be like trying to correlate activities of parts of the body to cardiac output and total oxygen consumption. Sokaloff's results were confirmed

by McElligott and Melzack (1967) using thermistor probes implanted in the brain to determine rCBF. The use of temperature probes to measure BF was challenged on the grounds that metabolic activity also produces heat, however, these arguments were logically disposed of by Melzack and Casey (1967) using peripheral nerve as a model. A large number of other studies using various techniques came to the same conclusion, supporting a localized functional vasodilation in cerebral tissue (Meyer and Goth, 1961; Baldy-Moulinier and Ingvar, 1968; Freeman and Ingvar, 1968; Bondy, 1973; Moskalenko, et al. 1974). Special mention should be made of recent work reported by Ingvar and co-workers from Lund, Sweden. Using humans, they first reported (Ingvar and Risberg, 1967) an increase in rCBF during mental effort as determined by external monitoring of xenon-133 clearance. This was followed by reports of characteristic rCBF patterns during: organic dementia (Ingvar and Gustafson, 1970), memorizing and reasoning (Risberg and Ingvar, 1973), and speech and reading (Ingvar and Schwartz, 1974). Indirect evidence for the close association of neuronal cells and vasculature in the brain was found by Barker (1972) using micro-anatomical methods in which India ink injected vasculature from slices of rat brain disclosed a reduction in the number of nerve cells per length of capillary from arterial to venous end. Barker hypothesized that this was due to a reduction in concentration of vital metabolites or build up of waste products as blood proceeded through the vascular tree.

In a review article Lierse (1963) summarized the position thus: 'Up to now, capillary density remains the only anatomical indicator of the oxygen consumption of limited regions of the brain.'

b. Autoregulation

The seeming rigidity of the skull led Alexander Monro (1783) to suppose that expansion of cranial contents was severely limited and that changes in the caliber of cerebral vessels was unlikely.

This observation was one of several which Monro made from post-mortem examinations and it was apparent that Monro together with other anatomists of his time recognized neither the origin nor significance of cerebrospinal fluid. Monro's general concept was confirmed by Kellie (1824 a and b) partly from the post-mortem observations of men who had succumbed to exposure and partly from experiments on dogs. This concept subsequently became known as the Monro-Kellie doctrine and exerted powerful influence over the interpretation of experimental results in the nineteenth century and for some years of the present century.

The perceptive work of Burrows (1846) acknowledged the condition of the rigid skull, while realizing the importance of the cerebrospinal fluid (CSF). Burrows emphasized that although the brain was incompressible this was not as important as the fact that changes in brain vascular volume could occur at the expense of other fluids. The incompressibility of the skull must

be qualified in considering the work of Magendie (1836) and many more recent investigators who have demonstrated that the occipito-atlantoid ligament possesses sufficient elasticity to transmit pulsations of the CSF, similar to the membranous covering of the anterior fontanelle in young children. Pressure-volume measurements made by Langfitt, Weinstein and Kassell (1965) indicate that the volume of an intracranial balloon can be increased some 2 to 7 ml before CSF pressure starts to rise. In this regard, studies have revealed that the escape of CSF around the cranial and segmental nerves may be demonstrated by dye injection (Key and Retzius, 1876; Weed, 1914). It would seem, therefore, that the vascular volume of the brain could be expanded and reduced within certain limits to accomodate functional loading.

As vessels penetrate the cerebral surface, they are sheathed by extensions of the pia mater, so that, vessels are surrounded by a subarachnoid space which contains CSF (Rosenblum, 1975). These extensions are known as the Virchow-Robin spaces and end at the capillary level. It is proposed that changes in total CBV will be compensated for by reciprocal changes in CSF volume; indeed, a circadian rhythm in CBV has been observed in the mouse with changes of up to about 20 µl per gram of tissue (Edvinsson, Nielsen and Owman, 1973).

Early studies of total brain blood flow indicated the total CBF remained constant over a wide range of arterial blood pressures, i.e. autoregulation occurred. However, this does not exclude

regional changes due to shifting of flow and volume within the brain. Autoregulation requires appropriate adjustment in vascular caliber with vessels constricting when the systemic pressure rises and dilating when the systemic pressure falls. Direct observation shows that the pial arterioles participate in this response (Fog, 1937; Rapela and Green, 1964). Pial arterioles (30-60 μ diam.) are not as sensitive to pressure reductions, but they dilate when mean arterial pressure (MAP) drops below about 80 mm Hg, whereas pial arteries (100 μ diam.) dilate even when the reduction in MAP is relatively minor. It appears that in the usual range of blood pressure (80-120 mm Hg.), the maintenance of flow in the face of reductions in pressure is achieved by adjustments in caliber of the larger vessels (Kety and Schmidt, 1948 b).

The mechanism by which the adjustment in vessel caliber occurs in response to changes in arterial pressure has not been clarified completely. Two general mechanisms, myogenic and metabolic, have been proposed. The myogenic hypothesis (Bayliss, 1902) states that the vascular smooth muscle is inherently responsive to stretch or changes in tension. An increase in transmural pressure causes increased contraction of the vascular smooth muscle and results in vasoconstriction (Fog, 1937; Meyer and Denny-Brown, 1957; Yoshida, et al., 1966; or Ekstrom-Jodal, et al., 1969). Evidence for the metabolic theory was first presented by Roy and Sherrington (1890); this theory proposes that changes in blood pressure produce transient changes in flow resulting in passive variation in vessel caliber. These

changes in flow, in turn, alter the concentration of vasoactive metabolites and the vascular smooth muscle subsequently responds to the new concentrations of metabolites (Carpi, 1972). Attempts to distinguish between these two mechanisms have been based on two types of experiments. One type is concerned with the speed of response. It has been shown under certain conditions that autoregulation is a fast process (Yoshida et al., 1966); however, the claim that metabolic mechanisms are slower due to the delay of diffusion has been challenged (Fieschi et al., 1968; Fieschi et al., 1969). The results of another type of experiment on changes in vessel diameter occuring in response to an increase in venous pressure are more helpful. Increases in venous pressure should constrict arterioles if the myogenic mechanism is operative; as increased pressure is transmitted to the arterial system and an increase in transmural pressure would result. On the other hand, the associated decrease in flow would result in dilation of the arterioles if the metabolic mechanism is operative. The evidence as reviewed supports the myogenic hypothesis, but the evidence is not complete (Kontos, 1975). As indicated by the finding of impaired autoregulation even though CO_2 reactivity was intact in patients with acute cerebrovascular lesions (Fieschi et al., 1968), the best conclusion may be that both mechanisms play a role in the autoregulation of CBF.

As described above, when autoregulation is operative a reduction of systemic blood pressure in healthy man and in

experimental animals to about half normal does not lower the CBF significantly. However, it should be noted that under hypoxic and hypercapnic conditions, autoregulation is impaired since the relationship between pressure and flow becomes passive, with hypotension producing a drop in CBF. Also, when hypotension is first present, hypoxia and hypercapnia fail to produce the usual increase in CBF (Davies, 1969).

c. Neurogenic control

The existence of autonomic receptors in smooth muscle of pial and intracerebral vessels has usually hinged on whether there is autonomic innervation. The argument may be academic since it was recently demonstrated (Owman et al., 1973) that in the case of the umbilical artery autonomic receptors are present without a nerve supply to the receptors. With the advent of the Falck-Hillarp fluorescence method for the histochemical demonstration of catecholamines, (Bjorklund, Falck and Owman, 1972) the presence of adrenergic innervation in the brain blood vessels is conclusive. The question is no longer <u>if</u> cerebral vessels are under neurogenic influence, but rather <u>how</u> this influence by adrenergic nerves operates in physiological control of cerebral blood flow. In a recent review, Rosenblum (1971) stated that there is no reason for researchers to "... believe that positive results (related to neurogenic mechanisms) are artifacts of the experimental situation, caused by variables not controlled by the investigators".

In several respects the intracranial vascular system is

uniquely situated and would be expected to be different: as already noted it is in a rigid compartment; further, owing to the presence of the blood brain barrier (BBB), circulating compounds may not have immediate access to vascular receptors (Oldendorf, 1974). However, BBB does not exclude action on exquisitely sensitive cells which are either stimulated by extremely low levels of agents or are more easily accessible through an opening in the BBB. Such openings have been demonstrated in the area postrema, supraoptic crest, subfornical organ, pineal body, pituitary gland and choroid plexus (Truex and Carpernter, 1969). Further unique characteristics of the cerebral circulation include the venous system (also heavily innervated by adrenergic nerves) which has a different construction compared to peripheral veins. Although the dura mater is closely applied to the inner surface of the skull this is not so for the spinal region. The epidural space, which encloses an abundant venous plexus and fatty areolar tissue, is interposed between dura and the bony vertebral canal and segmental ligaments. Maynard et al. (1957) have shown that intracerebral venules may be up to 30 μ in diameter before they acquire a muscular coat. Venules are generally indistinguishable from small capillaries having an endothelial layer resting on a basement membrane. Not only resistance vessels but also vessels with a caliber corresponding to that of conducting arteries receive autonomic innervation; in some areas that innervation is more extensive in distribution and density than in

vascular supply of any other organ of the body (Owman, Edvinsson and Nielsen, 1974).

The ultimate demonstration of physiologically important neurogenic activity may now be at hand as a result of two recently reported abstracts which support the hypothesis of Owman and co-workers (1974). Based upon histological and other in vitro work, the locus coeruleus (located in the brain stem) appears to be a center of neurogenic intracerebral microcirculatory control (this may be considered analagous to an intracerebral sympathetic ganglion). First, using microcannulae to stimulate the locus coeruleus with carbachol, Raichle and associates (1975) have produced a prompt reduction in CBF and an increase in BBB permeability. The intraventricular administration of the alpha-blocker phentolamine (25-100 μg) had the opposite effect which could imply that tonic sympathetic constriction was reduced. This suggests that the central noradrenergic system in the brain may have the dual function of regulating capillary permeability, as well as, blood flow. The second abstract presented at the same symposium was by de la Torre, Walker and Mullan (1975), who postulated a role of brain stem noradrenergic neurons (seen associated with intracerebral arterioles) as the source of innervation of blood vessels. This was based on microelectrode stimulation or ablation experiments done in conjunction with rCBF measurements.

d. Autonomic agents

Study of the reviews by Sokoloff (1959) and Carpi (1972)

indicates that the pharmacological evaluation of the effect of sympathetic amines on cerebral circulation has been at best controversial and at worst unintelligible. It is well established that marked changes in CBF (and CBV) are produced by alterations in pCO_2 and perivascular pH. Such chemical mechanisms do not, a priori, exclude neurogenic control; both mechanisms may even interact or exert their actions through a common final pathway. For instance, if increased CBF is needed, a simultaneous increase in 'sympathetic' tone could operate to limit the total intracranial blood supply to amounts necessary for overall activity while the local, metabolically mediated increase in flow is satisfied. The generalized constriction would prevent a concomitant elevation of intracranial pressure through an increase in cerebral blood volume due to the local vasodilation added to the present volume (Owman, Edvinsson and Nielsen, 1974). Direct and unequivocal evidence for a neurogenic mechanism in the brain circulation has been confined to only a few studies of the sympathetic system in which several anatomical, physiological and methodological considerations have been adequately controlled (D'Alecy, 1973; D'Alecy and Feigl, 1972; James, Miller and Purves, 1969). The locus coeruleus work cited above brings a new facet to the neurogenic control hypothesis which may necessitate a re-evaluation of its importance relative to other control mechanisms.

Evidence for the presence of adrenergic receptors in the brain vasculature is adequate (Nielsen and Owman, 1970;

Nielsen and Owman, 1971; Politoff and Macri, 1966; Uchida, Bohr and Hoobler, 1967); however, the exact role assigned to alpha or beta components is not clear. Rosendorff (1972), employing microinjections of transmitters and isotope clearance to measure rCBF in the hypothalamus, presented evidence which indicated that norepinephrine (NE) has a dilator effect in low concentrations and a constrictor effect in higher doses. Subsequent studies implied that the effect was similar when NE was endogenously released by tyramine (Mitchell, Scriven and Rosendorff, 1975). Appropriate blockers indicated that dilator effects are beta and constrictor effects are mediated by alpha receptors. This approach has one major weakness, which is probably more attendant to the beta mediated vasodilation; that is, studies in which NE was applied to neurons by microiontophoresis may increase (Boakes et al., 1968; Johnson, Roberts and Straughan, 1969) or decrease (Biscoe and Straughan, 1966; Phillis and York, 1967) the firing rate. Neuronal excitation has also been reported for isoprenaline administration (Johnson et al., 1969), and NE excitation may be blocked by both alpha and beta antagonists, but more consistently with beta antagonists (Brawley and Johnson, 1973). Still further alternatives come from the work of Stone (1971) who states that because of the similar latency and time course between small vessel constriction and excitation of central neurons by NE, the effects on nerves in some areas of the CNS may be secondary to effects on the vasculature. Indeed, depolarization produced by hypoxia

is well known, and some investigators have proposed that the oxygen supply to neurons is a major cell activity modulator (Davies and Bronk, 1957).

Experiments by Seylaz and co-workers (1973) using intra-arterial administration of isoprenaline showed that vasodilation was produced (and blocked by propranolol) in the caudate nucleus as measured by indwelling thermistor probes. These experiments would tend to support Rosendorff, if it can be substantiated that these drugs do not have an effect on nerve cell firing when given via this route. The critical point for comparison in these studies is that Rosendorff used microinjection while Seylaz introduced drugs intraarterially; both observed vasodilation with a beta agonist.

e. Papaverine

Papaverine is reported to be the only available pharmacologic agent that has repeatedly been shown to produce cerebral vasodilation (McHenry et al., 1970). Yet as stated by Davies (1969). "The use of vasodilators to improve the cerebral function is frought conceptually with at least two hazards; one, that they will lead to generalized vasodilation in the body as a whole and thus to diminished blood flow; two, that even if an increase in cerebral blood flow were to be shown as a result of their use, this might, as in the heart (with dipyridamole), be at the expense of ischemic areas in the brain".

The major thrust in papaverine research has concerned its use for the treatment of stroke. Again, the evidence does not

give us a clear understanding, but does indicate the direction of investigation which must be taken; in clinical situations there must be an evaluation of both changes in rCBF (not total CBF) produced by papaverine (or any other agent) and a quantification of the course of patient recovery with appropriate controls and statistical evaluation (this requires accurate diagnosis into subcategories of stroke). Certain types of stroke appear more susceptible to successful papaverine treatment, namely so-called "major-vessel occlusion" as opposed to small-vessel disease with infarction (Meyer et al., 1965; McHenry et al., 1970).

In an experimental model situation, Betz and Schmahl (1966, as reviewed by Carpi, 1972) compared the effects of papaverine on the circulation of symmetrical cortical areas in the cat upon occlusion of one carotid artery. When the occlusion caused no permanent change in homolateral cortical blood flow, the papaverine-induced increase of blood flow was identical in the two areas. However, if the occlusion caused a persistent reduction of the homolateral cortical blood flow, the effect of papaverine was reduced proportionally on this side. The disappearance of the normal vasodilator response to papaverine in brain areas with insufficient arterial inflow and the persistence of this response in normal areas suggests that papaverine causes an "intracerebral steal", with a further reduction of blood flow in the damaged areas. This would be consistent with most of the clinical studies cited which have a high incidence of infarction.

Nevertheless, this should not be regarded as evidence against the usefulness of papaverine in cerebral vascular insufficiencies (Meyer et al., 1965). Papaverine may have a deleterious effect on rCBF in cases where the drug cannot reach the locus of damage (as in small vessel occlusion with infarction), but certain types of large vessel occlusion appear to be relieved. Because most of the studies of papaverine's effects on cerebral circulatory insufficiency did not consider all the parameters necessary to determine its role in regard to alleviation of stressed tissues (with the exception of Betz and Schmahl, 1961), no conclusion on the possible mechanisms responsible for changes in cerebrovascular reactivity to papaverine in various types of cerebrovascular disease would be justified. Further evaluation of papaverine under controlled experimental conditions is clearly warranted.

2. Methods of rCBV or rCBF measurement

Almost thirty years ago Kety and Schmidt (1948) demonstrated that the Fick principle could be used in the measurement of cerebral blood flow. In this technique, they derived cerebral blood flow (CBF) by intermittently measuring the blood concentrations of an inert, nonmetabolizable gas (nitrous oxide) in the carotid artery and the jugular bulb while the subject breathed the inert gas. The resultant CBF values were expressed in ml blood flow per 100 gram brain per minute. Further, when CBF was multiplied by the difference in concentration between arterial and venous blood of metabolites, such as glucose, O_2

and CO_2, their metabolic rates could be estimated. Since that time the literature on CBF measurements has continued to expand (see reviews by: Carr and Fisher, 1970; Brock et al., 1969; Russell, 1971). Following the pioneering work of Kety there evolved an important distinction between total cerebral blood flow measurements and the regional cerebral blood flow (rCBF) measurements that were more recently introduced and developed by Lassen and co-workers (see e.g., Ingvar and Lassen, 1973).

The methods of Lassen utilized isotope clearance and required injection into the internal carotid artery with extracranial placement of a matrix of photoelectric tubes for regional counting. Recent reviews indicate that there is a high degree of interest in applying more sophisticated approaches to this concept of rCBF (Ingvar and Lassen, 1973; Kanno and Uemura, 1975; Waltz, Wanek and Anderson, 1972). An important clinical and research development was made with the application of the more readily available scintillation camera (especially the gamma camera developed by Anger, 1958) rather than dependence upon the classic array of phototubes which have only recently become comercially available at a great cost and with restricted utility (Cannon et al., 1974; Heiss, Prosenz and Roszuczky, 1972).

Regional cerebral blood flow can be calculated from isotope clearance data in two ways: mean blood flow (F) through the region in the view of the counter equals the product of the height (H) from the tracer washout curve and the partition

coefficient between blood and brain tissue (λ), divided by the area under the curve (A). The equation ($F = H\lambda/A$), based on the Fick principle, is similar to the Kety-Schmidt equations (1948 a). The curve can be alternatively expressed as two exponential functions. This compartmental analysis may be done, as described by Hoedt-Rasmussen (1967), if the half-times for decay of a fast and slow component of the washout curve are divided by gray and white matter partition coefficients to yield flow in fast and slow compartments. The assumption was made that these flows represent gray and white matter flow respectively.

The multi-focal rCBF technique has several advantages; 1) it measures many regions of the brain and thus detects local pathological or physiological changes, 2) it measures at least two compartments (believed to be gray and white matter) 3) it can distinguish between flow and size of those two compartments, and 4) it can be repeated after a short time because the isotope is readily cleared from the brain. There are also several disadvantages, the major ones being that it requires an internal carotid artery puncture; and secondly, its regionality is defined geometrically in only two planes (the x and y). The depth (or z plane) of the region can only be estimated by the degree of isotope radiation attenuation from tissue within the volume of detection (if there were essentially no attenuation the volume of tissue would be shaped as a truncate cone passing through the entire skull).

Questions have been raised about the use of xenon-133, an

isotope which, because of its low energy, undergoes considerable absorption in the head before it reaches the detectors and undergoes considerable Compton scatter, causing the regional probes to show overlap. At its best energy emission levels somewhere between 13% and 20% of the counts are the result of Compton scatter. Also, the detector is affected more by counts near the surface than from the depths, and it is likely that during the course of the flow study there is diffusion first from gray to white matter, as the gray matter saturates first; later the opposite occurs with isotope diffusing from white to gray matter as the gray matter desaturates first.

In spite of such problems with the xenon-133 clearance technique it can supply useful and unique information on regional cerebral blood flow. For example, it was used by Risberg, Ancri and Ingvar (1969) to correlate between rCBF and rCBV. This correlation is of fundamental importance in the present research project. Regional blood flow and rCBV were measured simultaneously in the same cerebral region in anesthetized cats with controlled respiration. Both values came simultaneously from the same scintillation-detector using the freely diffusible indicator xenon-133 for flow determinations and the intravascular tracer RISA-iodine-131 for volume estimates. A very high correlation was found between volume and flow changes ($r = 0.96$, $p < 0.001$). This finding indicates that variations of rCBV are accompanied by proportional blood flow changes.

This regional volume increase is hypothesized to take place via a dilation of the cerebral vascular bed or through recruitment of unopened vessels which become patent as flow increases.

With the advent of high resolution fluorescence x-ray detection the use of nonradioactive tracers (Hoffer et al., 1969) allowed the next break-through which stimulated the present studies (details of this phenomena are described below under 'methods'). The essential unique features of this approach are, 1) avoidance of emission from the scalp and skull because of inherent geometric characteristics, and 2) elimination of the problems attendant to isotope administration. Although it was claimed (Moody et al., 1971) that the tracer could be given by inhalation or intraarterial injection, the quantitation into meaningful data from inhalation clearance measurements has only recently been reported and that was for the radioactive isotope xenon-133 (Obrist et al., 1975). The use of either transit time or clearance time made estimates of the flow parameter only, while application to volume measurements was hypothesized later by Ter-Pogossian and his associates (see: Hoffer, Beck and Gottschalk, 1971). Preliminary results were first reported by Ter-Pogossian et al. (1971/1972) for a technique similar to that used in our studies. The key features of the volume determination are to allow the equilibration of a nonradioactive tracer that is restricted to the vascular space (administered by a relatively non-traumatic intravenous injection) and

to measure its concentration in a three dimensionally defined volume. Calculation of blood volume is made from a concomitant measurement of tracer concentration in a sample of blood. During the course of our research an extensive study of this method was published in a series of reports from Ter-Pogossian's laboratory (Grubb, Phelps and Ter-Pogossian, 1973; Grubb et al., 1973; Phelps, Grubb and Ter-Pogossian, 1973 a and b).

Recently, Kuhl et al. (1975) reported on measurement of rCBV determined by three dimensionally reconstructing technetium-99m brain scan data. In this method, a transverse section scan is made after the venous injection of technetium-99m-labeled red blood cells. Absolute tracer concentration is then calculated and a point-to-point estimate is produced that is analgous to an autoradiograph. When the concentration of activity is determined, the data are converted to a two-dimensional map of rCBV.

Comparison of the results from various studies of rCBV are presented in Table 1, the slopes of lines calculated from rCBV and either pCO_2 or MAP are presented because these parameters are of critical importance in quantitation of the rCBV. As a crude estimate, the CBV may be considered to be about 5% of the tissue volume.

TABLE 1. RELATIONSHIP of pCO_2 or MAP to rCBV

Animal	pCO_2 Slope	MAP Slope	Reference
Goat	0.043		Smith et al., 1971
Rhesus monkey	0.049		Phelps, Grubb & Ter-Pogossian, 1973
Rhesus monkey		- 0.015	Grubb et al., 1973
Baboon	0.049	- 0.013	Kuhl et al., 1975

MATERIALS AND METHODS

A. Theory of X-ray activation for rCBV measurement

X-ray activation (or x-ray fluorescence) is a well established technique for the nondestructive qualitative and quantitative analysis of elements. There has been recent interest in the application of this technique for *in vivo* studies in the field of medicine; the value of different approaches is evaluated in the reviews of Tinney (1971) and Woldseth (1973).

The technique of x-ray activation may be appreciated using the analogy of more familiar 'chemical fluorescence' commonly used in catecholamine detection. In the case of chemical fluorescence, an ultraviolet wavelength photon interacts with molecular orbital electrons to produce a fluoresced photon of lower energy which is characteristic of the molecule from which it arises. In x-ray fluorescence, the incident photon is of x-ray wavelength and interacts with K or L shell electrons of atoms to produce an x-ray of characteristic energy (and therefore wavelength) depending on the atom that is struck. (To eliminate possible misunderstanding, the terms 'x-ray' and 'gamma-ray' do not distinguish wavelength or energy but only relate to the origin of the photon; x-rays originate from electron orbital transitions, as in x-ray tubes, while gamma-rays arise from activity in the atomic nucleus.) In elements with an atomic number greater than 46, the L and M to K

transitions are accompanied by K-shell x-rays greater than 80% of the time. An L to K transition would produce a more energetic x-ray, called K_β, while a M to K transition would produce a K_α. Since the K-shell x-rays are unique in energy for each element, this provides a means of quantitatively detecting the presence of a particular element.

The incident photon must possess an energy equal to or greater than the K-shell binding energy of the element to be detected in order to remove the K-shell electron. In our work, the in vivo activation of an iodinated radiographic contrast material, meglumine (60%) and sodium (30%) salts of 3,5-diacetamido-2,4,6-triiodobenzoic acid (Hypaque-M, 90%), is used as a tracer to measure the amount of blood per volume of cerebral tissue. In this case the exciting photon beam must have an energy greater than 33.2 keV (the K-shell binding energy of iodine). The observed fluorescent spectrum of iodine consists of K_α (28.5 keV) and K_β (32.4 keV) x-rays, as well as, any scatter which results from the excitation source.

B. Instrumentation

1. Source of activation x-rays

The excitation source may be considered in terms of two major features. First, as alluded to previously, it must emit photons with an energy equal to or greater than the binding energy of iodine (33.2 keV). The closer the energy of the exciting beam is to the binding energy, which must at least be exceeded, the larger the probability for a useful photoelectric interaction (activated x-ray

production). However, the incident photon must also have enough energy to penetrate bone and soft tissue to reach the region of interest. Tinney (1971), by considering the K-shell x-ray fluorescence intensity to tissue surface dose, has recommended for an iodine tracer and the probable organ depths to be encountered that the optimum excitation energy be between 40 and 60 keV. The second aspect to be considered is the intensity of photon flux which must be great enough to produce statistically significant data. There are a number of available excitation sources such as long-lived radioisotopes which produce peaks of single energy radiation, x-ray generators fitted with secondary radiators which produce characteristic energy peaks. In this work the first two types of sources were used.

A monoenergetic excitation source would theoretically be ideal if the energy could be chosen to give optimum excitation. There are only a small number of monoenergetic isotopes, and their energy range is limited. A long half-life source of large enough mass to achieve the high photon intensity needed for in vivo studies has limitations because of problems in self-absorption and geometry. We used a disc shaped one curie source of americium-241 (59.5 keV gamma-ray), supplied by Monsanto Nuclear, for initial studies but found the beam intensity insufficient to produce statistically significant count rates in order to follow dynamic vascular events.

Later an x-ray generator, the Kristalloflex 2H d.c. generator manufacturered by Siemens was used. It had a 60 kilovoltage peak (kVp) output. This powered a 1,300 watt, tungsten target tube that had a true focus size of 10 by 0.75 mm^2. Calculations of the

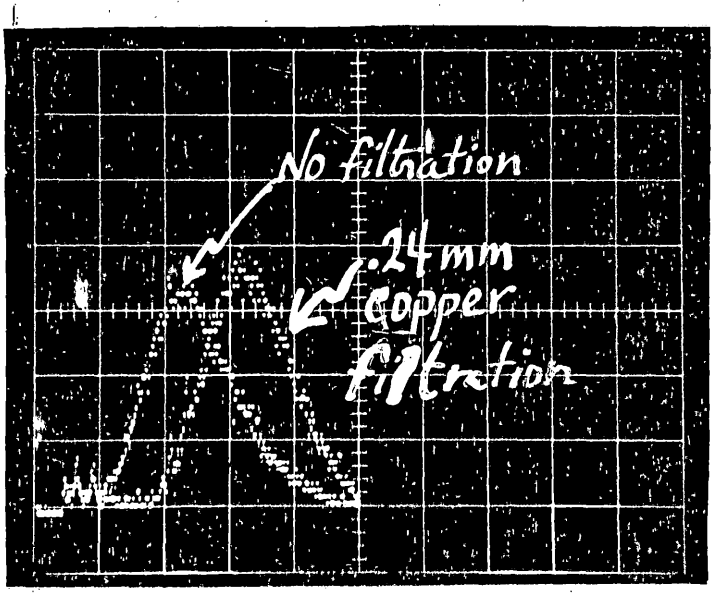
maximum allowable kVp-amperage combinations and data on the relationship between the tube current and photon output at 60 kVp are given in Table 3 (Results section). By selectively filtering the photon beam of the x-ray tube, the bremsstrahlung distribution can have its maximum energy shifted higher and above the binding energy for iodine activation. We used 0.48 mm of copper to filter the beam and effects on bremsstrahlung distribution are illustrated in Figure 1.

The x-ray generator offers a number of advantages over isotope sources: 1) high photon density from a small diameter source (it is estimated that even though the x-ray tube has a smaller source diameter, it produces about 10^4 times as many photons as the americium-241 source); 2) the energy output can be regulated by changing the potential on the x-ray tube or using different filters; 3) variable flux densities allow the use of higher flux densities in animals than would be safe in humans (however, the same system could be employed for human subjects by reducing the energy output); 4) problems of radioisotope storage are avoided.

2. Detection of fluorescent x-rays and data handling

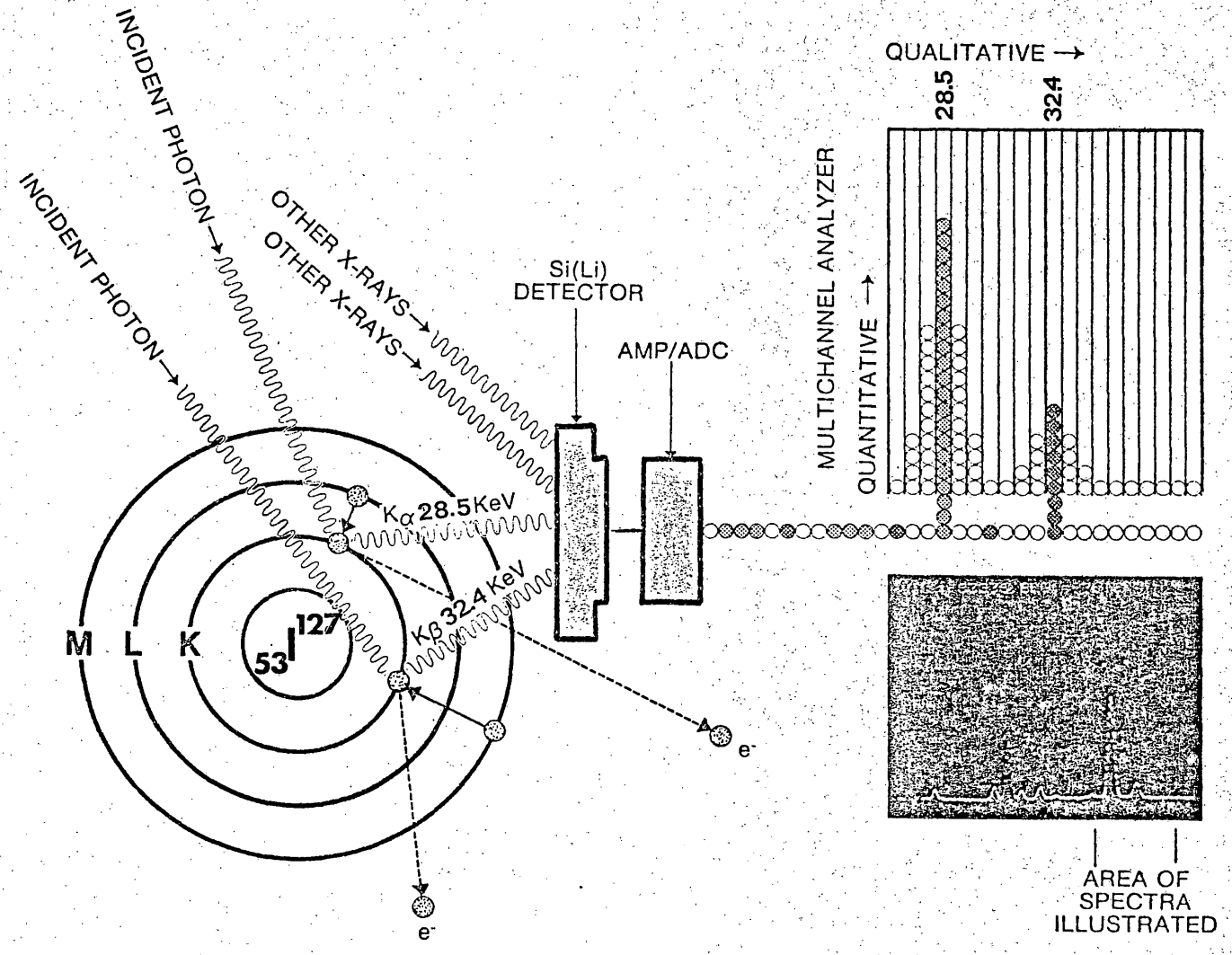
The detector used in these studies was a Nuclear Diodes lithium-drifted silicon (Si(Li)) semiconductor (or less accurately 'solid state') detector with a 200 mm^2 surface and accompanying FET preamplifier. By definition, a semiconductor is a very poor conductor of electrical charges. The use of a semiconductor as a radiation detector is based on its ability to absorb x-ray energy photons with effective ionization of the material, making it briefly conductive.

Figure 1 Spectrum of bremsstrahlung produced by scatter from plastic. Peak activity is shifted to higher energies when 0.24 mm copper filters out some lower energy photons.


No filtration
.24mm
copper
filtration

With a high voltage potential placed across the semiconductor crystal and negligible current transmission in the normal non-excited state, the absorption of ionizing photons allows the flow of pulses of current. Within limits, the degree of semiconductor ionization (or the amount of current flow) is directly proportional to the energy absorbed due to the incident radiation. This burst of charge after proper amplification forms the basis for quantitating photon energies. This amplified burst is then changed from analogue to digital values which are sorted by a multichannel analyzer (MCA) into a spectrum of pulse amplitudes which are proportional to the energy of the radiation being detected. This is schematically diagrammed in Figure 2, together with a representation of the origin of a fluoresced x-ray described in the previous section. If, as in our case, just one particular energy photon is required to be counted (the 28.5 keV, K_{α} x-ray from iodine), a device called a single channel analyzer is used to sort or filter out the pulses at some pre-set amplitude. The counting of a particular energy radiation takes place in the MCA after the detector has handled all photons that cross it. As illustrated in Figure 1, when the whole spectrum is counted, the greatest number of events arise from scatter; count rates up to 20,000 per second are not uncommon. Compatability among components and high performance characteristics must be obtained at all times so that meaningful counts are not lost. The original system employed contained an amplifier which was too slow to keep up with the count rates produced by an x-ray tube, the details are in Appendix A.

Figure 2 Schematic of the semiconductor detection and processing of a spectra from a source of fluoresced X-rays.


INCIDENT PHOTON
INCIDENT PHOTON
OTHER X-RAYS
OTHER X-RAYS
Si(Li)
DETECTOR
AMP/ADC
Kα 28.5KeV
Kβ 32.4 KeV
M
L
K
53 I 127
e⁻
e⁻
QUALITATIVE →
28.5
32.4
MULTICHANNEL ANALYZER
QUANTITATIVE →
AREA OF
SPECTRA
ILLUSTRATED

The resolution of our system for the 5.898 keV manganese K-shell x-ray is 584 eV full width at half maximum (FWHM). This high resolution of the Si(Li) detector, which is related to its ability to distinguish fluorescent x-ray from background (Woldseth, 1973) is necessary to quantitatively detect the low concentrations of iodine present as a tracer.

In order to define the area of interest geometrically, the detector and x-ray tube are fixed at right angles as diagrammed in Figure 3. The detector has a conical collimator which restricts the field of view to a 0.5 cm circle which widens along a 5 cm length to 1.5 cm at the detector window. A pencil beam of photons intersects this field of view after collimation by a cylinder 7 cm long and 0.55 cm in diameter. This cross-collimation gives a region of interest of about 0.1 cc.

Considering the small region of interest and relatively exact positioning necessary to define the volume of brain tissue intended for measurement, the x-ray tube and detector were mounted on a comercially available stereotaxic device. Specially machined interfaces were fabricated to connect the tube and detector to stock heavyduty manipulators obtained from the David Kopf Instrument Company. The stereotaxic frame and rabbit head holder were also stock items from Kopf (see Figure 4 for clarification).

The use of stereotaxic technique first described by Clarke and Horsley (1906) is the foundation for modern approaches in exploring the neurobiology of deep structures in the whole brain while it remains in situ. The principle relies on the localization of an intra-

Figure 3 Schematic of cross-collimation between the X-ray tube and semiconductor detector. Note also the placement of a monopolar EEG electrode and its ground in the ear. The EEG was recorded only in the rCBV experiments. Rendering by Lee Rose, Medical College of Georgia.

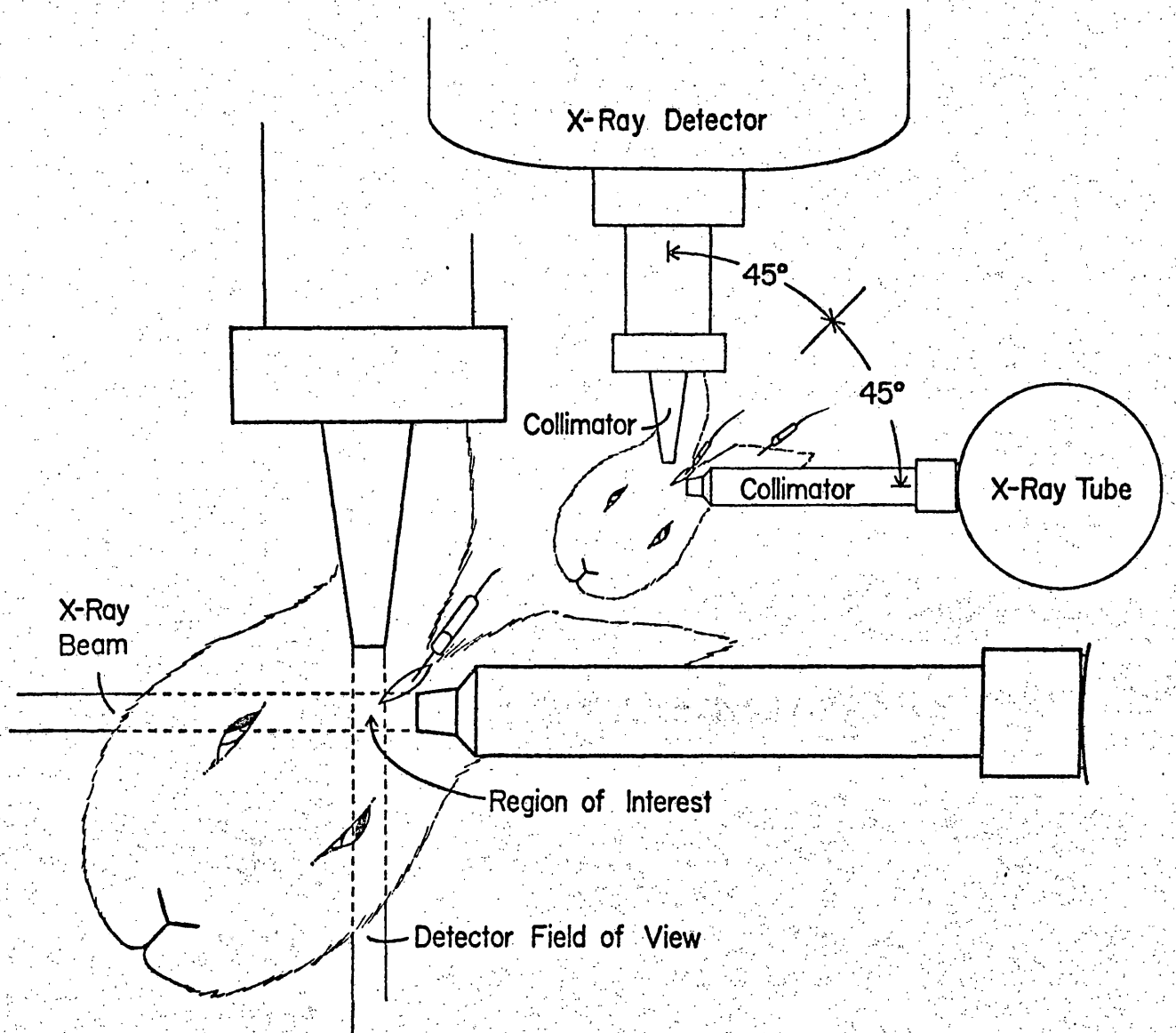
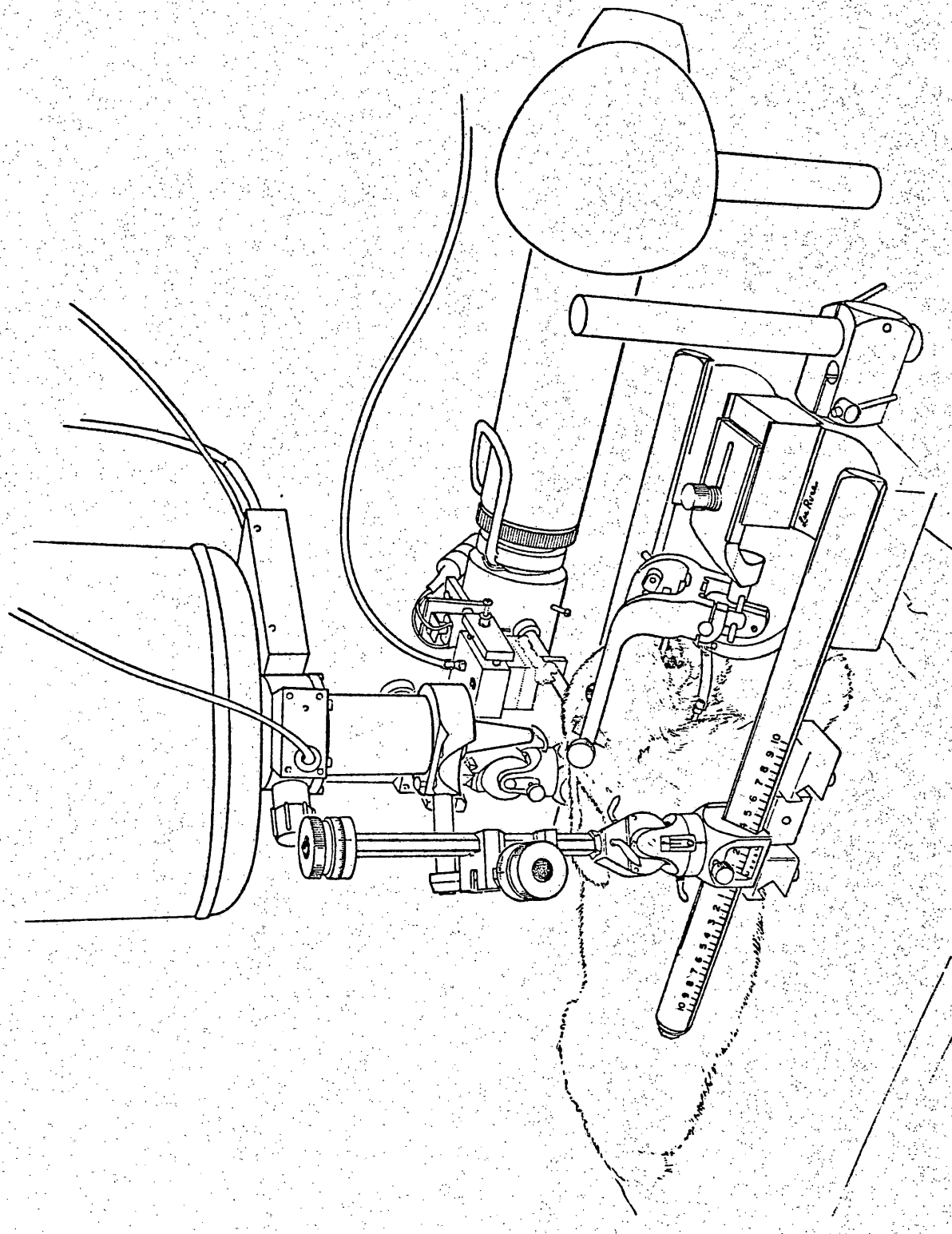

X-Ray Detector
45°
45°
Collimator
Collimator
X-Ray Tube
X-Ray
Beam
Region of Interest
Detector Field of View

Figure 4 Line rendering of the coupling between X-ray devices and the stereotaxic frame. The ellipsoid-shaped object on the right represents a Grass photic stimulator used for visual stimulation of the rabbit. The stereotaxic frame is a model #1630 (special, with 15 inch A-P bars and two sets of ear bar blocks) with two model #1760 manipulators that position the X-ray tube and detector. The rabbit head holder is a model #1630. Drawing rendered by Lee Rose, Medical Illustration Department, Medical College of Georgia.

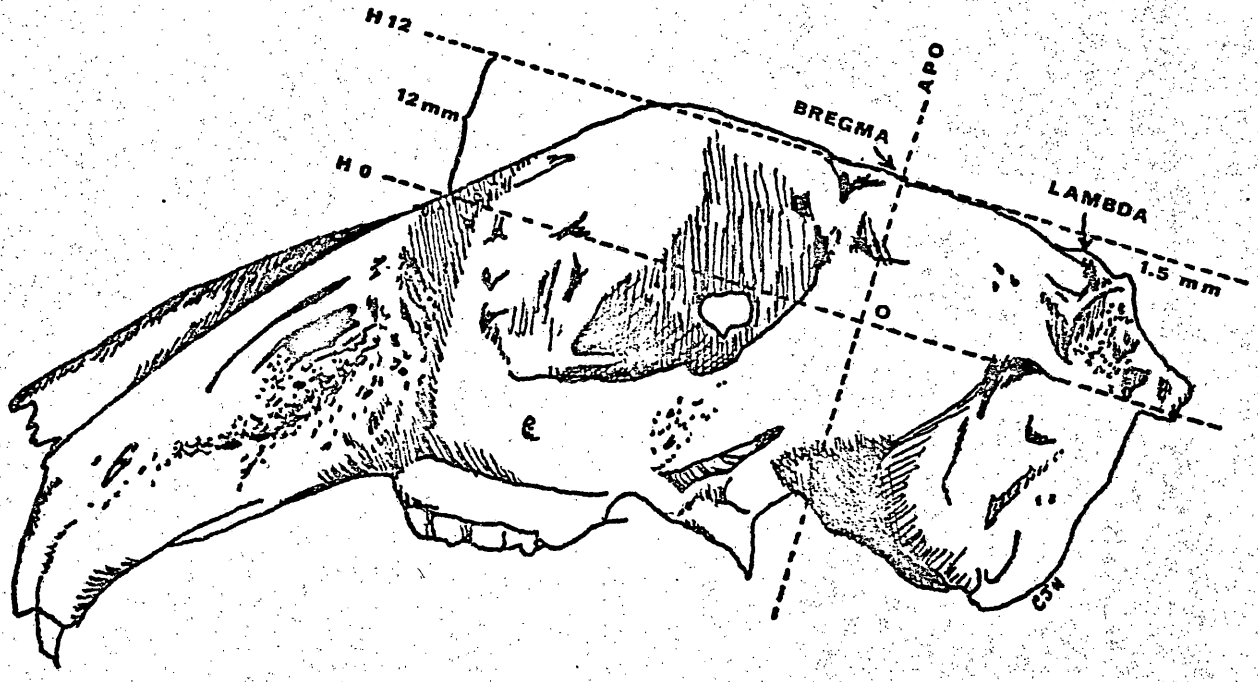


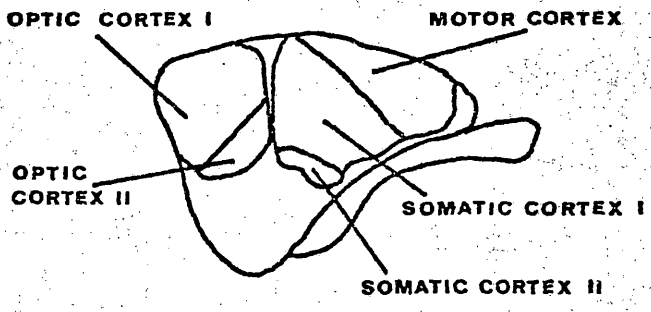
cranial region with reference to a three dimensional coordinate system determined by external landmarks on the skull. This system of three perpendicular planes is fixed by the positioning of the most readily identified plane, which is, in the case of the rabbit, the basal (see Figure 5) and is defined as being perpendicular to the sagittal plane (a plane running medially through the sagittal suture) and passing through the bregma and a point 1.5 mm above lambda (Bureš, Petrán and Zachar, 1967). Two references were used to ascertain the depth of cortex in different areas: one, a classical work including histology of serial sections from rabbit brain (Winkler and Potter, 1911); the other illustrated the rabbit brain in coordinates using the Horsley-Clarke system employed in this work (Sawyer, Everett and Green, 1954). Electrophysiological work of Thompson, Woosley and Talbot (1950) was consulted to determine anatomical areas of cortex that received visual input. A schematic of a summary from different researcher's maps of cortical regions in the rabbit is seen in Figure 6 (from Bureš, Petrán and Zachar, 1967). Estimates from these works and our own histology (to be discussed in Section E,1) yield values between 3 and 5 mm for the depth of cortex in the visual areas.

The datum acquired contain information on rCBV which is in the form of counts or pulses of x-ray photons originating from the activated tracer (Hypaque-M, which has 462 mg iodine per ml) and are summed for successive time periods to yield volume changes with respect to time. It is obligatory that the tracer remain in a defined vascular compartment; the iodine tracer used primarily

Figure 5 Rabbit skull and reference points for leveling. With the head leveled so the basal plane (H12) is passing through bregma and 1.5 mm above lambda, a perpendicular plane that passes through bregma will be the antero-posterior zero (AP0) plane.

Figure 6 Map of the projection areas of the cerebral cortex of the rabbit. Redrawn from Bureš, Pefráň and Zachar, 1967.


H12
12mm
H0
BREGMA
APO
LAMBDA
1.5 mm
O
C5N


OPTIC CORTEX I
MOTOR CORTEX
OPTIC
CORTEX II
SOMATIC CORTEX I
SOMATIC CORTEX II

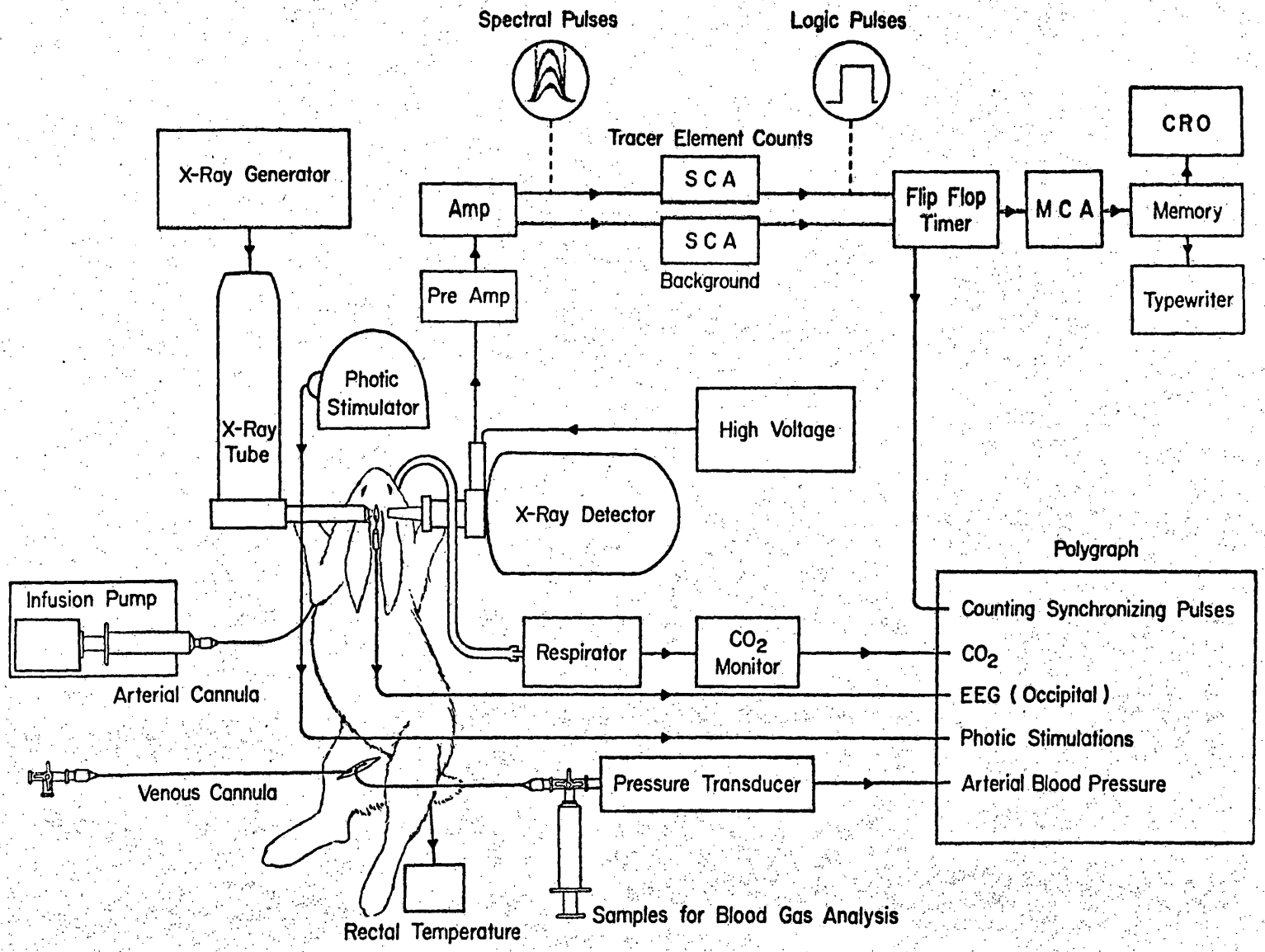
in these studies has been shown to be restricted to the plasma space in the brain when infused slowly (Studer and Potchen, 1971). Reference to Figure 7 will aid in following the steps necessary to process the raw pulse data from the detector to the final print-out of volume changes. First, the detector must have a high voltage bias of 400 volts (positive) before it will respond properly to x-rays. As already discussed above, the 'spectral' pulses (characteristic in amplitude for different energy photons) undergo amplification by both a preamplifier and an amplifier. The amplifier is set at a gain of 30 and a time constant of one microsecond (see Appendix A for amplifier characteristics). The amplified spectral pulses then proceed to the single channel analyzers (SCA) which respond to spectral pulses of the amplitude to which they are calibrated (see Appendix B for calibration with the iodine K_{α} x-ray). Each time one of the SCA receives a spectral pulse in the range for which it was set, it responds with a logic pulse (5V, 0.5 sec., 10 ohms) with characteristics that are compatible with the electronics of the multichannel analyzer (MCA). The MCA accumulates counts for periods determined by the timer (usually 24 sec) and temporarily stores them in a memory. At this same time a second SCA, set to receive counts in the energy range between the K_{α} and K_{β} x-ray from iodine, receives counts that are processed in the same way and are stored separately as background for time units identical to the tracer counts. From this memory (ferrite core, 1250 byte) a display may be photographed from an oscilloscope (analogue output) and/or printed out by a typewriter

Figure 7 Components of the X-ray activation system for blood volume determinations.

detector: Nuclear Diodes, Model X5-200-650-BCF
high voltage power supply: Nuclear Diodes Model #402-1
preamplifier: Nuclear Diodes, Model #107B
single channel analyzer (SCA): Tennelec, Model TC 441
single channel analyzer (SCA): Ortec Model #451
flip flop timer: a) Ortec timer model #719
b) Solid state flip flop designed and constructed by Biomedical Engineering Dept. of the Medical College of Ga.
multichannel analyzer (MCA): Nuclear Data,
memory: Nuclear Data, 1024
oscilloscope (CRO): Tektronix, type R564 B, storage

X-ray generator: Siemens, Kristalloflex 2H
X-ray tube: Siemens, type AG W30

photic stimulator: Grass, model #PS22
infusion pump: Sage, model #355
respirator: Harvard, model #607
pressure transducer: Statham, model P23AC
temperature unit: Yellow Springs Instrument Co., model #47
CO_2 monitor: Beckman, model LB1
polygraph: Grass, model #7


Spectral Pulses
Logic Pulses
Tracer Element Counts
X-Ray Generator
Amp
SCA
SCA
Background
Flip Flop Timer
MCA
CRO
Memory
Typewriter
Pre Amp
Photic Stimulator
X-Ray Tube
High Voltage
X-Ray Detector
Polygraph
Infusion Pump
Arterial Cannula
Respirator
CO_2 Monitor
Counting Synchronizing Pulses
CO_2
EEG (Occipital)
Photic Stimulations
Venous Cannula
Pressure Transducer
Arterial Blood Pressure
Rectal Temperature
Samples for Blood Gas Analysis

(digital output). The printed hard-copy is hand carried and entered into a programable calculator for processing (see Appendix C for calculator program) into data representative of consecutive blood volume changes. The data is also stored on magnetic tape by the calculator for possible later reevaluation.

The net peak count is the parameter that carries the blood volume information. This value is obtained from two measurements, the total count (N_T), and the background (N_B). Both observations carry a statistical uncertainty. The net count (N_O) is obtained by subtracting N_B from N_T. The deviation of the net count may then be obtained from the theory of combination of independent errors:

$$\sigma_{(N_O)} = \sqrt{\sigma_T^2 + \sigma_B^2} \qquad \text{Eq. 1}$$

and the relative percent error (% E):

$$\%E = \frac{100}{\sqrt{N_T}} \cdot \frac{\sqrt{B\ (B + 1)}}{(B - 1)} \qquad \text{Eq. 2}$$

where $B = N_T/N_B$, or the total peak to background ratio. Equation 2 yields the error at the one standard deviation level (or 67% confidence limit) while in most of the evaluations of BV the two standard deviation level (95% confidence limit) was the criteria for a 'significant' change. Due to the ability of the semiconductor system to detect a range of the spectrum simultaneously, the background was taken from a region of the spectrum adjacent to the iodine peak. Since N_T and N_B are obtained during the same time

(t) the relation may be expressed in terms of intensity: $I_T = N_T/t$ and $I_B = N_B/t$. A determination of the information rates (I_T and I_B) permits an evaluation of the peak to background ratio (B) and from the requirement for a certain precision, an estimate may be made of the required analysis time (for a more detailed account of counting statistic considerations, see Woldseth, 1973).

An example of the calculator output generated from a standard iodine solution contained in a rabbit skull phanton is found in Figure 8. The values listed under 'significance' represent the number of standard deviation units found between adjacent counts, during a 24 second acquisition time (24 sec was typically used in all experiments).

C. The experimental animal

At various stages, these studies employed the cat, dog or rabbit, but generally preparation was similar, with pentobarbital anesthesia and gallamine (Davis and Geck Company) for immobilization. Rabbits ranged in weight from 3.0 to 4.2 kg and received 30 mg/kg pentobarbital via the ear vein, 0.5 mg/kg gallamine and a single dose of 2,000 IU of heparin (Upjohn Co.,) after surgery. Cats ranged from 3.2 to 4.3 kg and received 25 mg/kg pentobarbital and 5 mg/kg gallamine. Dogs were anesthetized with 30 mg/kg pentobarbital and immobilized with 0.4 mg/kg gallamine. Any surgical wounds were infiltrated with lidocaine (2% Astra Laboratories) or topical benzocaine (20% Arnar-Stone Laboratories, Inc.) was applied. Demetrescu and Julian, 1974 found that benzocaine does not interfere with cortical activity. Until the time of their use, animals

Figure 8 Data printout from a rabbit skull phantom containing 0.06% iodine. Note that the greatest value is 0.6 standard deviation units which reflects the contribution of random errors. Random errors may accrue from fundamental random effects in the instrumentation, generally denoted as noise.

TOTAL COUNTS

5920	5920	6006	5863	5934	5976	5744	5949	6089	5844
5732	5829	5934	5863	5690	5894	5804	5800	5828	5806
5754	5812	5790	5927	5829	5865	5760	5804	5820	5831
5749	5753	5799	5693	5707	5710	5813	5812	5677	5770
5694	5558	5643	5698	5661	5633	5564	5761	5543	5553
5520	5469	5423	5426	5599	5488	5578	5600	5619	5453
5623	5590	5551	5371	5578	5574	5471	5554	5460	5614
5554	5346	5477	5536	5403	0	0	0	0	0

BACKGROUND COUNTS

4543	4543	4300	4314	4426	4422	4266	4436	4446	4339
4269	4276	4212	4417	4378	4372	4273	4267	4347	4304
4296	4232	4209	4190	4241	4328	4349	4400	4435	4221
4246	4425	4327	4394	4225	4200	4254	4140	4329	4181
4364	4258	4333	4151	4145	4178	4174	4285	4246	4288
4121	4194	4077	4146	4286	4265	4258	4307	4305	4259
4097	4104	4266	4183	4156	4124	4228	4152	4245	4308
4089	4282	4280	4010	4213	0	0	0	0	0

SIGNIFICANCE

0.0	0.2	-0.4	0.2	0.1	0.6	0.3	0.3	-0.6	-0.3
0.2	0.3	-0.2	-0.4	0.5	-0.2	-0.0	0.1	-0.1	-0.1
0.1	-0.1	0.4	-0.3	-0.1	-0.1	0.1	0.0	0.0	-0.2
0.0	0.1	-0.3	0.0	0.0	0.3	-0.0	-0.4	0.2	-0.2
-0.3	0.2	0.1	-0.1	-0.1	-0.2	0.5	-0.6	0.0	-0.1
-0.1	-0.1	0.0	0.4	-0.3	0.2	0.1	0.0	-0.4	0.4
-0.1	-0.1	-0.4	0.5	-0.0	-0.3	0.2	-0.2	0.3	-0.1
-0.6	0.3	0.1	-0.4	-12.1	0.0	0.0	0.0	0.0$$$$$$	

were housed in the Medical College of Georgia vivarium facilities and fed standard laboratory chow and water ad libitum.

Special mention should be made regarding the rabbit, which was used in the majority of these studies. Longo (1962), in his extensive volume that relates drugs effects to changes in the EEG of rabbits, has reported on a number of quaternary ammonium compounds. A desynchronization analogous to that induced by acetylcholine can be elicited by administering, via the carotid, a number of these substances: tetramethylammonium hydrate (1-2 μg), decamethonium (2-5 μg), d-tubocurarine (2-5 μg) and succinylcholine (3-7 μg); however, it was found that gallamine produced no changes in the EEG pattern (Longo, 1955) which implies a lack of CNS effect. For this reason, gallamine was chosen for immobilization in the present studies.

Other animal preparation unique to a given experiment will be elaborated in the appropriate sections which follow.

D. Experimental classifications and protocols

1. Clearance studies of tracer

The rate of iodinated contrast material (Hypaque-M, 90%) clearance from the vascular system was evaluated in dogs by X-ray fluorescence using the one Ci amerecium-241 source for activation. The collimated gamma-ray source was positioned with the detector on the attached gingiva adjacent to the mandibular canine teeth. Femoral arterial and venous cannulas were inserted for blood pressure and tracer administration or blood sampling respectively.

2. Blood volume in cerebral cortex

A summary of the experimental apparatus may be seen in Figure 7. The X-ray activation and detection aspect of this system has been described previously (Section B,2). The physiological parameters monitored include arterial blood pressure from a femoral artery, rectal temperature and expired per cent CO_2. The animal was also prepared by administering artificial ventilation (25 ml tidal volume and 21 breaths per minute) through a tracheal tube, and infusion cannula in both the right carotid artery and the femoral vein. The carotid artery cannula was inserted in the manner described by Capon (1960) to allow perfusion of the ipsilateral vertebral artery. Lastly, a monopolar EEG electrode was placed in a hole drilled in the exposed calvarium over the occipital cortex using the positioning outlined by Longo (1962).

Because of the narrow range of weights, all rabbits received a uniform 10 ml dose of Hypaque-M, 90% (Winthrop Laboratories) tracer, infused at 1 ml/min, into the femoral vein. Other drugs administered include epinephrine (Parke-Davis), papaverine-HCl (Sigma Chemical Co.) and propranolol (Ayerst) which were made up to the appropriate concentration in 0.9% saline (the epinephrine also contained 10^{-4} M ascorbic acid as an antioxidant).

The experimental protocol is summarized in the following eight steps:

1. Infuse Hypaque; IV, 1 ml/min, 10 ml total volume; wait 20-30 min; start BV recording
2. Photic test; 6 HZ for 240 sec
3. Infuse Epi; IA, 2 µg/ml at 0.5 ml/min (0.5 µg/min) for a total of 6 ml
4. Photic test; 6 HZ for 240 sec
5. Infuse papaverine-HCl; IA, 5 mg/ml at 0.5 ml/min (2.5 mg/min) for a total of 2 ml
6. Photic test; 6 HZ for 240 sec
7. Infuse Epi; IA, 2 µg/ml at 0.5 ml/min (0.5 µg/min) for a total of 6 ml
8. Photic test; 6 HZ for 240 sec

The photic stimulation consisted of illumination of the anterior aspect of the head of the rabbit with a Grass photo stimulator (model #PS 22) at maximum intensity.

3. Blood volume in the hindlimb

Blood volume changes were evaluated in the left hindlimb of rabbits using a radioisotope labeled human serum albumen (HSA) tracer and the same detector system described previously (Section B, 2) for the fluorescent x-ray technique. This alternate method was employed because the activated tracer (Hypaque) escaped into the interstitial fluid while the HSA-technectium-99m (HSA-TC-99m) remained in the vascular space (see Appendix D for more details on this tracer). The size of the detector collimator was increased to 3 cm internal diameter in order to record changes in a larger volume of tissue. The SCA's were set to accept the 140 keV photon from TC-99m. Blood flow was measured simultaneously with BV by dissecting out the femoral artery and positioning an appropriately sized electromagnetic flow probe around it (equipment from Carolina Medical Electronics, Inc., Model 501). The response of the vascular bed was determined after occluding the artery to produce

a state of hypoxia. Occlusion was produced by tension on a loop of 4-0 silk suture placed around the artery. This occlusion supplied an effective functional load to the limb as evidenced by reactive hyperemia upon the return of flow.

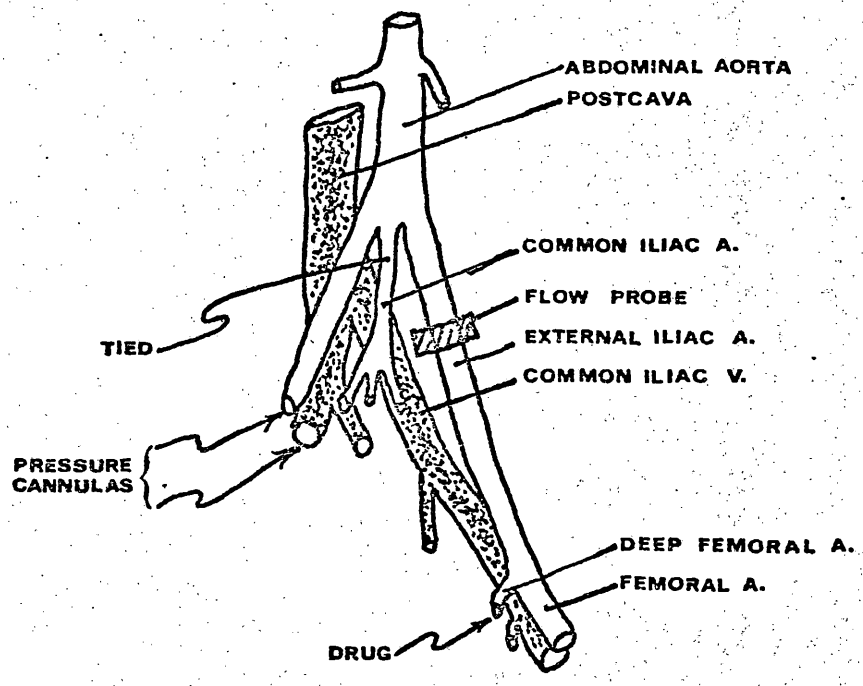
4. Blood flow in the hindlimb

Blood flow studies were conducted using the hindlimb of immobilized (gallamine, 5 mg/kg) cats ranging in weight from 3.2 to 4.1 kg. Various concentrations (diluted in normal saline) of the vasoactive compounds acetylcholine-Cl (Ach) and dopamine-HCl (DA) were given by close arterial injection into the deep femoral artery. Figure 9 illustrates the positioning of the infusion cannula as well as the pressure and flow transducers. The effect of these drugs alone on the flow rate were determined as controls, followed by measuring flow changes produced after a single IV injection of different doses of pentylenetetrazole (PTZ), or physostigmine. The PTZ (analeptic agent) was used in an attempt to produce a pharmacological functional load.

E. Evaluation of cerebral perfusion studies

1. Histology

Histology of India ink injected vasculature was performed on rabbit brains to evaluate the extent of possible disruption of the microvasculature exposed to osmotic (from the Hypaque) and radiation (X-ray tube) stresses (see Rapoport, Hori and Katzo, 1972). At the termination of several experiments, an injection of India ink (about 1 ml) was given IV and a period


ABDOMINAL AORTA
POSTCAVA
COMMON ILIAC A.
FLOW PROBE
EXTERNAL ILIAC A.
COMMON ILIAC V.
TIED
PRESSURE CANNULAS
DEEP FEMORAL A.
FEMORAL A.
DRUG
1 cm

of 20 minutes allowed to elapse before sacrifice. Brains were removed as rapidly as possible (under 4 minutes) and placed in 150 ml of 10% buffered formalin (1% $CaCl_2$ buffer). This solution was changed daily for 5 days after which the specimen was left undisturbed for at least one month before tissue sectioning. Tissue was dehydrated in ehtanol, cleared in xylene and embedded in parafin (Paraplast) using a standard technique (see Appendix F for details). Sections 20 μ thick were cut on a Porter-Blum microtome. Cut sections were placed on a pool of albumin on microscope slides, and then warmed on a hot plate (about 45°C) for 5 minutes. The excess albumin was drained off and the slides upended to dry. The slides were placed in an incubator (50°C) overnight before staining with Hematoxylin-Eosin as outlined in Appendix E.

2. Arteriography

Two arteriography studies were performed on rabbits in which the carotid artery cannula, intended to perfuse through the vertebral arteries, was injected with 5 ml of Renographin-60 to ascertain the validity of this route for perfusion of the brain stem. One right-lateral (R-Lat) and one anterior-posterior (A-P) view study was performed using a Philips-Muller Super 100 3 phase x-ray unit equipped with a Elema-Schönander high speed film changer. The film changer was adjusted to deliver 2 films per sec for 3 sec, then 1 film per sec for 8 sec. The x-ray tube (1.2 mm focal spot) delivered 22 mAs (40 kVp, 1/40 sec) for the R-Lat study and 25 mAs (45 kVp, 1/40 sec)

for the A-P study. X-ray film was exposed using a grid and developed by a Kodak automatic processor. By changing the subject to film distance (focal-film distance, 42"; focal-object distance, 30") it was possible to magnify the image (in this case, 1.4 times).

RESULTS

A. Tracer Pharmacology

In order to evaluate the characteristics of the X-ray activation equipment a number of experiments were conducted to determine stability, reproducibility and resolution of the system.

Illustrated in Figure 10 is a series of 24-second interval count rates obtained during a 6.8 minute span from a 0.3% iodine standard placed in a rabbit skull phantom. From this data, a mean count rate of 12,204 with a standard deviation of $\pm$ 174 was calculated. A count rate greater than 12,552 or less than 11,856 ($\pm$ 348 or $\pm$ 2 standard deviations) would be expected, purely by chance, less than 5 times out of 100 trials. This measure of the repeatability of the count rate was used to determine the predictability of a change in the concentration of iodine which would reflect a blood volume change.

A linear relationship between tracer concentration (expressed as per cent iodine) and measured fluoresced X-ray count rate is represented in Figure 11. The values in this graph fall away from the linear regression line when above 0.2% iodine, but this concentration is never reached in the blood stream. The correlation coefficient calculated for *in vivo* concentrations of iodine was found to be close to perfect (+ 0.99). In the linear regression formula used in Figure 11 the X value is the iodine concentration and the Y

Figure 10 Graph of count rate obtained by irradiating a 0.3% iodine solution in a rabbit skull phantom. Vertical bar indicates a plus and minus one standard deviation and the horizontal line is the mean.

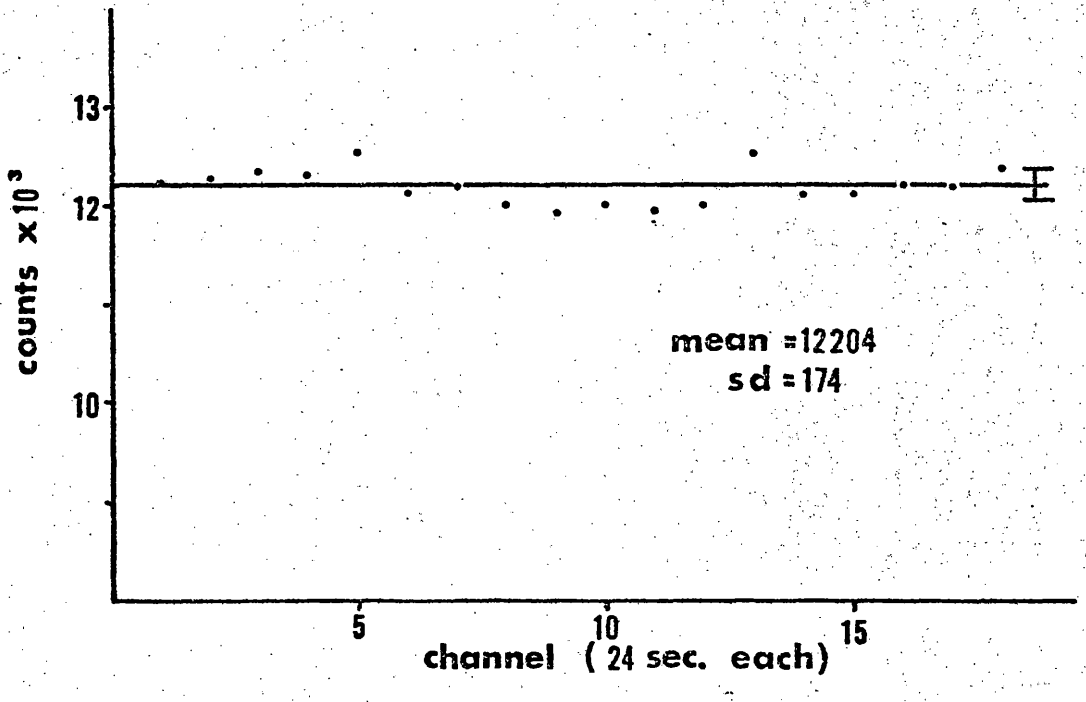
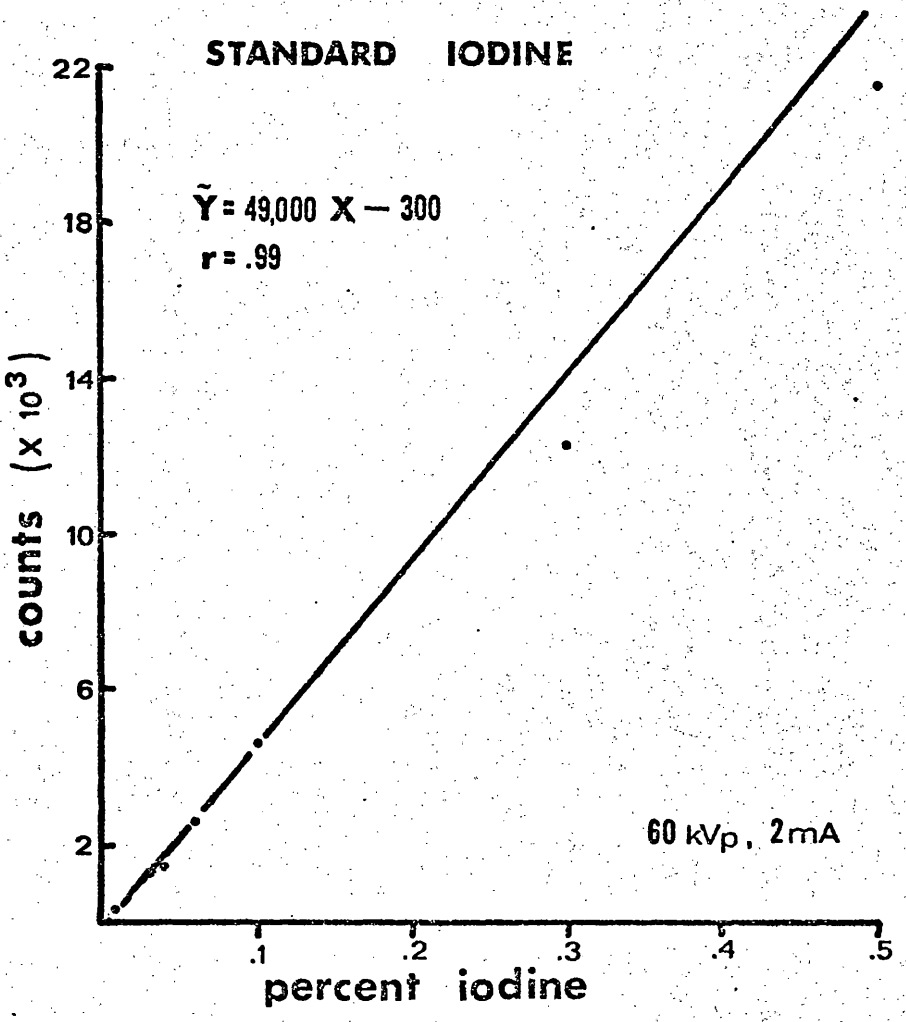

13
12
10
counts x10³
mean =12204
sd =174
5
10
15
channel (24 sec. each)

Figure 11 Graph of different concentrations of iodine versus the count rate produced. Each value is the mean of at least 18 repetitions of a 24 second count interval. Counts were obtained from iodine solutions placed in a rabbit skull phantom to reproduce the geometry found in the experimental situation.


STANDARD IODINE
$\tilde{Y}$ = 49,000 X − 300
r = .99
22
18
14
10
6
2
counts (X 10^3)
.1
.2
.3
.4
.5
percent iodine
60 kVp, 2mA

value is the count rate. Since this linear relationship exists at normal blood concentrations of iodine it is also possible to determine the minimum detectable blood volume change (expressed as a change in the diameter of a cylinder which represents a blood vessel).

Table 2 contains the mean count values obtained for a series of standard iodine concentrations on two different days. All comparable means fall within a two standard deviation range of each other at the different measuring times. As shown in Table 2, for count rates obtained from iodine concentrations less than 0.06% (experimental range) it may be calculated that there is an increase of about 44 counts per additional 0.001% of iodine. Further, using a maximum computed standard deviation of 70 (see Table 2) it may be stated that with all other conditions equal, when consecutive measurements are made, a given count rate of $\pm$ 140 with respect to the previous count rate reliably indicates an increase or decrease in iodine concentration 95% of the time (two standard deviations). Therefore, a change of $\pm$ 0.003% in iodine concentration is detectable at the 95% confidence limit (140 divided by the 44 counts per 0.001%). This detectable change of 0.006% ($\pm$ 0.003%) may be alternatively considered as a change of 6 parts in 100,000. Considering that the volume of a cylinder changes with the square of its radius ($V = \pi r^2 l$), a change of 0.006% in volume would be reflected as a change of 0.11% in radius (if the length is uniform). In other words, if the vessels expand 0.11% in radius, they will increase the amount of iodine in the field of the detector by a statistically significant amount.

TABLE 2. STANDARD IODINE SOLUTIONS MEASURED ON TWO DIFFERENT DAYS

Parameter	Per cent Iodine					
	0.50	0.30	0.10	0.06	0.04	0.01
Day one						
mean	21646	12204	4576	2524	1624	375
SD	328	174	86	69	66	46
Day two						
mean	19857	11116	4515	2052	1445	384
SD	252	216	140	70	61	43

The measure of uniformity within samples (the standard deviation) obtained on a given day is supplemented by comparison with samples obtained on a different day to determine to what degree they may be described as coming from the same population distribution. The t-ratio for non-independent means was used to compare the results obtained on different days and again the null hypothesis is rejected ($P < .01$), therefore, measurements made on different days of the same iodine solutions are comparable because they are members of the same population distribution.

In Table 3 is shown the mean count rates obtained from an iodine solution when the X-ray tube was operated at 60 kVp with variations in mA from 1 to 15. The tube is rated for a maximum power output of 1300 watts (kVp x mA = watts); therefore, 15 mA at 60 kVp is a safe level to continuously operate the generator and tube. The flux density produced (measured as the count rate) increases linearly as the current of the tube is increased at a set voltage level. It was decided to do most of the blood volume studies between 5 and 10 mA at either 50 or 60 kVp, thus producing about 1 Rad per hour of surface dose radiation as measured by a ratemeter (Victoreen model).

Figure 12 illustrates the spatial resolution of the detector and its collimator when a 'point' source (1.7 mm diameter) of iodine is activated. The three peaks in Figure 12 quantitate the field of view for the detector at increasing distances (0.0, 0.5 and 1.0 cm) from the collimator. As would be expected the field of view widens as distance from the collimator increases, but this would be limited to the diameter of the activating X-ray beam.

The initial pharmacological studies carried out in this research project involved the effects of the iodinated contrast material (Hypaque-M, 90%) on the experimental animal. Using mongrel dogs, the clearance of Hypaque-M, 90 was determined from about 0.1 ml volume of soft tissue consisting of mandibular gingiva adjacent to the canine teeth. Accumulation of counts, indicating the concentration of tracer in the tissue, during 100 second intervals is shown in Figure 13. Also shown is a regression line which was

TABLE 3. EFFECT OF CHANGING GENERATOR mA ON FLUORESCED X-RAY OUTPUT USING A STANDARD IODINE TARGET

mA at 60 kVp	Output (counts/10 sec.) Mean	SD
1	1048	42.2
2	1581	38.9
3	2132	52.3
4	2379	78.1
5	2538	43.6
6	2635	61.9
7	2972	52.7
8	3376	63.7
9	3727	58.0
10	4163	44.8
11	4506	117
12	5042	127
13	5221	50.6
14	5651	76.0
15	5998	111

linear regression: $Y = 338\ x + 820$, $r = .99$
Y is mA values and X is output in counts per second

Figure 12 Spatial resolution of X-ray tube and detector system. A 'point' source of iodine (1.7 mm diameter) was moved across the field of view of the detector at intervals indicated on the abscissa with count rates recorded on the ordinate. The distances the point source was from the tip of the collimator are shown above each curve.

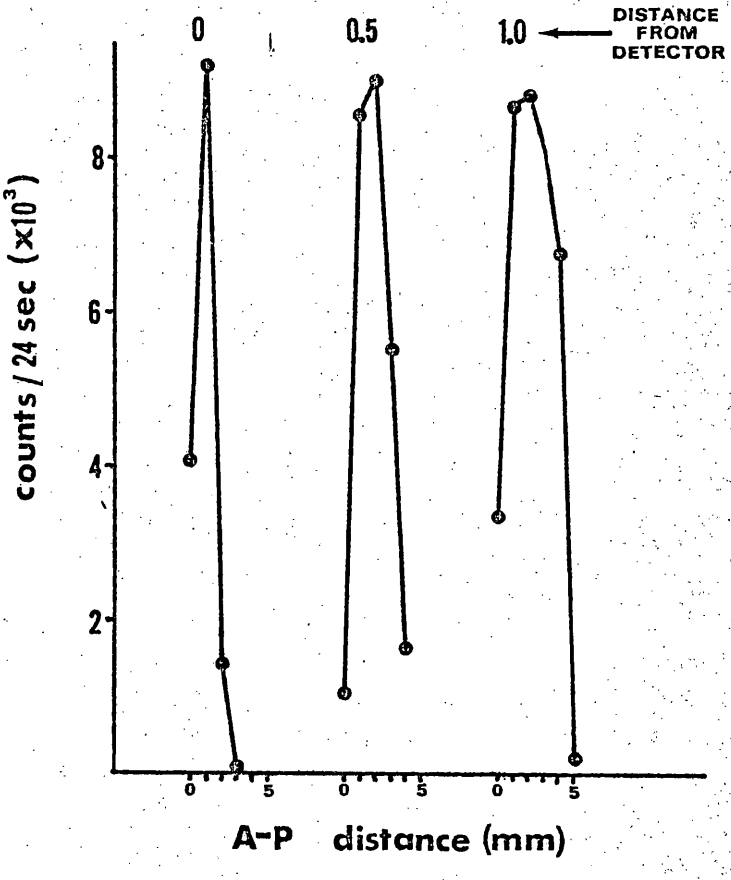
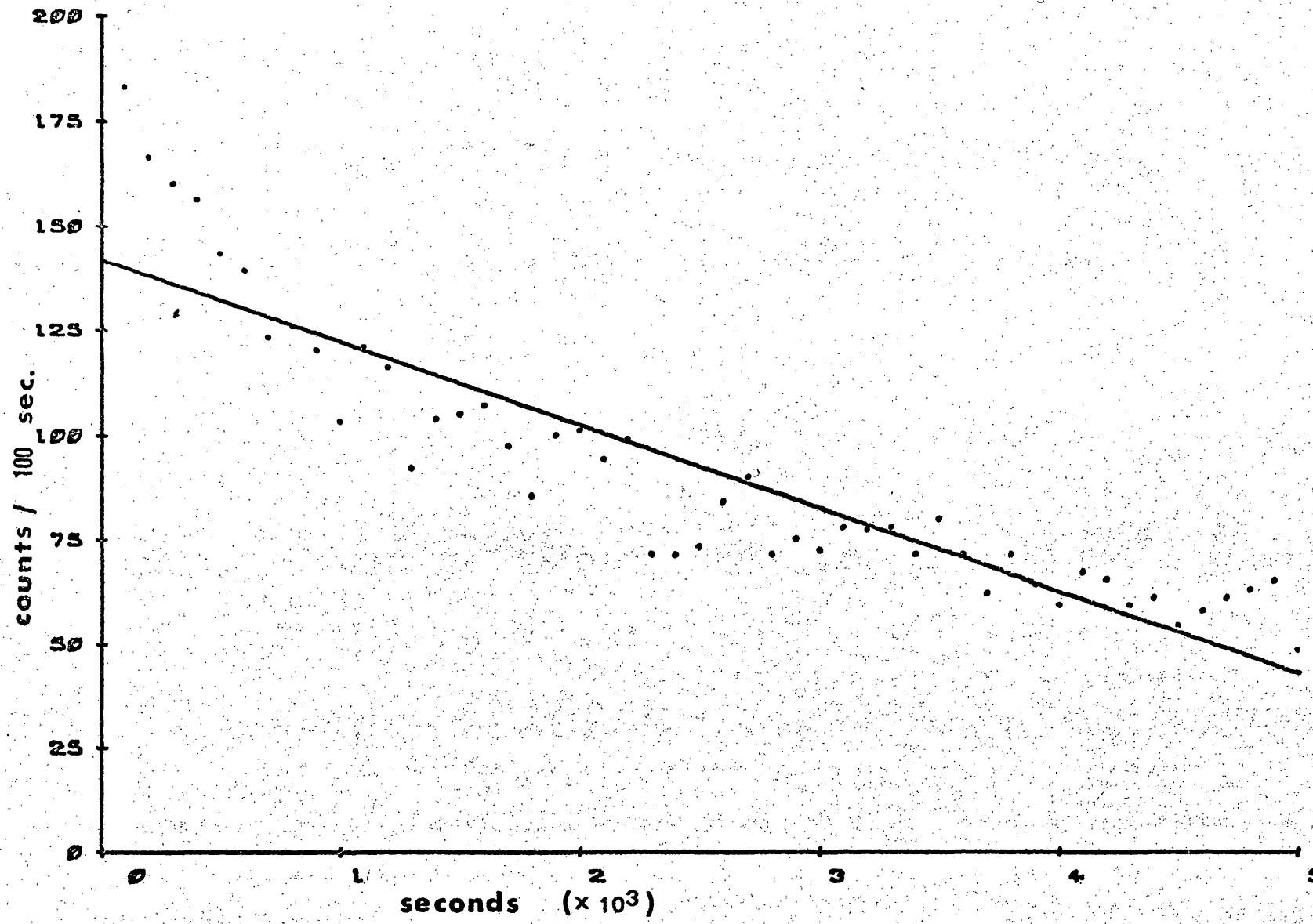
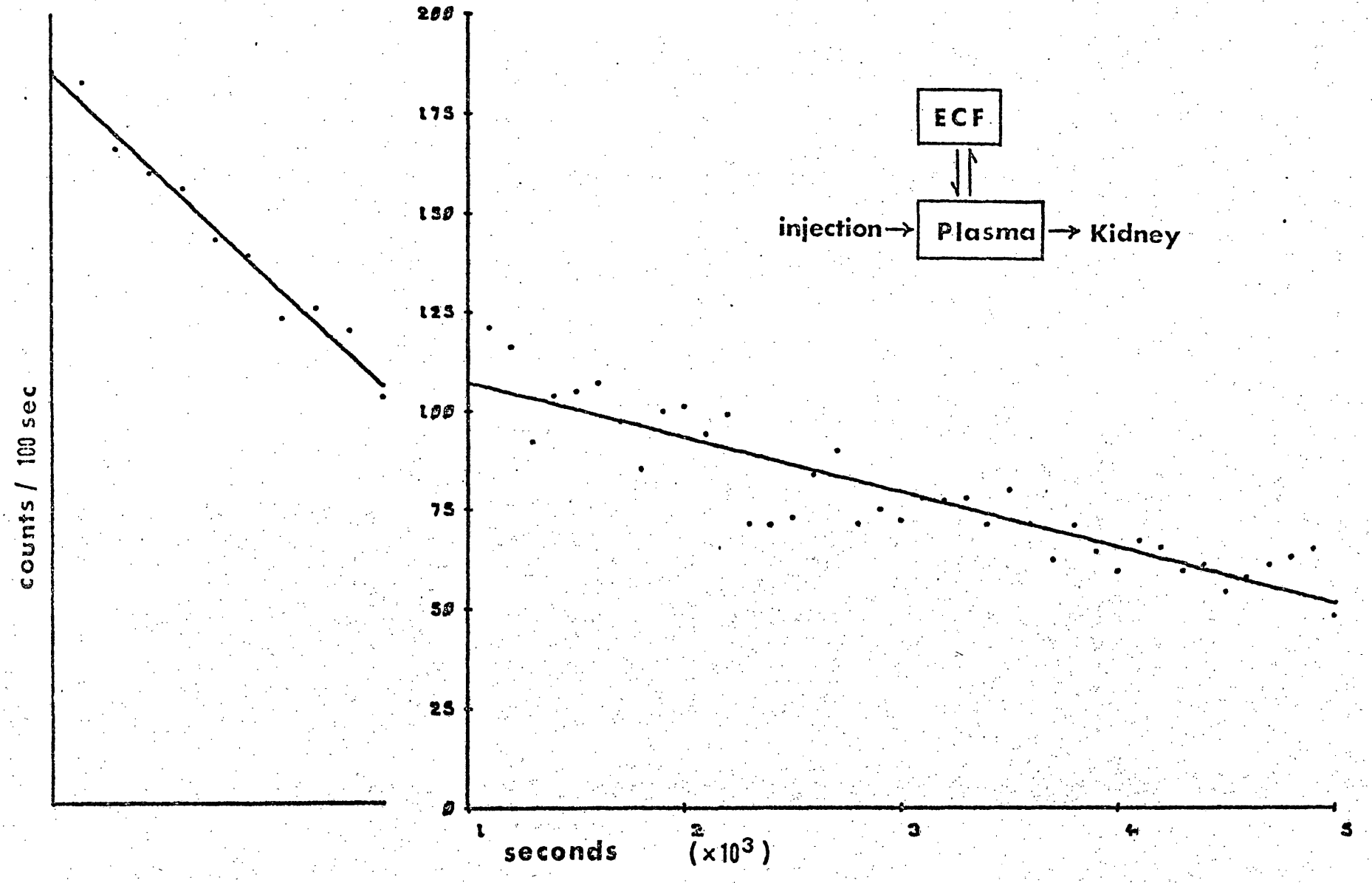

0
0.5
1.0
DISTANCE FROM DETECTOR
counts / 24 sec (x10³)
8
6
4
2
0
5
0
5
0
5
A-P distance (mm)

Figure 13 Scatter diagram of the concentration of iodine in a volume of gingival tissue in the dog with respect to time. Line is the least square best fit for all the values.


200
175
150
125
100
75
50
25
0
counts / 100 sec.
0
1
2
3
4
5
seconds (x 10^3)

drawn using a Wang 600 calculator with an appropriate commercially prepared program. It is evident that the early phase of the clearance curve is uniformly steeper than indicated by the regression line. Thus assuming a two compartment clearance, a linear regression line was generated for an early (first 1,000 seconds or 16.6 minutes) and a late phase of the clearance as shown in Figure 14. Although the distinction between early and late phases is based upon the 'viewers' judgement, the commonly used technique of "data stripping" as described by Soloman (1949) employs similar judgements. Grubb et al. (1974) have reported that cerebral autoregulation is lost for about 15 minutes after the IV injection of iodinated contrast agents; this is the same period of time shown in our data for the fast clearance portion of the washout curve. Increased femoral artery blood flow was observed by Sako (1963) up to 30 minutes after injection of angiographical contrast agents. Changes in hemodynamics after contrast agent injection are of ill-defined origin, although Grubb et al. (1974) considers the observed changes in serum osmolarity to be important. Hypaque-M, 90 is, like other contrast agents, hypertonic and produces a transient rise in serum osmolarity. The reported increase in serum osmolarity in conjunction with our clearance studies led us to consider the fluid compartment relationship illustrated in the insert of Figure 14 as a representation of the fate of the tracer. Upon injection into the plasma space the contrast agent will continuously be cleared by the kidneys and for a period of time will diffuse into the extracellular fluid (ECF) space. When iodinated contrast material in the ECF space

Figure 14 Two-compartment scatter diagram of the concentration of iodine in a volume of gingival tissue in the dog with respect to time. Each line is the least square best fit regression line for the values indicated. Each point is the mean of four determinations. ECF = extracellular fluid.


counts / 100 sec
200
175
150
125
100
75
50
25
0
1
2
3
4
5
seconds (×10³)
ECF
injection
Plasma
Kidney

is in equilibrium with iodinated contrast material in the plasma space the clearance curve will represent only the kidney clearance rate.

To gain further insight into the hemodynamic effects of administered Hypaque-M, 90 the hematocrit of serial samples of femoral artery blood was estimated with a Clay-Adams 'Readacrit' centrifuge. The results from 5 rabbits which were sampled from 4 to 7 times in the course of an experiment are graphed in Figure 15. Although there was a considerable range in initial hematocrit readings (32.0 - 40.5) the trend was clear; just after Hypaque injection there was a hemodilution effect lasting up to about 12 minutes followed by a trend toward hemoconcentration.

Blood gases and pH were determined at convenient intervals during 5 experiments to insure that these important physiological parameters were within physiological limits. Table 4 summarizes these findings with special reference to values before and after the infusion of Hypaque. Animals number 3 and 5 underwent the most dramatic changes (pO_2 changes of -35.6% and -15.5% respectively) after Hypaque infusion; however, these values after infusion may still be considered physiologically normal. Data of rabbit arterial blood compiled from many sources by Altman and Dittmer (1971) showed ranges for pCO_2 between 22 and 51 mm Hg and for pH between 7.21 and 7.57 in normal animals. The extremely high range of pO_2 values in animals 3 and 5 (Table 4) is related to ventilation for a period on pure oxygen.

B. Regional cerebral blood volume studies

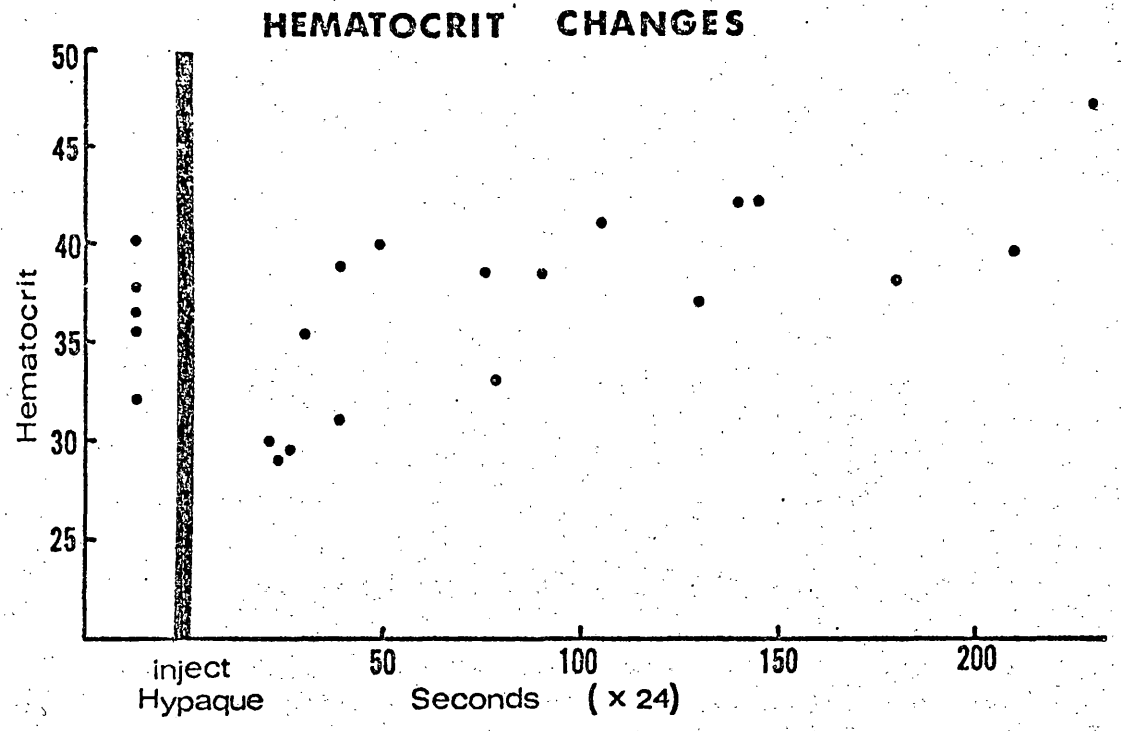
Experiments, outlined in detail in the methods section, to determine the regional cerebral blood volume (rCBV) changes in a 0.1 cc

TABLE 4. Arterial Blood Gases and pH of Rabbits During Cerebral Blood Volume Experiments

	n	mean ± SD	range	before**	after**	% change
Corrected* pCO_2						
1.	5	54.5 ± 1.3	53.5 - 56.7	53.4	54.3	1.5
2.	6	33.8 ± 3.4	29.9 - 38.2	33.3	33.2	- .3
3.	7	40.5 ± 4.3	34.1 - 46.3	39.1	41.6	6.4
4.	4	32.9 ± 5.0	35.3 - 35.7	35.3	35.7	1.1
5.	4	44.9 ± 7.5	36.6 - 54.6	36.6	42.6	16.4
Corrected* pO_2						
1.	5	35.3 ± 3.2	31.8 ± 40.2	31.8	33.8	6.3
2.	6	61.8 ± 7.5	47.2 ± 67.9	62.8	64.8	3.2
3.	7	49.5 ± 25.5	28.0 ± 99.6	99.6	64.1	-35.6
4.	4	58.5 ± 7.6	50.5 ± 68.5	55.8	59.2	6.1
5.	4	61.6 ± 38.4	35.2 ± 118.5	50.3	42.5	-15.5
pH						
1.	5	7.299 ± .04	7.231 - 7.340	7.340	7.324	-.20
2.	6	7.454 ± .03	7.420 - 7.506	7.449	7.457	.10
3.	7	7.364 ± .08	7.225 - 7.451	7.421	7.354	-.90
4.	4	7.434 ± .08	7.320 - 7.493	7.493	7.475	-.24
5.	4	7.285 ± .11	7.147 - 7.400	7.400	7.345	-.74

*corrected according to Severinghaus (1966)
**before and after is relative to the infusion of Hypaque

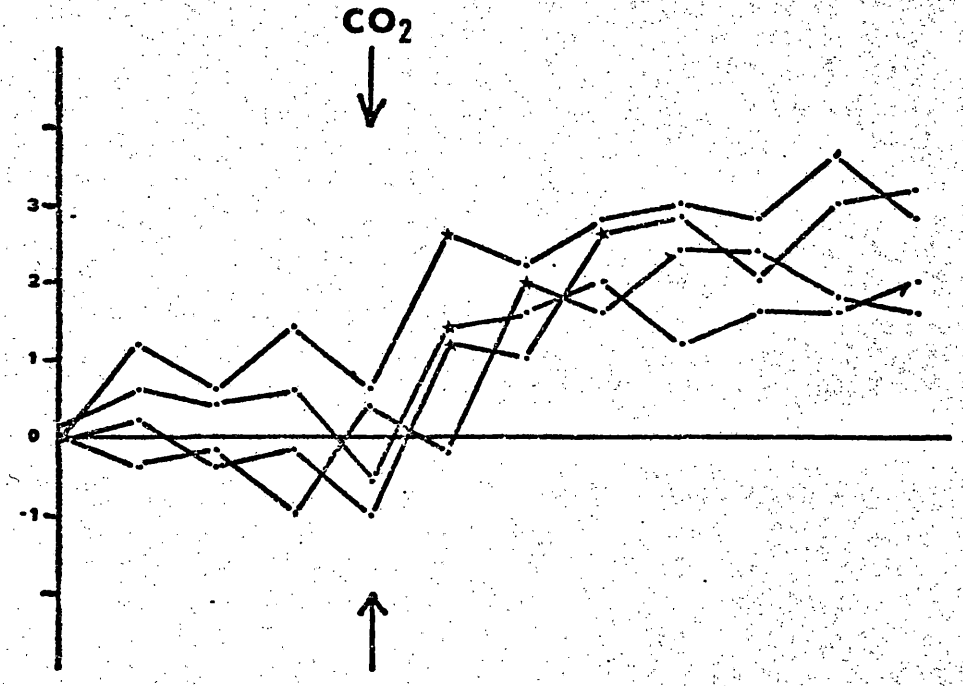
Figure 15 Scatter diagram of the hematocrits recorded in the course of five blood volume experiments.


HEMATOCRIT CHANGES
Hematocrit
50
45
40
35
30
25
inject
Hypaque
50
100
150
200
Seconds (x 24)

volume of occipital cortex tissue were performed to evaluate the effect of a functional load and the influence of selected vasoactive agents on the changes produced by a functional load. The procedure allows the determination in qualitative terms of an increase or decrease in the relative BV within the total tissue volume (0.1 cc) under observation. The changes reported are qualitative values integrated over a 24-second interval. Animals were infused with the X-ray tracer material (Hypaque-M, 90) before any studies were performed. The Hypaque-M, 90 was slowly (1.0 ml/min) infused intravenously to obtain a blood concentration estimated from the body weight to be about 0.06 per cent. The advantages this method has over others include: 1) the repetitive sequential measurements obtainable (a value every 24 seconds), thus providing a temporal aspect to the measurement, and 2) the relatively non-invasive nature of the determination due to unneeded surgical or mechanical intervention. Also, the tentative relationship between volume and flow (discussed in the introduction) may be used to interpret the meaning of rCBV changes.

The first regional cerebral blood volume experiments were evaluations of the response to inhaled 5% CO_2. A consistent vasodilation and concomitant rCBV increase was demonstrated (4 trials) during the first or in one case the second 24 second counting period (see Figure 16). The delay in the one trial is attributed to the time necessary for the gas to traverse the ventilation apparatus and reach the lungs. It is also due in part to variability in manually turning on the gas mixture and marking the polygraph record. The

Figure 16 Relative rCBV changes during inhalation of 5% carbon dioxide by rabbits. Starred data points indicate a greater than 2 standard deviation units change from the previous value. Each point is 24 seconds apart.


CO2
3
2
1
0
-1

generalized and extensive cerebral vasodilation associated with increased carbon dioxide tensions in the blood were not considered a very demanding test of the capability of this detection method. The crucial experiments were the demonstrations of rCBV increases in the visual cortex during exposure of the rabbit to light flashes. It was noted that the light intensities used (setting number 16 on a Grass Photic Stimulator) were intolerable to this investigator when viewed from the distance used (30cm); however, the rabbit has a more lateral location of the orbits and a state of maximum visual stimulation was desired, so this intensity was employed.

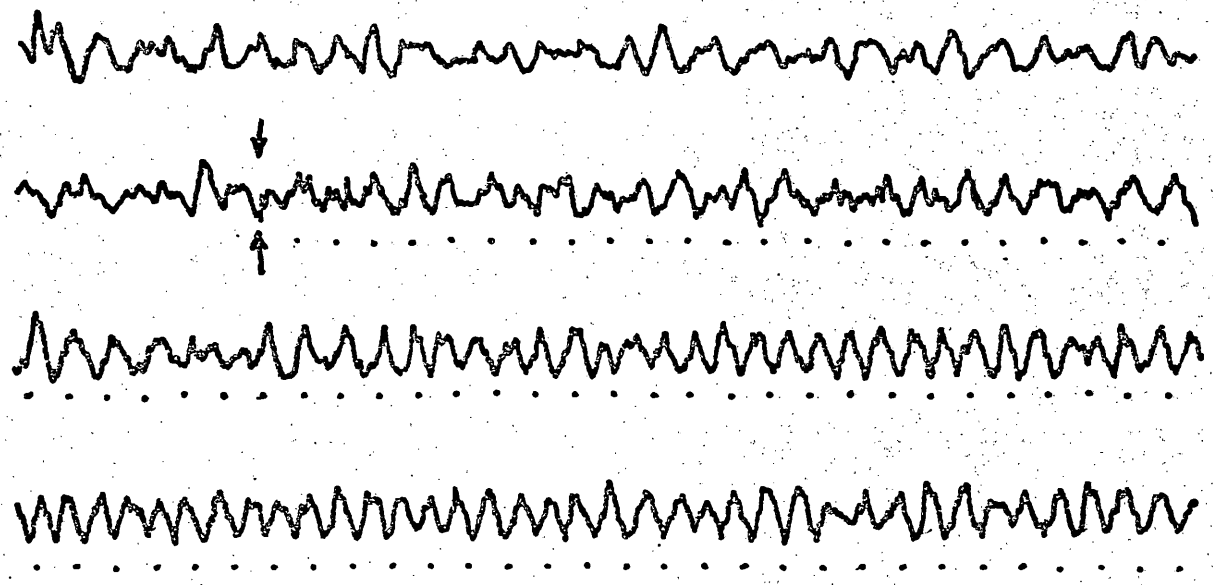
Figure 17 (upper half) represents the occipital lobe EEG record observed during the onset of a photic stimulation experiment. The upper example of Figure 17 illustrates the entertainment or 'following' effect the light flashes have on the EEG, while the example below it shows, in addition, the phenomena of recruitment evidenced by the increase in trace amplitude after photic stimulation was started.

In an attempt to demonstrate the cortical localization of the photically induced rCBV increase to the visual cortex, measurements were made of the general somatic area (refer to Figure 6 for anatomical location) during stimulation with negative results in three trials using two animals. Recording the EEG from the general somatic area revealed no evoked potentials as found on the occipital cortex during photic stimulation.

A further measure of the physiological condition of the preparation

Figure 17 Electroencephalograms (redrawn) from occipital lobe unipolar electrodes placed into the bone of the calvarium. Solid circles below the records indicate a light flash (rate of 6 flashes per second). The two groups of four lines of record each are from different rabbits. Note that the EEG becomes more regular with the onset of light flashes ('entrainment'), as well as, a distinctively increased amplitude in the lower set of records.

4/4/75



4/2/75

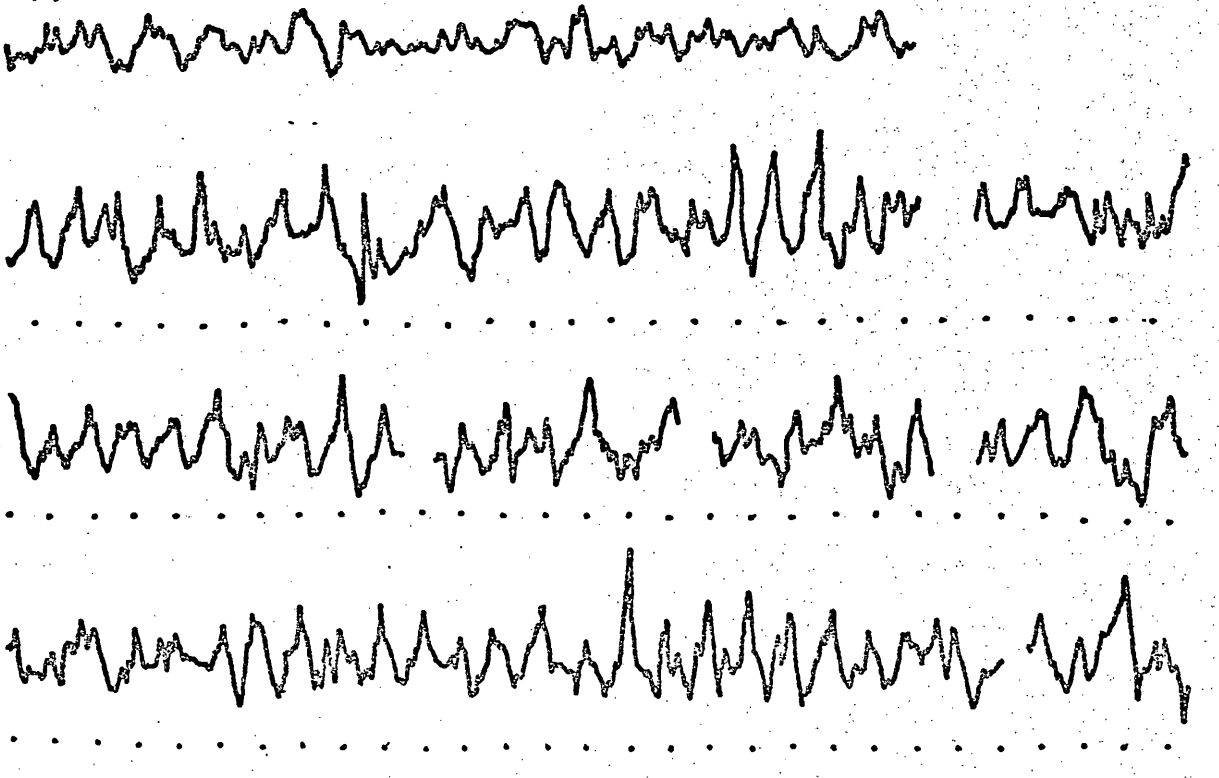
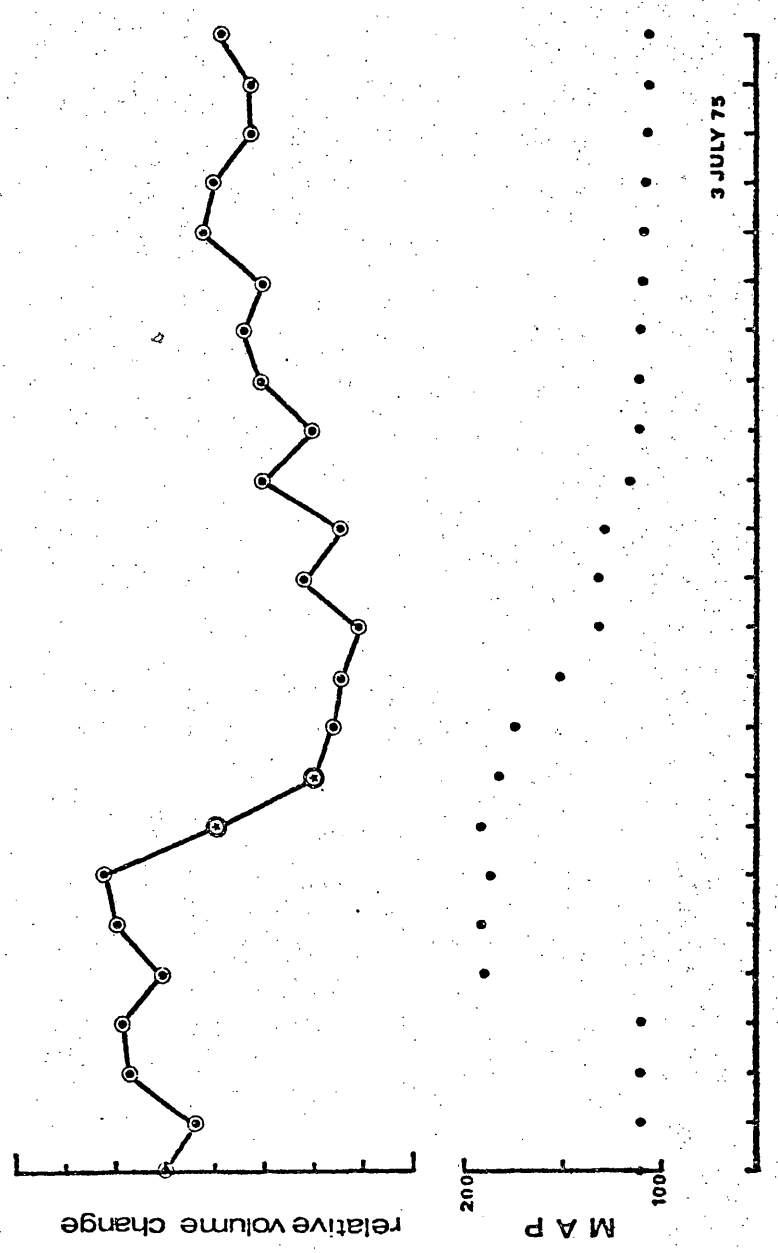


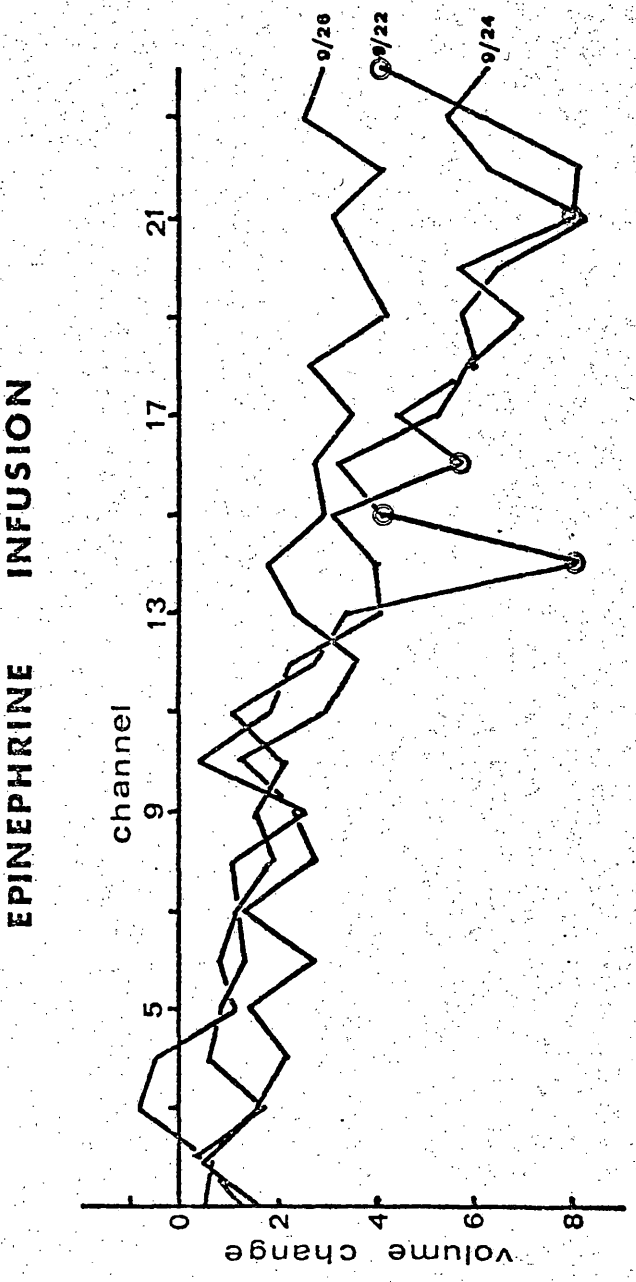
Figure 18 Graph of relative cerebral blood volume changes during a hypertensive incident in the rabbit. Starred data points indicate a greater than 2 standard deviation units change from the previous value. MAP is mean arterial pressure.


relative volume change
M A P
200
100
3 JULY 75
24 sec. intervals

is a test for the presence of the autoregulatory response. This was carried out by determining the rCBV response to an increase in systemic blood pressure. A decrease in volume would indicate normal autoregulation. The blood pressure increase was produced by an infusion of norepinephrine intravenously. Figure 18 shows a typical response to the transient hypertension. A decrease in rCBV in response to an increase in systemic blood pressure was demonstrated 7 times in 8 attempts. All preparations examined had previously had time to recover from the Hypaque infusion (30 to 60 minutes) and had not deteriorated or exhibited hypotension (described as a low mean arterial pressure with loss of auto-regulation).

Prior to the evaluation of the effect on the functionally induced occipital lobe rCBV changes by a vasodilator (papaverine), and a naturally occuring catecholamine (epinephrine), the responses to arterial infusions of papaverine and epinephrine alone were compared to controls. Drug infusions lasting approximately 6 minutes produced rCBV changes as diagrammed in Figures 19 and 20. Control values (obtained from periods of no stimulation in different experiments) are shown above the responses to papaverine in Figure 20. A tendency exists for statistically significant increases in rCBV to appear during the papaverine infusion (8.3%, increases in rCBV x 100/total number of measurements) and a tendency for decreases during the epinephrine infusion (4.5%). These values may be compared to a 2% chance for any change to occur spontaneously in the control situation. Further, there is a trend for all the rCBV

Figure 19 Graph of relative cerebral blood volume changes during an epinephrine infusion. Circled data points indicate a greater than 2 standard deviation units change from the previous value. Epinephrine is infused at a 2 ug/ml concentration at a rate of 0.5 ml per minute starting at the beginning of the graph. Each channel is 24 seconds long. The volume changes indicated are relative values, each determined in relation to the immediately preceeding absolute value.


EPINEPHRINE INFUSION
9/20
9/22
9/24
channel
0
5
9
13
17
21
volume change
0
2
4
6
8

values to decrease during the epineprhine infusion (negative slope) while the papaverine values tend to appear more like the control values in slope (see Figure 20).

Photic stimulation was employed as a regional functional load, which hypothetically produces vascular responses associated with metabolic (or chemical) changes accompanying induced activity. The rCBV response to this stimulation was determined following successive infusions of epinephrine and papaverine. This data is summarized in Table 5. Two important relationships are evident: 1) there was a significant ($p < .02$) decrease in the latency of the rise in rCBV with photic stimulation during epinephrine infusion as compared to non-infusion periods. The rCBV response to photic stimulation was brought about on the average 37 per cent faster after epinephrine infusion; 2) the response pattern to photic stimulation after the second epinephrine infusion (following the papaverine infusion and photic stimulation) was different from the first trial for each animal. Referring to Table 5, it is seen that this situation produced a single increase in rCBV while decreases were produced in three animals. The data where photic stimulation was administered after papaverine was noteworthy in that only chance single increases or decreases were observed. Some changes are to be expected because of statistical uncertainty coupled with a background of spontaneous rCBV changes (see Figure 20), but many more changes were demonstrated during photic stimulation without papaverine (see Table 5). The well-accepted cerebral vasodilator effects of papaverine (McHenry et al., 1970) in normal brain tissue may be expected to dilate

Figure 20 Graph of relative cerebral blood volume changes during controlled periods (above) and papaverine infusions (below). Starred data points indicate a greater than 2 standard deviation units change from the previous value. Values are 24 seconds apart. Papaverine was infused at 2.5 mg per minute starting at the first value shown. The relative volume changes indicated are determined in relation to the immediately preceeding absolute value.

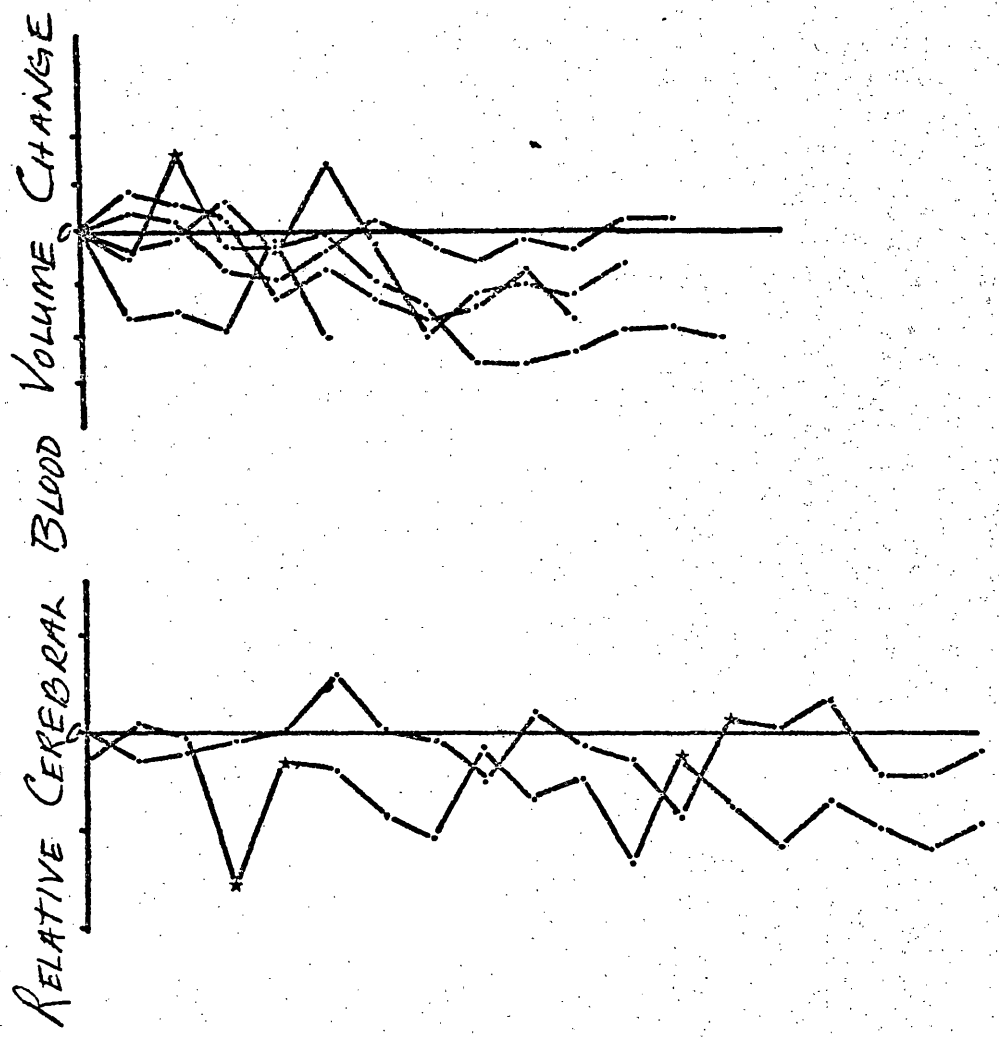

RELATIVE CEREBRAL BLOOD VOLUME CHANGE

TABLE 5. Summary of Temporal Relationship Between Photic Stimulation and rCBV Change After Drug Infusions

		Time to significant change (sec.)			
Study	Direction of Change	Photic Stimulation (P.S.)	P.S.* & Epinephrine	P.S. & Papaverine	P.S. & Epinephrine
1	increase	192	48	NR	NR
	(decrease)	(24)	(NR)	(NR)	(24)
2	increase	192	120	NR	NR
	(decrease)	(216)	(NR)	(NR)	(NR)
3	increase	120	96	72	NR
	(decrease)	(NR)	(NR)	(NR)	(120)
4	increase	168	24	NR	192
	(decrease)	(NR)	(NR)	(216)	(168)
5	increase	240	48	NR	NR
	(decrease)	(NR)	(216)	(NR)	(NR)
Mean (n)	increase	182(5)	67(5)	72(1)	192(1)
	(decrease)	120(2)	216(1)	216(1)	104(3)

*Differences between photic stimulation (P.S.) and epinephrine infusion with P.S. was significant for all trials to the 98% confidence limit (Students t-test, t = 3.86, P.02 = 3.74, statistics of non-independent scores were used).

NR = no response in that category.

vessels to such a degree that a metabolic load could result in no further increase, or possibly a delay in vasodilation beyond the observation period.

Because the photic stimulation was administered immediately after the drug infusions some experiments were carried out where photic stimulation was given during the epinephrine infusion. As indicated in Figure 21, a rise in rCBV was recorded in the first 24 second interval. This rapid response was observed in both of two trials. Mechanisms for the distribution of epinephrine (enzyme degredation and tissue uptake) would be expected to rapidly change its vascular smooth muscle concentration at receptor sites. These factors may be the reason that the response to photic stimulation was faster during infusion than during the post-infusion period as presented in Table 5. The effects produced by papaverine are not so transient as epinephrine. Gottstein (1962) has stated that it is among the longest acting of the vasodilator drugs, and for this reason it was not thought necessary to examine the photic stimulation response during papaverine infusion.

The most pronounced rCBV increase in response to photic stimulation is diagrammed in Figure 22. Many more photic stimulation responses are tabulated in Table 5 but the record diagrammed in Figure 22 has a distinguishable feature. There is an unusual continuing trend of increase, or no change between data points after the starred point (statistically significant value). Since each point denotes the change in count rate from the previous point an extended increase is rarely seen. The graphical representation of BV data more often has a saw-tooth appearance (see Figures 18, 19 or 20).

Figure 21 Graph of relative rCBV changes during photic stimulation in a rabbit arterially infused with epinephrine. Starred data points indicate a greater than 2 standard deviation units change from the previous value. Epinephrine infused via vertebral arteries at 1 μg per minute.

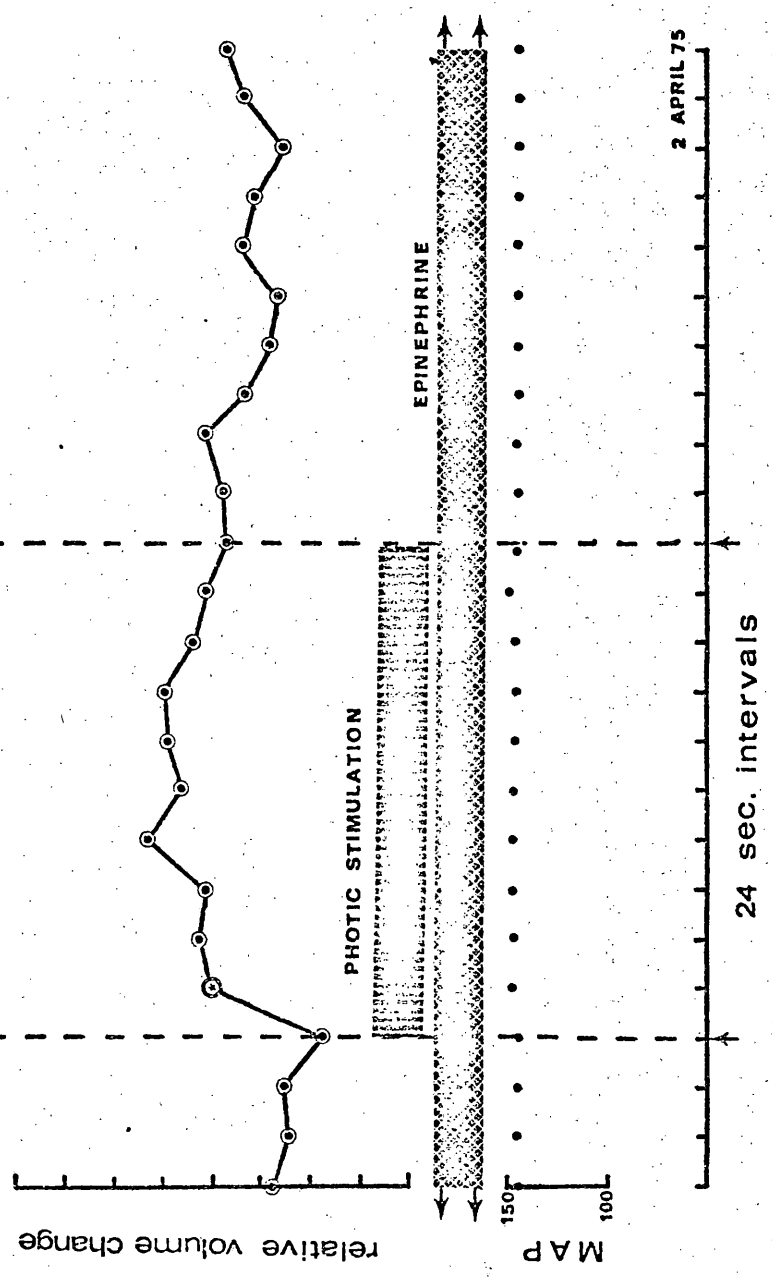
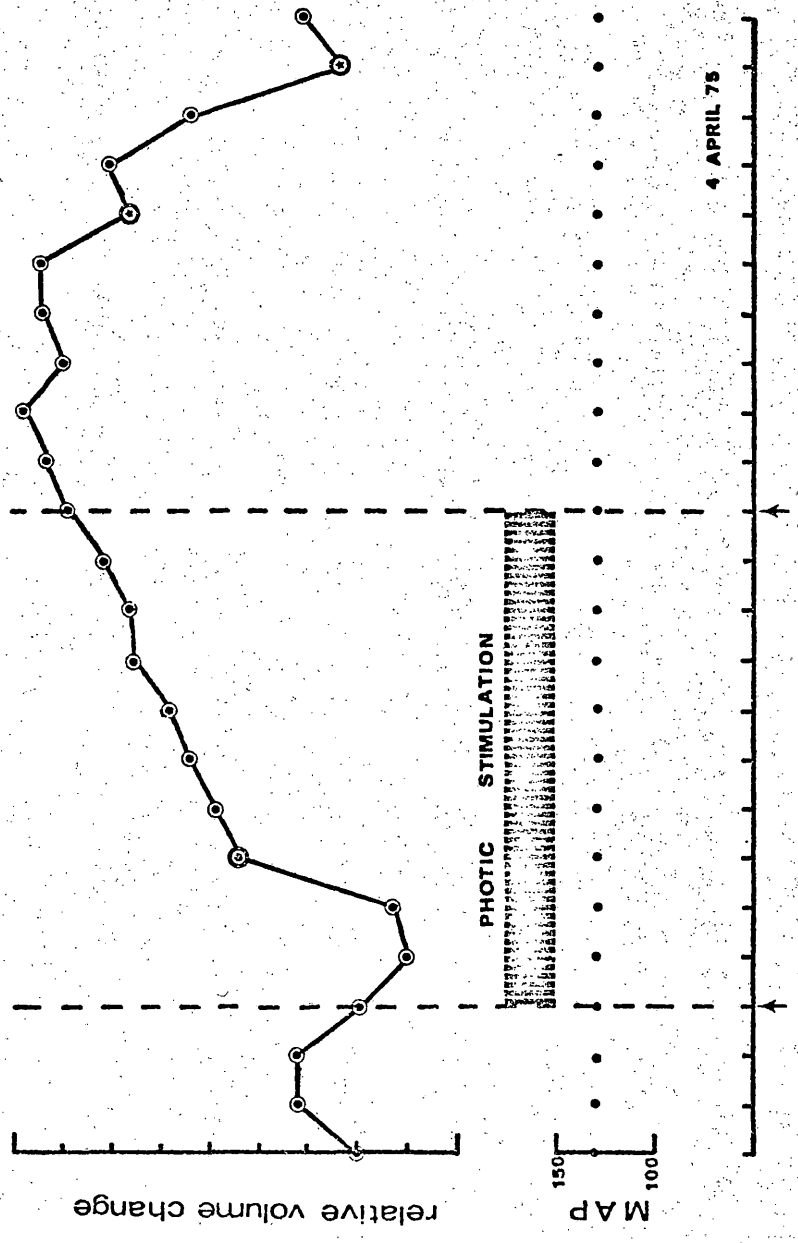

relative volume change
PHOTIC STIMULATION
EPINEPHRINE
MAP
150
100
24 sec. intervals
2 APRIL 75

Figure 22 Graph of relative rCBV changes during photic stimulation of the rabbit. Starred data points indicate a greater than 2 standard deviation units change from the previous value.


relative volume change
MAP
150
100
PHOTIC STIMULATION
4 APRIL 75
24 sec. intervals

C. Studies with the hindlimb

Carpi and Cartoni (1972) conclude in their review that non-convulsant doses of the analeptic pentylenetetrazol (PTZ) increase CBF both passively, through a rise in arterial pressure, and actively, through a cerebral vasodilator response. The active component is thought to be related to the increased functional and metabolic activity of the brain, as revealed by the increased EEG frequency and unchanged cortical pO_2 or cerebral A-V oxygen difference. Although photic stimulation was used as a specific physiological stimulus of cortical activity in the present research, most other investigators used an analeptic as a non-specific pharmacological activator (Carpi and Cartoni, 1972). Therefore, it was thought important to investigate the blood flow response of a simpler system (the hindlimb) to PTZ with or without the vasodilator acetylcholine (Ach).

Blood flow was measured at the femoral artery to determine the change in response to the close arterial injection of the vasoactive agent acetylcholine (Ach) before and after pentylenetetrazol (PTZ) administration. In Table 6 the analeptic PTZ is shown to potentiate the vasodilation produced by Ach in the highest dose (7.5 µg/ml) at both the one and two µg PTZ level. The 2 µg PTZ level also increased the response to the intermediate (0.75 µg/ml) dose of Ach.

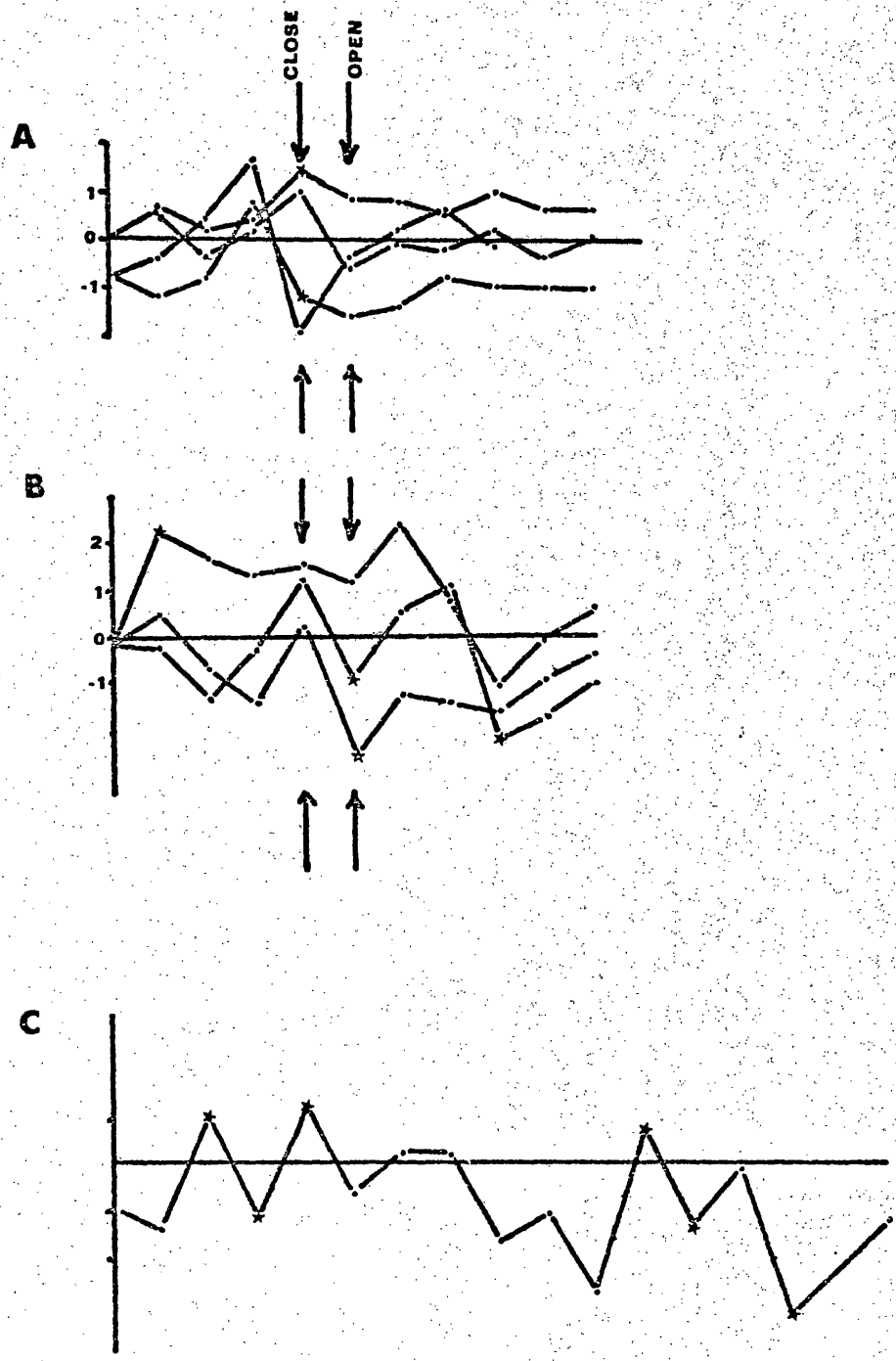
In a further attempt to evaluate the hemodynamic effect of a functional load on the hindlimb of the rabbit, the femoral artery was occluded for 24 seconds and the BV and BF response measured. Figure 23A represents the BV changes observed. It is evident that the BV response to the occlusion was variable. Although during the

TABLE 6. Pentylenetetrazol Effect on Acetylcholine Vasodilation in the Cat Hindlimb

Acetylcholine (μg/ml)	Mean Change in Flow (ml/min) control	1 μg PTZ*	2 μg PTZ*
0.075	8.67 ± 4.4 (4)	8.1 (2)	8.8 (3)
0.75	15.3 ± 1.5 (4)	13.1 (2)	22.1 (2)
7.5	17.2 ± 0.7 (4)	29.7 (3)	30.3 (2)

Values are mean ± standard deviation (number of samples)
*PTZ = pentylenetetrazol

Figure 23 Blood volume changes in the hindlimb of the rabbit during various operations. A, femoral artery occluded at arrow marked 'closed' and reopened 24 seconds later at arrow marked 'open'. B, same as 'A' but animal first treated with dibenzyline. C, measurements of BV change during epinephrine infusion. Starred data points indicate a greater than 2 standard deviation units change from the previous value. Each point is 24 seconds apart.


CLOSE
OPEN
A
1
0
-1
B
2
1
0
-1
C

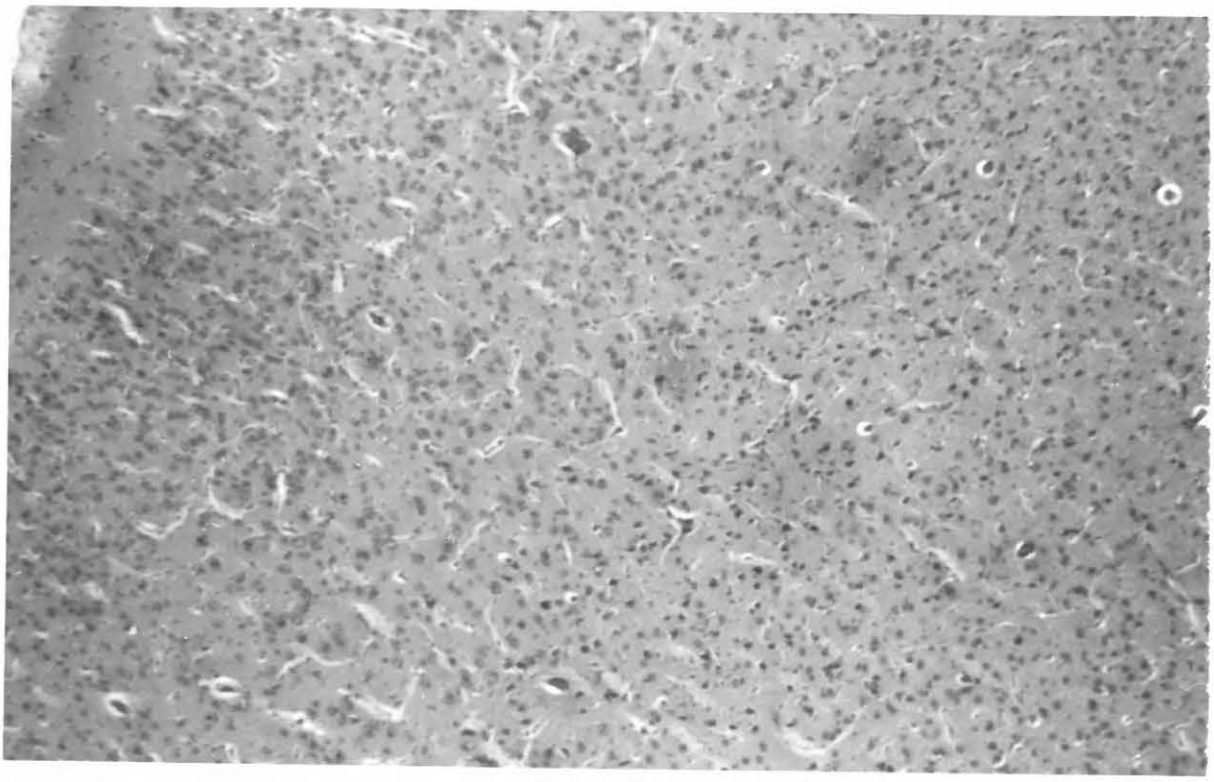
occlusion the flowmeter always indicated no flow in the femoral artery, the BV change during the occlusion may have been increased or decreased. Likewise, after the occlusion, while the BF always exhibited a transient increase over the preocclusion level, the BV might increase or decrease. Due to this unpredictability a subsequent series of BV experiments was conducted in which rabbits were given 30 mg dibenzyline by intravenous infusion (450 μg/min). In these experiments (see Figure 23B) the BV significantly fell during the occlusion in two of three experiments. Part C of Figure 23 shows the BV changes recorded during an epinephrine infusion (2 μg/min). In six of the 15 intervals shown there was a statistically significant change in BV indicating an unusually high incidence of spontaneous vascular activity.

D. Histology

In the course of the BV measuring experiments India ink was injected into the brain to evaluate patency or trauma to cerebral vasculature. The photomicrographs of histologic sections in Figure 24, A and B, are two magnifications (4x and 40x) from the same field of cerebral cortex tissue. Blood vessels are noted for their appearance as dark irregular but generally elongated areas surrounded by a clear zone. The darkest area is believed to be the India ink filled lumen of the vessel. Section of cortex examined were uniform in appearance with no local abnormalities observed. Theoretically, damage could have been produced by the ionizing radiation from the X-ray beam.

With the high magnification (100X) photomicrograph of Figure 25,

Figure 24 Photomicrographs (low magnification) of occipital cortex from the brains of rabbits injected with India ink before sacrifice. Sections (12 microns thick) were stained with Hematoxalin-Eosin. A. Magnification 4X, enclosed area is same field of view seen below in B, at 40X magnification.



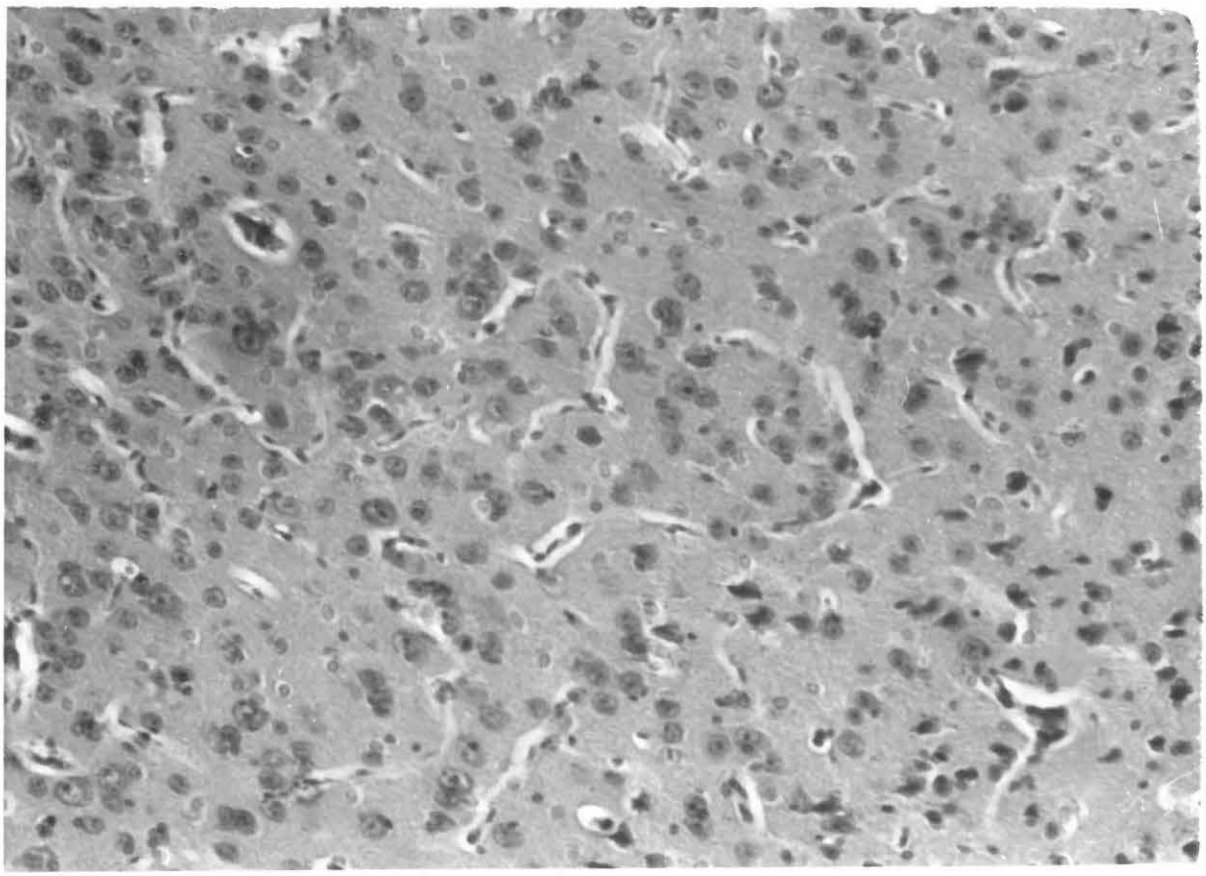
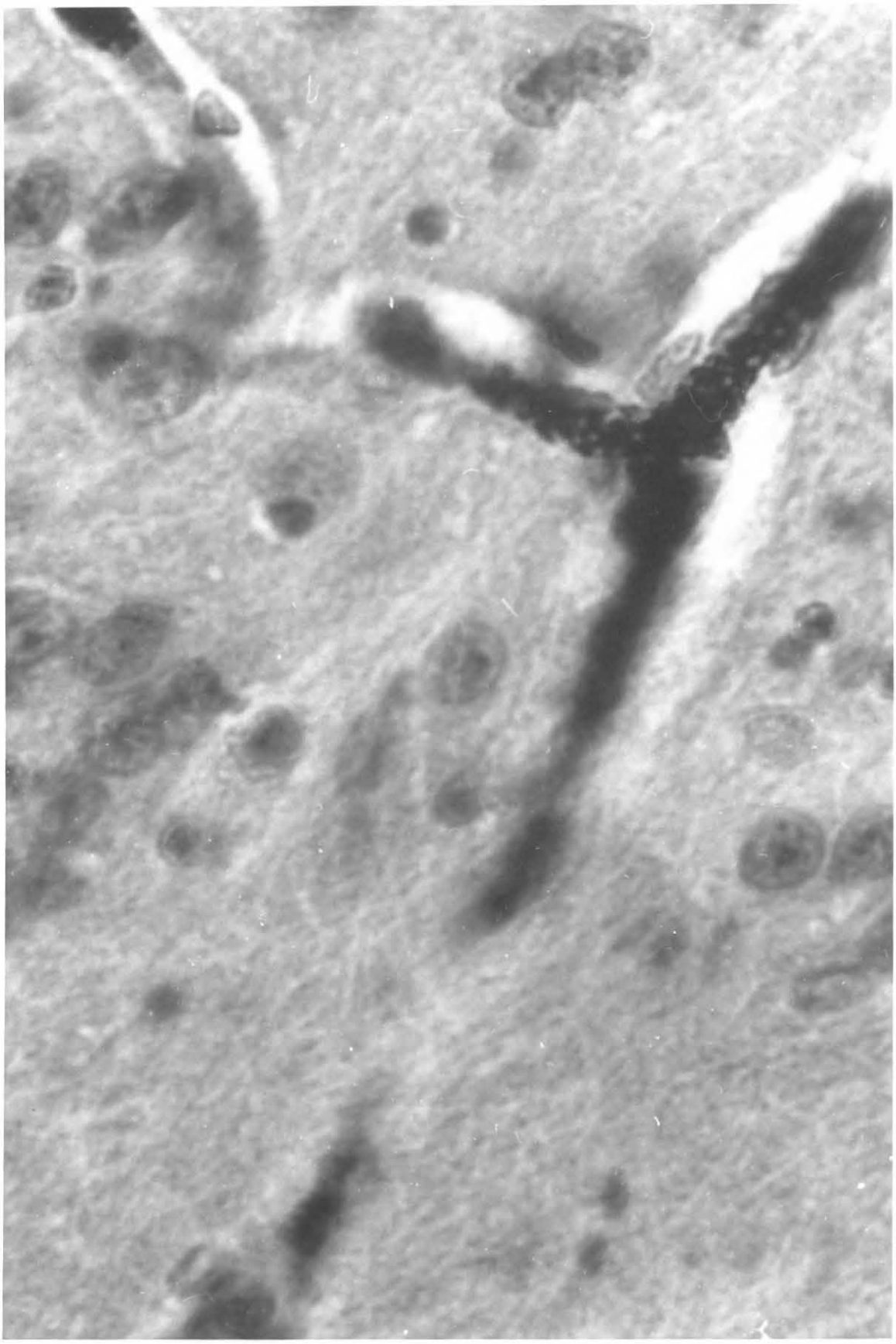


Figure 25 Photomicrograph (100X) of occipital cortex section from the brain of a rabbit injected with India ink before sacrifice. Sections was stained with Hematoxalin-Eosin.



the globular appearance of the vessel lumen contents are apparent. The clear zone surrounding the vessel is also more distinct with nuclear structures evident occasionally. The presence of these nuclei suggest that this clear zone may be a region of swollen endothelial cells. The fact that no India ink granules are seen outside the vascular lumen attests to the tight junction between the endothelial cells.

E. Arteriography

A series of X-ray arteriograms were made to assure that the cannula placed into the carotid artery was delivering fluid into the vertebral arteries. Figure 26 is one of the right lateral exposures. The lower arrow in the figure points to where the vertebral artery leaves the aortic arch and begins ascending toward the brain stem. The upper arrow points to the beginning of a 'capillary blush' (diffuse whitening) characteristically found prior to the venous phase of circulation. This is interpreted as positive evidence that the brain stem is being arterially perfused. In Figure 27 an anterior-posterior view shows similar details. The sharply opaque object in both exposures is a pair of forceps used to hold the skin flap and cannula in place.

Figure 26 Photograph of right lateral view arteriogram of a rabbit. This is part of a series in a dynamic study.

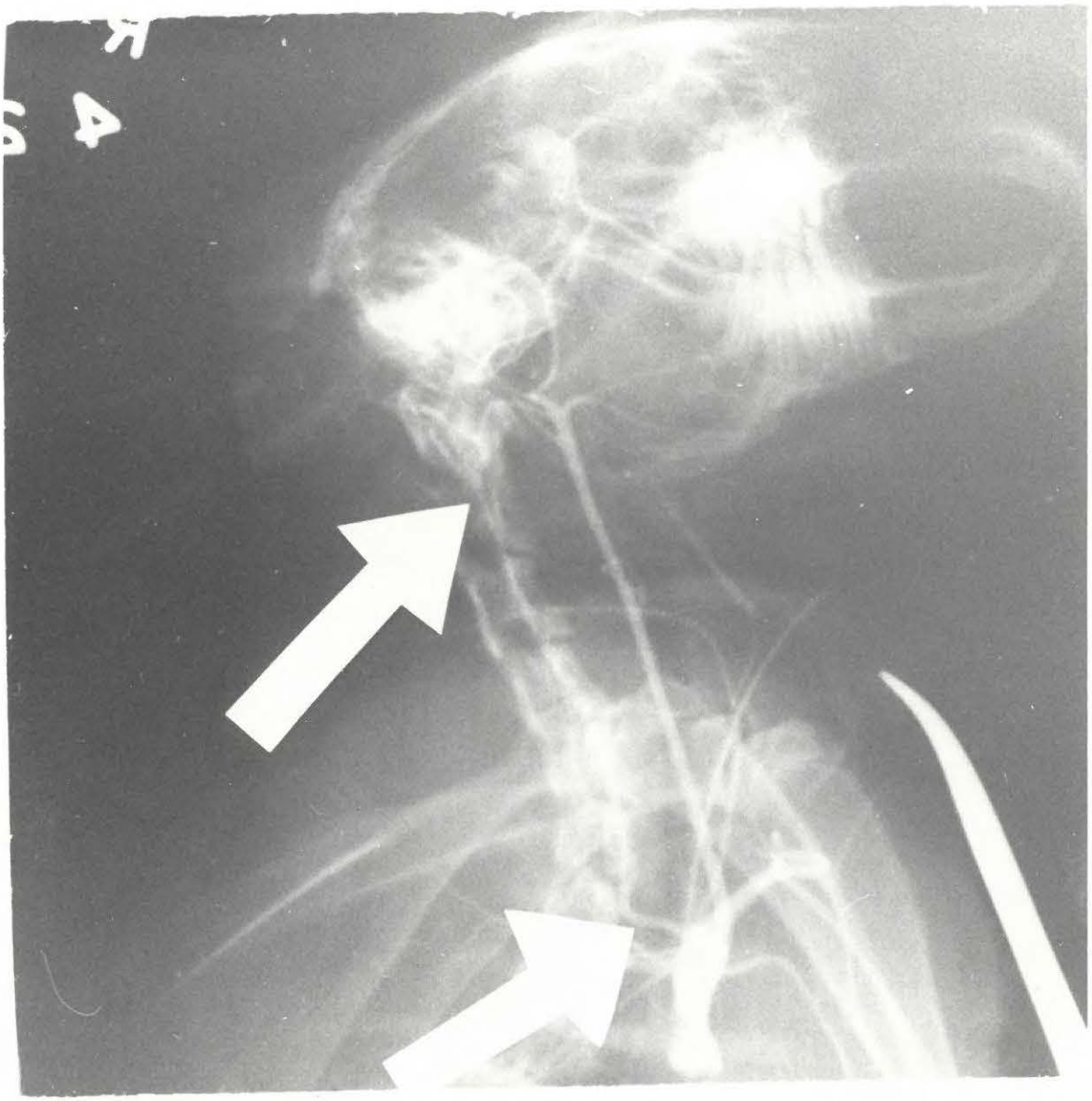
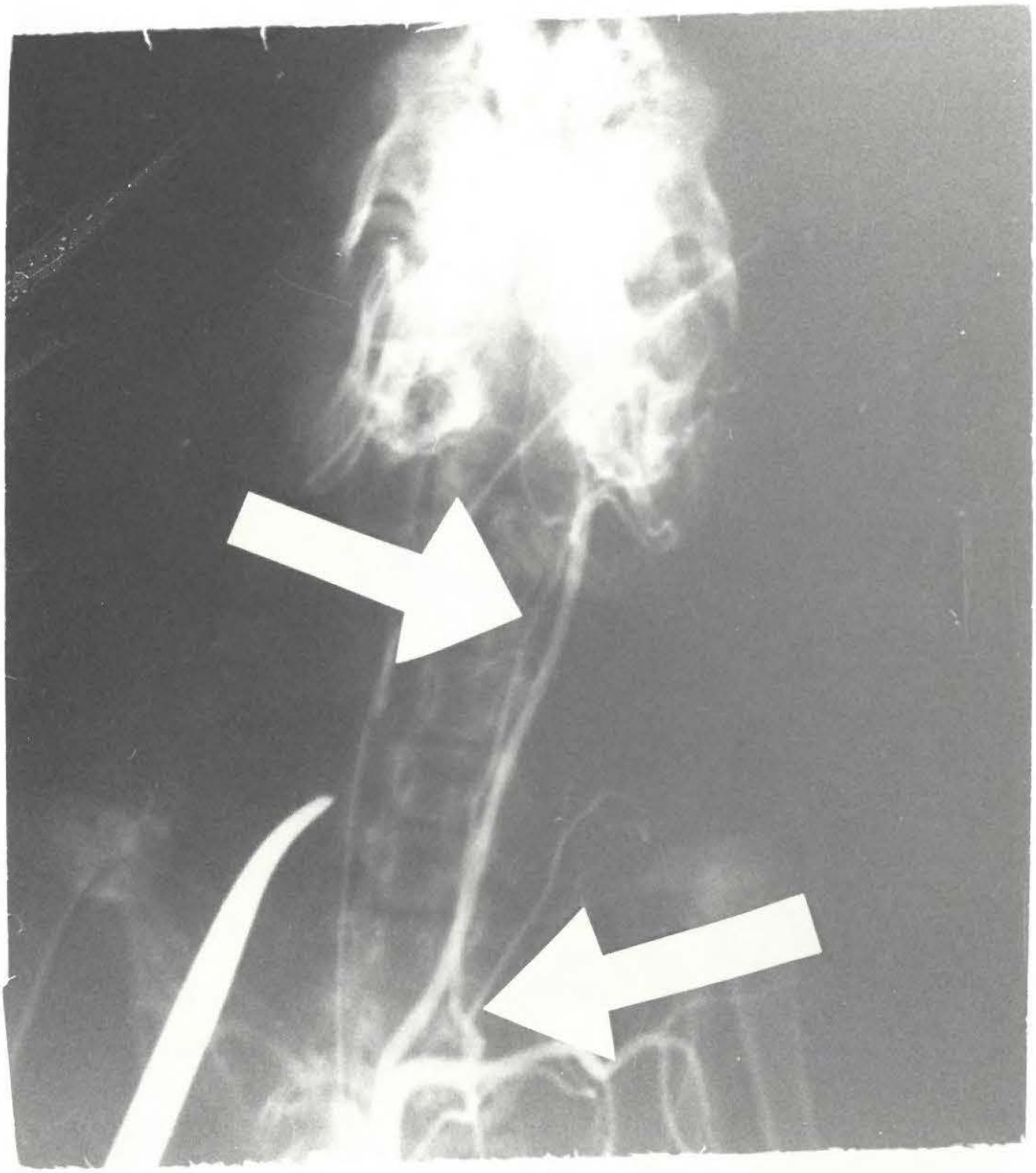


Figure 27 Photograph of an anterior-posterior view arteriogram of a rabbit. This is part of a series in a dynamic study.



DISCUSSION

A. General overview

The importance of a functional load and its concomitant metabolic activity in regard to other vasoactive determinants such as the presence of epinephrine or papaverine is addressed in this research. The assumption that local blood flow regulation is related to the functional load of a tissue involves a metabolic or biochemical process which links them. This research project was designed primarily to consider one of the points where dissociation between rCBF and functional activity may be arising, namely the nature of vascular control exerted by epinephrine and papaverine on the functionally produce vasodilation. The premise that carbon dioxide and the related pH are key metabolic determinants of rCBF regulation is experimentally supported according to Purves (1972) in his textbook of cerebral circulation. It seems reasonable teleologically that the cellular production of carbon dioxide in direct proportion to functional activity links pCO_2 with the blood flow which in turn supplies the tissue with needed metabolites.

Because our methods did not measure rCBV quantitatively, the degree of change is not known, but the method is unique in showing temporal relationships between stimulus and vascular response. Continuing measurement at 24 second intervals of relative rCBV changes

allows analysis of this temporal aspect, whereas conventional rCBF techniques using isotope washout are estimates integrated over 100 to 600 seconds with a single value obtained for a given flow situation. Employing the relatively non-invasive methods previously described, the effect of epinephrine and papaverine infusions together with a localized functional load in visual cortex were examined in regard to moment to moment changes in rCBV in the present research.

B. Photic stimulation as a functional load

This author believes a clearer relationship between the site of vasoactive stimulation and the resulting vascular response is necessary for understanding regional vascular control. The possibility that selected areas in the brain stem sensitive to carbon dioxide are involved in the reflex regulation of CBF was explored by Shalit et al. (1967). This mechanism would allow correlations between inspired (or systemic) carbon dioxide and CBF to be interpreted on a neural reflex rather than local metabolic basis. Shalit and co-workers (1967) showed that lesions in certain defined areas of the brain stem caused a large reduction in the vascular response to carbon dioxide, compared with the control or with that observed when lesions were made in other parts of the brain stem or brain. These experiments can be criticized on the grounds that CBF was not actually measured but derived from the reciprocal of the A-V oxygen difference (with the assumption that cortical oxygen consumption had not changed). Further, it could be objected that the reduced vascular response to carbon dioxide was an artifact depending on initial surgical manipulation rather than in response to the brain stem lesion. This objection could not explain

why some lesions were effective and others only a few millimeters away were not. Also, these workers showed that if the brain stem lesion was caused by local cooling, the normal vascular response to carbon dioxide was restored upon rewarming. Considering these experimental results it may be interpreted that part of the cerebral vascular response to carbon dioxide is reflex in origin and that the pathway involved can be interrupted by localized brain stem lesions. Other investigators have arrived at similar conclusions with somewhat less elegant experiments: James, Millar and Purves, 1969; Lambertson et al., 1959; Geiger, 1958.

For reasons discussed above the use of a sensory evoked functional load to the cortex (such as photic stimulation of the optic sensory cortex) was considered ideal for studying local vascular responses. Although complications from activation of reflex systems by the visual stimulus used in these experiments is not completely removed, the functionally evoked activity is a stimulus more consistent with obtaining data concerning a local vascular regulatory theory.

To evaluate the regional nature of the visually evoked load to the optic cortex, measurements of rCBV changes in somatic cortex were performed during photic stimulation. As expected rCBV in somatic cortex did not respond to the functional load evoked in the visual modality nor did the EEG of that region manifest the increased amplitude or following tendency demonstrated in optic cortex during photic stimulation (see Figure 17). It should be noted that since no response was obtained in the somatic cortex with photic stimulation, further evaluation of this area in the presence of drug infusions and

functional load was not considered necessary. In retrospect, this experiment may yield positive results as norepinephrine infusions have been reported to produce general spreading of EEG activity evoked through a single sensory modality (Rothballer, 1959).

C. The functional load and epinephrine

Relationships between functional load and the sympathomimetic epinephrine on a cerebral vascular region have not been reported in terms using an approach comparable to the one employed in this research. The classic experiments have shown a relationship between inhaled carbon dioxide (considered a metabolic vasoactive mediator) and either arterial infusions of sympathetic amines or cervical sympathetic chain stimulation. Within limits the results are in qualitative agreement and identify a clear interrelationship, but deviate in the degree of effect. The limits are that only studies employing the washout of a diffusable tracer measured by external detectors to determine rCBF are considered for comparison. Purves (1972) considers this method the most reliable measure of flow in the brain. Alternate methods generally require extensive surgery in attempts to isolate portions of the cerebral circulation. This is thought to be contributory to the conflicting results and controversy that continues to prevail with regard to these studies.

Purves and James (1969), using an isotope clearance technique, observed that the vasodilation response to carbon dioxide (above about 40 mm Hg) in the sheep fetus and newborn lamb, and in the adult baboon (James, Millar, and Purves, 1969) was essentially counteracted when the cervical sympathetic nerves were stimulated. In a recent report,

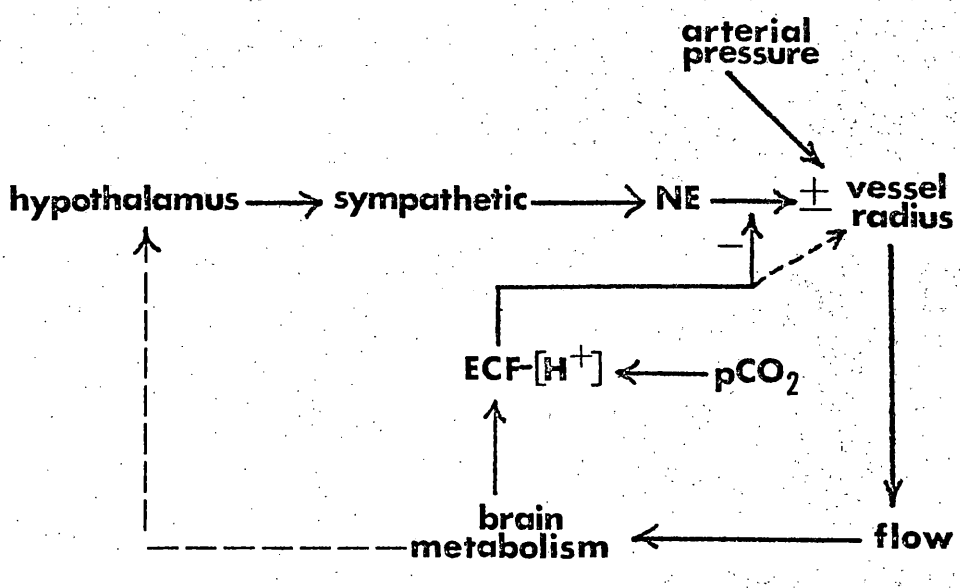
Harper (1975), using the same parameters of stimulation (pulse width, frequency and voltage) on the sympathetic nerve as used by Purves and co-workers, demonstrated that only a 25 per cent reduction in CBF was produced in baboons with hypercapnia (pCO_2 about 60 mm Hg). Harper explained that this quantitative discrepancy was due to the temporal muscle and scalp which were not removed by Purves and co-workers resulting in contamination of the xenon-133 clearance curve by extracranial vascular phenomenon Harper (1975).

These combined results demonstrate that the vasoconstrictor activity mediated by the sympathetic system is not constant but varies with carbon dioxide level. This, in turn, may explain many discrepant results obtained by early (and some current) workers. Conclusions that the sympathetic system had little or no effect on cerebral flow could be a result of the abnormally low pCO_2, since the pCO_2 tends to fall during long experiments. The present research demonstrates two further properties of the metabolic-sympathetic interaction. First, that a vascular response can be produced by the local metabolic demands produced by photic stimulation in visual cortex (see Table 5). This information has been previously demonstrated by other investigators using various methods different from those used in the present research (Sokoloff, 1961; Bondy, 1973). Furthermore, the present studies demonstrate that this response is induced more quickly in the presence of infused concentrations of circulating epinephrine (see Table 5). This is in agreement with chronic sympathectomy experiments where reduced cerebral vascular reactivity to carbon dioxide was shown (Stone, Raichle, and Hernandez, 1974). These authors also demonstrated

that catecholamines were depleted by the sympathectomy. The model of rCBF proposed by these authors depends on interactions between norepinephrine (NE) and extracellular fluid hydrogen ion concentration as diagrammed in Figure 28. They suggest that the local level of NE will depend on the degree of activation of the sympathetic nerves which are in turn dependent on some area of the brain stem. The assumption is made that there is a tonic level of activity imposed on the cerebral vessels. Some evidence for this tonic activity was referenced by these authors and recently more verification has been found (Harper, 1975; Raichle et al., 1975; James, Millar and Purves, 1969; Hernandez, Raichle and Stone, 1975; Edvinsson, Owman and West, 1972). A local factor that will modulate the radius of the vessel could be the extracellular fluid hydrogen ion concentration which would be subject to alteration from the altered pCO_2. As the hydrogen ion concentration increases, the release of norepinephrine is reduced (minus sign in Figure 29) with the radius increasing to some new level. (This hypothesis is supported by Puig and Kirpekar (1971) who demonstrated that (H+) increase caused reduced norepinephrine release). Although this scheme employs NE as a key mediator, the substitution of epinephrine may be at least possible.

The presence of epinephrine (Epi) in the systemic circulation originating from release by the adrenal medulla is well known. The presence of Epi in noradrenergic autonomic nerves due to uptake mechanisms in the nerve endings has also been described (Innes and Nickerson, 1970). It is now evident that Epi is more ubiquitous in the CNS than previously thought due to the discovery in the brain

Figure 28 A proposed local control mechanism for cerebral blood flow regulation. (From Stone, Raichle and Hernandez, 1974). NE, norepinephrine; ECF, extracellular fluid; H+, hydrogen ion concentration. Dotted lines are tentative relationships.


arterial pressure
hypothalamus
sympathetic
NE
± vessel radius
−
ECF-[H+]
pCO2
brain metabolism
flow

of the enzyme phenylethanolamine-N-methyl transferase (PNMT), which converts NE to Epi. Using chemical techniques, PNMT was demonstrated in the olfactory bulb and tubercle, hypothalamus and in the brain stem (Pohorecky et al., 1969); also immunochemical methods have demonstrated the presence of Epi in neurons transversing the hypothalamus and brain stem (Hokfelt et al; 1973). Even more pertinent are the findings of Saavedra, Grobecker and Axelrod (1976) which revealed higher than normal concentrations of PNMT in brain stems from hypertensive rats.

The study of patients with idiopathic orthostatic hypotension or Shy-Drager syndrome has uniformly indicated that CBF continuously responds to normal fluctuations of carbon dioxide concentration (Gotoh et al, 1972; Meyer et al., 1973; Caronna and Plum, 1973). It was also demonstrated that there was variability in the retention of autoregulation. Shy-Drager syndrome is considered to be the manifestation of the loss of function from post-synaptic cervical sympathetic nerves. These results have been used as evidence for both the unrelatedness of chemical or metabolic regulation to autoregulation and, more pertinent to this discussion, the independence of metabolic regulation from autonomic nervous influence. An important note of caution was appropriately interjected with conclusions based on the Shy-Drager syndrome, that is, no histochemical or other microtechnique has been used to substantiate the degree of post-synaptic nerve degeneration in any of these studies. This author feels that an important locus of pathology may be found in the brain stem, in keeping with the proposal by Owman and co-workers (1974) of an intracranial sympathetic system. This hypothesis was more fully explored in the

introduction to this dissertation.

D. The functional load and papaverine

The question of whether vasodilators improve rCBF in ischemic or areas of compromised CBF has been hotly debated. Objections have also been raised against the therapeutic use of vasodilators in occlusive cerebrovascular disease. It has been observed that these agents may divert blood from the ischemic area, where vascular reactivity is depressed or absent, by lowering the cerebral vascular resistance (CVR) in surrounding areas of the brain where a normal vascular reactivity is still present and so produce what has been termed a "cerebral steal" (Lassen, 1966; Shalit et al., 1967; Waltz, 1968).

The opposite view considers the possibility that vasodilators might improve CBF in ischemic areas of the brain. It has been shown that CBF and oxygen delivery were increased as well as clinical improvement in stroke patients after large doses of papaverine given during a ten-day therapeutic period (Gilroy and Meyer, 1966). Other evidence shows that the increase in CBF produced by papaverine was accompanied by an increase in cerebral metabolic rate for oxygen (Geraud et al., 1965); this suggests that the increase in CBF was effective in regional areas of ischemia.

It may be concluded from our study that papaverine indeed does improve the perfusion of the brain to the extent that local dilation (increase in rCBV) was no longer demonstrated in the face of a metabolic load (see Table 5). This implies that the brain is perfused in excess of its needs after papaverine. The results of other workers

demonstrating a "steal" of flow from ischemic regions of brain may be dependent on the ability of papaverine to reach the locus of ischemia. In our studies, the lack of response to photic stimulation after papaverine either with or without a subsequent epinephrine infusion (see Table 5) is in agreement with recent *in vitro* work by Keatinge and Graham (1974). These investigators state that papaverine greatly depressed the stable contraction produced in sheep carotid arteries by norepinephrine.

E. Cerebral vasculature and functional load

Roy and Sherrington (1890) are generally credited with being the first to propose that blood flow in the brain is regulated by increased metabolic activity. It is interesting to note that changes in brain volume were used as an index of blood flow in their studies. Since Langendorf and Seelig (1886) had shown brain tissue to become acid when rendered ischemic; Roy and Sherrington prepared a filtrate from the homogenized brain of an exanguinated dog; when the filtrate was injected intravenously they observed an increase in brain volume. Assuming that this represented an increase in blood flow, they concluded that cerebral vessels responded to acidification of surrounding "lymph", which normally occurs as a result of increased cellular metabolic activity.

In reviewing the literature on the interrelationship between cerebral functional activity and circulation, Carpi and Cartoni (1972) proposed a combined neurogenicbiochemical hypothesis which was compatible with research from both viewpoints. Strychnine or brain stem stimulation consistently produced an increase in total or rCBF (Ingvar

and Soderberg, 1956, 1958; Geiger, 1958; Langfitt and Kassel, 1968) providing"... indirect evidence for a neurogenic mediation of these cerebrovascular responses" (Carpi and Cartoni, 1972). In discussions of 'functional' and 'neurogenic' determinants of CBF an ambiguity exists; however, it is evident that brain function is nervous activity, and the term 'neurogenic' embodies the concept of 'functional' in the case of this organ.

Some data has been interpreted as conflicting with the concept that an increase in cerebral functional activity (as indicated by convulsive or activated EEG patterns) is accompanied by increase production of vasodilatory metabolites. In experiments by Mchedlishvili et al. (1970), cortical spike activity in rabbits induced by local application of strychnine was followed in its early phases by an increase in rCBF. In the later phases, there was discontinuity between the EEG and vascular reactions. Dissociation of EEG, metabolic (biochemical) and vascular phenomenon by pharmacological agents has been reported by others. For example, the EEG convulsive response and rCBF increase caused by bemegride were both abolished by chlordiazepoxide (Aizawa et al., 1966); nevertheless, measurements of extracellular sodium revealed concentrations even below the reduced levels of ongoing activity in a majority of the cases. With the knowledge that decreased extracellular $[Na^+]$ is associated with electrical activation or metabolic inhibition, it was concluded that chlordiazepoxide had its primary action through metabolic inhibition since electrical activity was blocked (Aizawa et al., 1966). This author would regard comparable studies of potassium concentrations as more revealing. The resting nerve membrane is normally 50 to 100 times as

permeable to potassium as to sodium. Therefore, potassium diffuses with relative ease through the resting membrane, whereas sodium diffuses only with difficulty. Since potassium can diffuse through the membrane very easily in comparison with sodium, the Nernst equation predicts that it is almost entirely the concentration difference of potassium across the membrane that determines the magnitude of the resting potential.

In another study, convulsant doses of pentylenetetrazol produced expected EEG responses in rats treated with the insecticide lindane, but motor convulsions and, more importantly, changes in cerebral metabolism were suppressed (Coper, Herken and Koransky, 1958). Although there was no flow studies performed during these experiments, the pH changes and CO_2 production were as expected from an activated EEG. In short, the two aforementioned reports are inconclusive on the fundamental issue of dissociation between cellular activity and rCBF response.

Non-convulsant doses of pentylenetetrazol, such as used in the cat hindlimb studies in the present work have also been shown to increase CBF. Jasper and Erickson (1941) contend that this increase in CBF is due to the hypertensive effects of subconvulsant doses of pentylenetetrazol; and indeed total cerebral electrical activity is not significantly altered and there is an alkaline shift in cortical pH indicating luxury perfusion. Other data (Ingvar, 1965) seem to suggest that the BF increase from pentylenetetrazol is due to an active cerebral vasodilation in excess of the metabolic needs; however,

no variation of cortical pO_2 accompanied pentylenetetrazol induced vascular and EEG changes.

The increased vasodilatory response to Ach and dopamine found by this author in the hindlimb after subconvulsive doses of pentylenetetrazol (see Table 6) could form the foundation for an alternative hypothesis. Vascular responsiveness to physiologically produced agents may be exaggerated in a manner similar to that found in the hindlimb when Ach and DA were administered. Relavent to this hypothesis, Jasper and Erickson (1941) noted that after a subconvulsive dose of pentylenetetrazol, it was possible to precipitate a typical convulsive discharge by administering epinephrine intravenously.

Ingvar (1969) and Freeman and Ingvar (1968) demonstrated the best correlation between blood flow and EEG when both measurements were taken from the same brain region. Control values were obtained in cats under nitrous oxide anesthesia. Regional CBF was measured by krypton-85 clearance and the EEG spectra were characterized by manual frequency analysis. Cortical activity was alternated by thiopental (5 mg/kg) with a reduced rCBF, and increased by subconvulsive pentylenetetrazol (6-10 mg/kg) with an increase in rCBF. These findings support the hypothesis that rCBF is locally adapted to the functional activity. Krupp and Carpi (1964) and Krupp (1966) however, demonstrated EEG activation without an increase in CBF after the administration of various barbiturates. Increasing doses of pentobarbital, hexobarbital and butethal caused increasing inhibition of the dilating response to carbon dioxide. Only pentobarbital (which was used in this author's experiments) led to a simultaneous inhibition of both the EEG activating and cerebral vasodilating effects of carbon dioxide.

Hexobarbital had the least effect on carbon dioxide-induced vasodilation, but markedly obtunded the electrographic reaction to carbon dioxide. Butethal significantly depressed the vascular reaction without altering the EEG response. Krupp (1966), therefore, asserts that there is no causal relationship between CBF and electrical activity.

Freeman and Ingvar (1968) showed that cerebral hypoxia produced a combination of high CBF (3.25 ml/g/min) with predominant slow wave activity in the EEG, these disturbances were explained in terms of the loss of autoregulation in cerebral vessels. Alternatively this author thinks that the type of EEG activity may in some cases be as important as the quantity. This would be in keeping with a neurogenic regulation of the CBF with the EEG reflecting at least in part activity in the vascular neurogenic nerves. In this regard, an increase in CBF was demonstrated prior to desynchronized sleep, immediately before the characteristic desynchronization of the EEG (Kanzow et al., 1962). Similarly, Moskalenko et al. (1974) concluded that increased rCBF due to a functional load from evoked potentials in cortex were due partly to neurogenic mechanisms because regional pO_2 levels increased during the stimulus before carbon dioxide changes occurred, indicating anticipation of the functional needs of the tissue. In this study a further characterization of the rCBF mechanism was made on the basis of vascular responsiveness with and without anesthesia (pentobarbital 35 mg/kg). A two-fold response to functional load was found in recordings of rCBF and regional pO_2 which converted to a single response after anesthesia. The component lost after anesthesia may represent the neurogenic component while

the remaining response was the locally mediated one.

From these findings it can be concluded that electrical activity of the brain and CBF may not always be correlated, although correlation is observed under certain conditions. When dissociation is observed between CBF and tissue activity, it is important to detail a site of action for the dissociating influence. If this is done it may be possible to describe regional CBF regulation in terms of functional load with the exceptions also explained by effects on mechanisms in this model.

F. Possible complications due to evoked functional load and anesthetics

Sharpless and Jasper (1956) as well as Hernandez (1961) demonstrated that responses in both direct and collateral sensory pathways to non-meaningful stimuli; as used to induce the metabolic load in our experiments, were attenuated quickly by repetition (this is called habituation). Such attenuation is reversed by drug induced depression of reticular formation (RF), lesions in RF or introduction of a 'meaningful' or novel stimulus in the same modality. Visual evoked potentials in cortex and the lateral geniculate body could be reduced by stimulating RF before the attenuation due to habituation, but after habituation the potentials at the same sites are amplified. It may be safe to assume that the photic stimulation (8 flashes per second) used in the present research would cause adaptation quite rapidly. Since small doses of barbiturate have not been shown to alter habituation, but rather to block arousal from RF stimulation, it was concluded that RF mechanisms for arousal are not related to habituation (John and Killam, 1959).

Pharmacological studies by the Killams (Killam and Killam, 1959) support the concept that the RF acts to provide a filtering mechanism for sensory input which might protect the organism from excessive input along sensory pathways. Studies with immobilized cats indicated that anesthetics depress the mechanism by which the reticular formation enhances filtering of sensory inflow (Killam and Killam, 1958 and 1959). The mechanism appears to be illustrated clinically by subjective reports of enhanced sensory perception during drowsiness or light anesthesia. Takaori et al. (1966) have confirmed the findings in the encéphale isolé cat with pentobarbital, the anesthetic used in the surgical preparation of animals for the present research. Chin et al. (1965) was able to demonstrate, in cats with chronic electrode implants, that pentobarbital (10 mg/kg) was capable of markedly depressing the inhibitory effect of RF stimulation on responses to a click recorded in auditory cortex, medial geniculate nucleus and dorsal cochlear nucleus. In some experiments, this dose of pentobarbital was sufficient to facilitate the previously inhibited sensory responses. This effect was proposed to be due to an unopposed facilitory influence from local areas in RF less affected by the pentobarbital, or to the release of tonic inhibitory influences on sensory transmission during wakefulness, as suggested by Hagbarth and Kerr (1954).

French et al. (1953) proposed RF depression to be the basic mechanism of anesthesia. This was based on findings that ether and pentobarbital blocked EEG arousal by RF stimulation and selectively depressed potentials evoked in the RF by sensory stimulation.

Low doses of pentobarbital and ether resulting in blockade of EEG arousal patterns, induced by sensory and RF stimulation, was confirmed and extended by the Arduinis (1954). Also, in the cat, data were confirmed by King (1956) and Domino (1955), and in the spinal cat by Bradley and Key (1958). Contrary data is proposed by Gangloff and Monnier (1958) from work in rabbits where doses of phenobarbital (20 to 25 mg/kg) failed to depress the threshold at which RF stimulation caused EEG arousal, even though these doses produced behavioral sleep. Gangloff and Monnier found their results to be consistent with the previous accounts when higher doses of phenobarbital were used. In a review by Killam (1962) this difference is ascribed to species specificity.

On the basis of the above accounts it is considered that the photic stimulation of the rabbit will produce a functional load in visual cortex. Our animals were usually on the verge of recovery from the initial 25 mg/kg pentobarbital when they were placed in the stereotaxic holder and given gallamine for immobilization.

SUMMARY

The relationship between a localized functional load to visual cortex produced by photic stimulation after vertebral artery infusions of epinephrine and papaverine was explored in regard to regional cerebral blood volume (rCBV). In order to evaluate rCBV changes in a small volume of unperturbed tissue, an X-ray technique using non-radioactive tracer (Hypaque) was refined from concepts reported by Moody et al. (1971). Studies evaluating the method and tracer excretion as well as arterial route of drug infusions were preliminary to pharmacological experiments.

Monitoring rCBV in 0.1 cc of cortical tissue revealed vasodilation produced after an average of 182 seconds of photic stimulation. After epinephrine (Epi) was infused, the time to a vasodilatory response to photic stimulation was decreased to an average of 67 seconds ($P < 0.02$). After infusion of the vasodilator papaverine the increase in rCBV usually produced by photic stimulation was abolished, which is possibly due to luxury perfusion produced by papaverine. The extended effect of papaverine was also able to prevent vasodilation produced by photic stimulation after a subsequent epinephrine infusion.

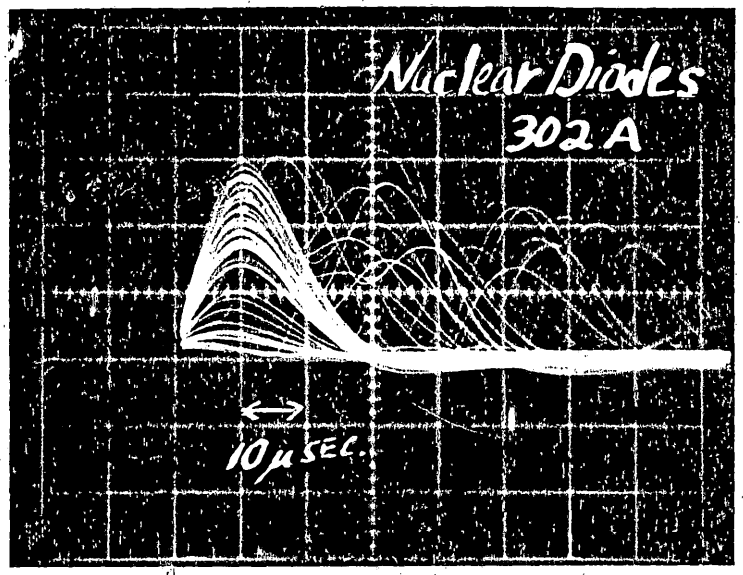
The analeptic pentylenetetrazol (used by many as a pharmacological functional load) was shown to potentiate the vasodilator effects of acetylcholine (Ach) in the hindlimb of the rabbit.

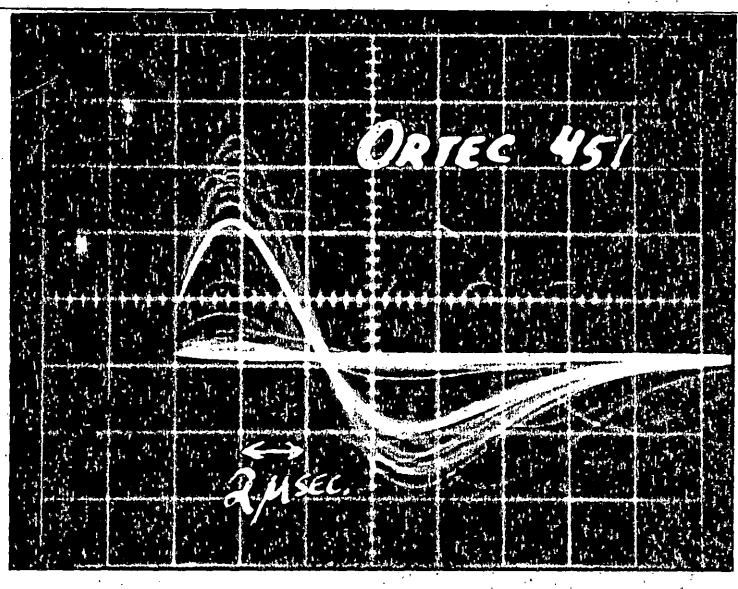
In conclusion, a functional load produced by physiological means appears to cause a vascular response more quickly in the presence of higher concentrations of Epi as shown in the brain.

APPENDIX A

Output characteristics from two commercially available linear amplifiers used in nuclear instrumentation are represented in Figure 29. The outputs were generated by exposing a semiconductor detector connected to the amplifiers to the fluoresced X-rays generated from an iodine solution. The amplitude of the generated pulse is supposed to be directly proportional to the energy of the X-ray that produced the original event at the detectors surface. The major difference between the two amplifiers is the time it takes to output a pulse corresponding to an inputted pulse. Note the different time scale used for the two photographs in Figure 29. The time it takes for a signal to reach its peak and start down toward the baseline is called 'rise time'. The rise time for the Ortec Model 451 is about three microseconds while the Nuclear diodes model 302A takes about 13 microseconds. Because the Nuclear Diodes amplifier takes about 10 microseconds longer, it can process far fewer pulses before it exhibits 'pulse pile-up' or the loss of counts due to more than one pulse being counted as one. The high count rates our detector system encountered needed the 'faster' amplifier to accurately represent the X-ray events.

Figure 29 Output characteristics from two solid-state amplifiers. Input signals were generated by a Si(Li) detector exposed to fluoresced X-rays from an iodine solution. Note the different scales on the abscissa (time coordinate).

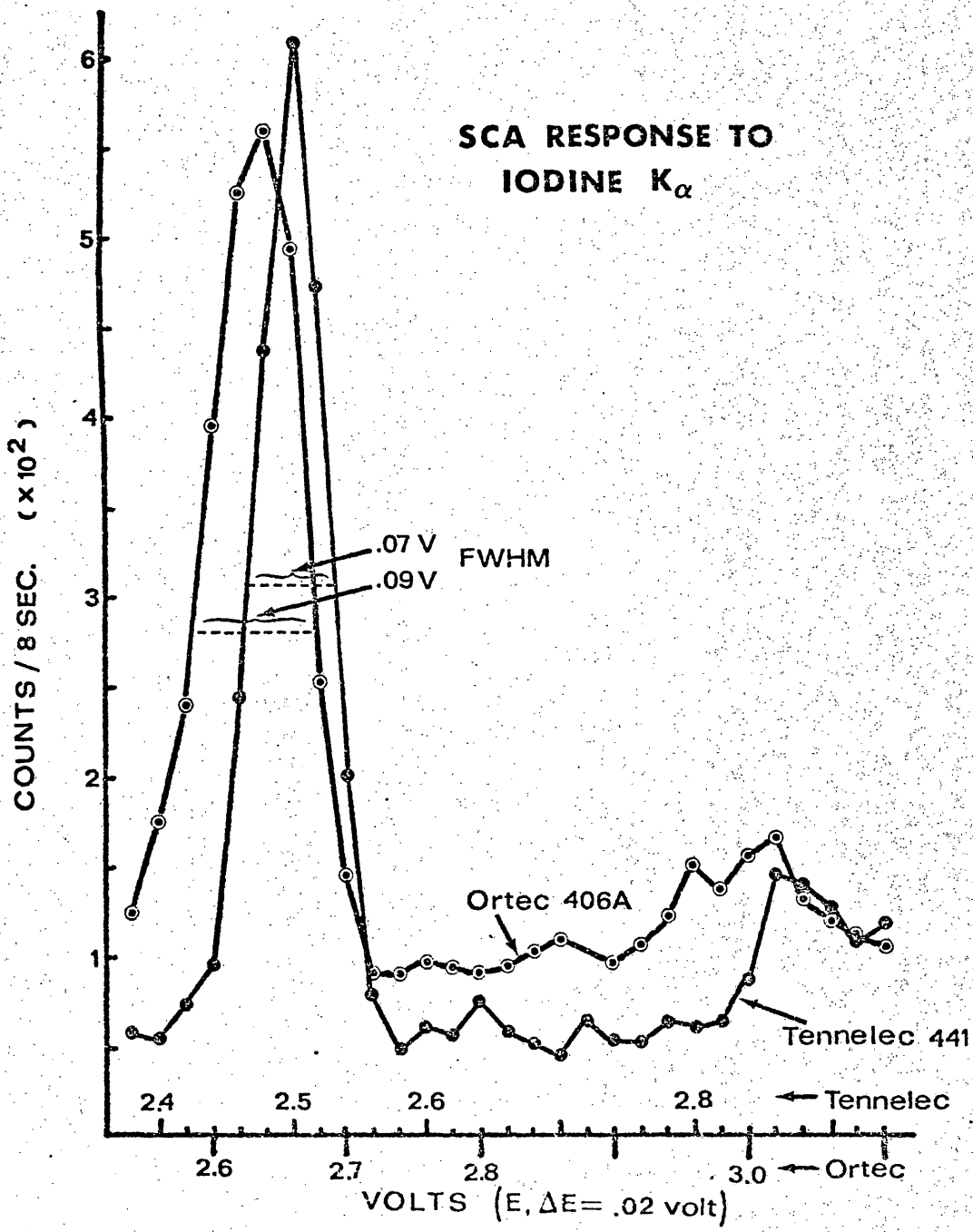

Nuclear Diodes
302 A
10 μ SEC.


ORTEC 451
2 μ SEC.

APPENDIX B

The calibration curve determined for the two single channel analyzers (SCAs) used in this study in response to the K_{α} X-ray of iodine is represented by Figure 30. The function of the SCA is to allow only pulses of a predetermined voltage pass through them. The purpose of the curve in Figure 30 is to determine the voltage setting on the SCA "window" which corresponds to the voltage of the input pulse produced by the iodine X-ray. The SCA has two settings, a window control which is a 10 turn potentiometer variable from 0 to 1 volt (designated ΔE), and a lower level control which is a 10 turn potentiometer adjustable from 0 to 10 volts (designated E) upon which the window rests. Only pulses with a voltage between E and E + ΔE are allowed through the SCA to be counted by other devices. As shown in Figure 30, by setting the window (ΔE) at 0.02 volts and varying the lower level control in 0.02 volt steps a spectrum of activity is delineated. The large voltage peaks on the graph represent the quantity of iodine X-rays detected in an 8 second interval. The FWHM (full width at half maximum) values shown in Figure 30 were used as the window widths for the SCAs in the present experiments. For further details on SCAs the book by Woldseth (1973) is recommended.

Figure 30 Calibration curves of the SCAs for the iodine K_{α} X-ray. "E" is voltage of lower level discriminator and "Δ E" is the voltage for the window control.


SCA RESPONSE TO
IODINE K_α
COUNTS / 8 SEC. ($\times 10^2$)
6
5
4
3
2
1
.07 V
.09 V
FWHM
Ortec 406A
Tennelec 441
2.4
2.5
2.6
2.8
Tennelec
2.6
2.7
2.8
3.0
Ortec
VOLTS (E, ΔE= .02 volt)

APPENDIX C

This program was written by Dr. Jon Trueblood, Medical College of Georgia, to be used on the Hewlett-Packard 9830A for obtaining the significance levels between consecutively accumulated blood volume measurements. Data was manually inputted from the typewriter output of the MCAs. This program demands the use of the advanced statistical ROM.

```
 10     DIM  DS(255), PS(255), US(255), VS(255), Z(255), Q(10)
 15     DISP  "PRINT ONLY SIGNIFICANCE MATRIX";
 16     INPUT Q$
 20     L = Ø
 30     For  I = 1 to R
 40     For  J = 1 to 1Ø
 50     L = L + 1
 60     U(L) = T(I,J)
 70     V(L) = B(I,J)
 80     IF  V(L) = Ø  THEN 1ØØ
 90     Go to 11Ø
100     V(L) = 1
110     Q = U(L)/ V(L)
120     D(L) = U(L)
130     D(L+1) = Ø
140     IF  Q  1.Ø1 THEN 17Ø
150     P(L) = (SQR(Q*(Q+1)))/(SQR U(L)*(Q-1))
160     Go to 18Ø
170     P(L) = 1
180     NEXT J
190     NEXT I
200     N = Ø
205     IF  Q$ = "Y" THEN 22Ø
210     PRINT LIN2 "BGRD TOTAL TOT_BGRD.  %E CHANGE SIGMA
        SIGNIFICANCE", LIN 1
215     PRINT "ROW 1"
220     FOR K = 1 to L
230     N = N+1
240     F = D(K+1) - D(K)
```

```
250    G = P(K)* D(K)
260    Z(K) = F/(G+0.ØØ1)
265    IF Q$ = "Y" THEN 33Ø
270    WRITE (15,28Ø) V(K), U(K), D(K), 1ØØ* P(K), F,
       G,Z(K)
280    FORMAT 6F7.Ø, F7.1
290    IF N = 1Ø THEN 31Ø
300    NEXT K
310    PRINT
312    IF K = L THEN 33Ø
315    PRINT "ROW"; (k/1Ø)+1
320    N = Ø
330    NEXT K
335    PRINT LIN 2, "SIGNIFICANCE"
340    FOR I = 1 to 1Ø*R STEP 1Ø
350    WRITE (15,37Ø) Z(1), Z(I+1), Z(I+2), Z(I+3), Z(I+4);
360    WRITE (15,37Ø) Z(I+5), Z (I+6), Z(I+7), Z(I+8), Z(I+9)
370    FORMAT  5F7.1
380    NEXT I
390    END
```

APPENDIX D

Procedure for electrolysis labeling of human serum albumin (HSA) with technetium-99m is taken essentially from Dworkin and Gutkowski (1971). The zirconium electrodes were obtained from New England Nuclear, radiopharmaceutical division as part of a kit, catalog number NRP-175. The technetium-99m was gratis from the Nuclear Medicine Department, Medical College of Georgia.

1) Inject 3-7 ml sodium ^{99m}Tc-pertechnetate into a shielded reaction vial (serum vial) containing 1 ml of .85 N HCl.

2) Inject 0.1 ml $\pm$ .01 HSA (25%) and swirl.

3) Insert zirconium electrodes through septum of reaction vial.

4) Adjust a d.c. power supply to deliver 100 $\pm$ 5 mA across the zirconium electrodes.

5) Invert vial and swirl during electrolysis for 42 $\pm$ 3 seconds.

6) Continue agitation for 10-15 sec. after current is discontinued.

7) Allow to stand for 30 minutes prior to addition of buffer solution.

8) Inject buffer solution (6 mg Na_2CO_3 and 38 mg $NaHCO_3$ in 2 ml water), hold plunger on syringe tightly because of building pressure from evolved carbon dioxide.

9) Carefully remove syringe plunger from barrel to vent CO_2, then remove syringe.

10) Use preparation within 8 hours.

APPENDIX E

Rabbit brain tissue embedding and staining (Hematoxalin-Eosin) sequence. These techniques were recommended by Dr. Thomas Rosenquist of the Medical College of Georgia.

I. Tissue embedding

A. tissue fixed for at least one month in 10% formalin buffered with 1% $CaCl_2$

B. clearing

1.	50% ethanol	1 hour
2.	70% ethanol	2 hours
3.	95% ethanol	2 hours
4.	95% ethanol	2 hours
5.	100% ethanol	2 hours
6.	100% ethanol	overnight
7.	xylene	2 hours
8.	xylene	2 hours
9.	xylene	overnight
10.	parafin	2 hours } in oven set at just
11.	parafin	2 hours } the melting point
12.	parafin	2 hours } of the parafin ($\sim$39°C)

All solutions were in volumes approximately 20 times that of the tissue.

II. Staining of brain sections (Hematoxylin-Eosin)

Because the sections (20 μ) were considered relatively thick, we used a time for exposure to Hematoxylin which is less than what is typically employed.

	Solution	Time
1.	xylene	3 minutes
2.	xylene	3 minutes
3.	100% ethanol	1 minute
4.	100% ethanol	1 minute
5.	95% ethanol	1 minute
6.	70% ethanol	1 minute
7.	distilled water	3 rinses
8.	hematoxylin	3 minutes
9.	tap water	several (about 2 minutes)
10.	distilled water	3 minutes
11.	Putt's Eosin	20-30 seconds
12.	distilled water	3 rinses
13.	70% ethanol	1 quick rinse
14.	95% ethanol	1 quick rinse
15.	100% ethanol	1 rinse
16.	100% ethanol	30 seconds
17.	100% ethanol	45 seconds
18.	xylene	3 minutes
19.	xylene	3 minutes

BIBLIOGRAPHY

Aizawa, T., F. Muramatsu, K. Hamaguchi, M. Tomita, R. Kakimi, and M. Toyoda. 1966. Study of the cerebral circulation, metabolism, and electrical activity: effects of chlordiazepoxide in the normal and convulsive cats. Jap. Circul. J. 30:13-20.

Altman, P.L., and D.S. Dittmer (eds.). 1971. Respiration and circulation, p. 225. Federation of American Societies for Experimental Biology, Bethesda, Maryland.

Anger, H. 1958. Scintillation camera. Rev. Sci. Instrum. 29:27-33.

Arduini, A., and M. Arduini. 1954. Effect of drugs and metabolic alterations on brain stem arousal mechanism. J. Pharmacol. 110: 76-85.

Baldy-Moulinier, M., and D.H. Ingvar. 1968. EEG frequency content related to regional blood flow of cerebral cortex in cat. Exptl. Brain Res. 5:55-60.

Barker, J. 1972. Blood flow limitation of neuronal population density along capillaries of rat brain. Fed. Proc. 31:401(abs).

Bayliss, W.M. 1902. On the local regulations of the arterial wall to changes of internal pressure. J. Physiol. 28:220-231.

Betz, E., and F. Schmahl. 1966. Durchblutung und sauerstoffdruk in der gehirnrinde bei carotisdrosselung und ihre beeinflussung durch pharmaka. Pflügers Arch. Ges. Physiol. 287:368-384.

Biscoe, T.J., and D.W. Straughan. 1966. Microelectrophoretic studies of neurones in the cat hippocampus. J. Physiol. 183:341-359.

Björklund, A., B. Falck, and C. Owman. 1972. Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines, pp. 318-368. In methods of investigative and diagnostic endocrinology, Berson (ed.), North-Holland-Publishing Co., Amsterdam.

Boakes, R., P. Bradley, N. Brooks, and J. Wolstencroft. 1968. Characteristics of the response of brain stem neurones to noradrenaline. Br. J. Pharmacol. 32:417P.

Bondy, S.C. 1973. The regulation of regional blood flow in the brain by visual input. J. Neurolog. Sci. 19:425-432.

Bradley, P., and B. Key. 1958. The effect of drugs on arousal responses produced by electrical stimulation of the reticular formation of the brain. Electroenceph. Clin. Neurophysiol. 10:97-110.

Brawley, P., and E. Johnson. 1973. Noradrenaline excitations: Neuronal not vascular. Nature, New Biol. 242:245-246.

Brock, M., C. Fieschi, and D. Ingvar (eds.) 1969. Cerebral blood flow. Clinical and experimental results. Springer-Verlag, New York.

Bureš, J., M. Petrán, and J. Zachar. 1967. Electrophysiological methods in biological research. Academic Press, New York. 824 p.

Burrows, G. 1846. On disorders of the cerebral circulation; and on the connection between affections of the brain and diseases of the heart. Longman, London.

Cannon, P.J., R.R. Sciacca, J.C.M. Brust, P.M. Johnson, and S.K. Hilal. 1974. Measurement of regional cerebral blood flow with ^{133}Xenon and a multiple-crystal scintillation camera. Stroke 5:371-383.

Caronna, J., and F. Plum. 1973. Cerebrovascular regulation in preganglionic and postganglionic autonomic insufficiency. Stroke 4:12-19.

Carpi, A. 1972. Pharmacology of the cerebral circulation. Pergamon Press. New York, 370 pp.

Carpi, A., and C. Cartoni. 1972. Central nervous system stimulants, p. 181-201. In A. Carpi (ed.) Pharmacology of the cerebral circulation. Pergamon Press, New York.

Carr, C., and K. Fisher. 1970. A study of new methods of measuring cerebral circulation. Life sciences research office, FASEB, Bethesda, Maryland.

Chin, J., and K. Killam. 1965. Comparison of chlorpromaxine, trifluoperazine and pentobarbital on conditioned arousal to reticular stimulation in cats. Psychopharmacologia 8:41-59.

Clarke, R., and V. Horsley. 1906. On a method of investigating the deep ganglia and tracts of the central nervous system (cerebellum). Brit. Med. J. 2:1799-1800.

Coper, H., H. Herken, and W. Koransky. 1958. Die freien Nucleotide des Gehirns im generalisierten Krampfanfall. Arch. Exp. Path. Pharmak. 234:455-473.

D'Alecy, L. 1973. Sympathetic cerebral vasoconstriction blocked by adrenergic alpha receptor antagonists. Stroke 4:30-37.

D'Alecy, L., and E. Feigl. 1972. Sympathetic control of cerebral blood flow in dogs. Circulat. Res. 31:267-283.

Davies, H.D. 1969. Cerebral blood flow, p. 234. In Drugs and cerebral function. Cerebral function symposium, W. Smith (ed.) Charles C. Thomas, Springfield, Illinois.

Davies, P., and D. Bronk. 1957. Oxygen tension in mammalian brain. Fed. Proc. 16:689-692.

De la Torre, J., R. Walker, and S. Mullan. 1975. Evidence for noradrenergic innervation of intracerebral blood vessels. Neurosciences Abs. 1:676.

Demetrescu, M., and R. Julien. 1974. Local anesthesia and experimental epilepsy. Epilepsia 15:235-248.

Domino, E. 1955. A pharmacological analysis of the functional relationship between the brain stem arousal and diffuse thalamic projection systems. J. Pharmacol. 115:449-463.

Dworkin. H., and R. Gutkowski. 1971. Rapid closed-system production of ^{99m}Tc-albumin using electrolysis. J. Nuc. Med. 12:562-565.

Edvinsson, L., K.N. Nielsen, and C. Owman. 1973. Circadian rhythm in cerebral blood volume of mouse. Experientia 29:432-433.

Edvinsson, L., C. Owman, and K. West, 1972. Intracranial pressure in conscious rabbits after decentralization of the superior cervical sympathetic ganglia. Acta. Physiol. Scand. 85:193-200.

Ekström-Jodal, B., E. Häggendal, N. Nilsson, and B. Norbäck. 1969. Changes in the transmural pressure-the probable stimulus to cerebral blood flow autoregulation, pp. 89-93. In Cerebral Blood Flow, M. Brock., C. Fieschi, D. Ingvar, N. Lassen, and K. Schurmann (eds.) Springer, Berlin.

Fieschi, C., A. Agnoli, N. Battistini, L. Bozzao, and M. Prencipe. 1968. Derangement of regional cerebral blood flow and of its regulatory mechanisms in acute cerebrovascular lesions. Neurology 18:1166-1179.

Fieschi, C., A. Agnoli, L. Bozzao, N. Battistini, and M. Prencipe. 1969. Discrepancies between autoregulation and CO_2 reactivity of cerebral vessels, pp. 120-122. In Cerebral Blood Flow, M. Brock, C. Fieschi, D. Ingvar, N. Lassen and K. Schurmann (eds.), Springer, Berlin.

Fog, M. 1937. Cerebral circulation. The reaction of the pial arteries to a fall in blood pressure. Arch. Neurol. Psychiat., Chicago, 37:351-364.

Freeman, J., and D.H. Ingvar. 1968. Elimination by hypoxia of cerebral blood flow autoregulation and EEG relationship. Expt. Brain Res. 5:61-71.

French, J., M. Verzeano, and M. Magoun. 1953. A neural basis of the anesthetic state. Arch. Neurol. Psychiat. 69:519-529.

Gangloff, H., and M. Monnier. 1958. Effect of phenobarbital on evoked activity following stimulation of cortical and subcortical structures in the unanesthetized rabbit. J. Pharmacol. 122:23A.

Geiger, A. 1958. Correlation of brain metabolism and function by use of a brain perfusion method in situ. Physiol. Rev. 38:1-20.

Géraud, J., A. Bèst, A. Rascol, M. Delpha, and J. Marck-Vergnes. 1965. Application de la méthode au Kripton 85. Pharmacologie de la circulation cérébrale. Presse Méd. 73:1577-1582.

Gilroy, J., and J. Meyer. 1966. Controlled evaluation of cerebral drugs in the progressive stroke, pp. 197-202. In Cerebrovascular Disease, fifth conference, C. Millikan, R. Siekert, and J. Whisnant (eds.), Grune and Stratton, New York.

Gotoh, F., S-I. Ebihara, M. Toyoda, and Y. Shinohara. 1972. Role of autonomic nervous system in autoregulation of human cerebral circulation. Eur. Neurol. 6:203-207.

Gottstein, U. 1962. Der hirkreislauf unter dem einfluss vasoaktiver Substanzen, Hütig, Heidelberg.

Grubb, R.L., M. Hernandez-Perez, M. Raichle, and M. Phelps. 1974. The effects of iodinated contrast agents on autoregulation of cerebral blood flow. Stroke 5:155-160.

Grubb, R.L., M.E. Phelps, M.E. Raichle, and M.M. Ter-Pogossian. 1973. The effects of arterial blood pressure on the regional cerebral blood volume by X-ray fluorescence. Stroke 4:390-399.

Grubb, R.L., M.E. Phelps, and M.M. Ter-Pogossian. 1973. Regional Cerebral blood volume in humans; X-ray fluorescence studies. Arch. Neurol. 28:38-44.

Hagbarth, K., and D. Kerr. 1954. Central influences on spinal afferent conduction. J. Neurophysiol. 17:295-307.

Harper, A.M. 1975. Autonomic control of cerebral blood flow, p.27-47. In Cerebral Vascular Diseases. Whisnant, J.P., and B.A. Sandok (eds.). Grune and Stratton, New York.

Heiss, W., P. Prosenz, and A. Roszuczky. 1972. Technical considerations in the use of a gamma camera 1,600-channel analyzer system for the measurement of regional cerebral blood flow. J. Nuc. Med. 13:534-543.

Hérnandez, R. 1961. Reticular mechanisms of sensory control. p. 497-520. In Sensory Communication, W. Rosenblith (ed.) M.I.T. Wiley, New York.

Hérnandez-perez, M., M. Raichle, and H. Stone, 1975. The role of the peripheral sympathetic nervous system in cerebral blood flow autoregulation. Stroke 6:284-292.

Hedt-Rasmussen, L. 1967. Regional cerebral blood flow. Acta. Neurolocica Scandinavica (Suppl 27) 43:13-79.

Hoffer, P.B. and A. Gottschalk. 1971. Fluorescent thyroid scanning: Scanning without radioisotopes. Radiol. 99:117-123.

Hoffer, P.B., R.E. Polcyn, R. Moody, J.J. Lowe and A. Gottschalk. 1969. Fluorescence detection: application to the study of cerebral blood flow. J. Nuc. Med. 10:651-653.

Hokfelt, T., K. Fuxe, M. Goldstein, and D. Johansson. 1973. Evidence for adrenaline neurons in the rat brain. Acta. Physiol. Scand. 89: 286-288.

Ingvar, D.H. 1965. Discussion on: Measurements of blood flow within circumscribed parts of the brain. Acta. Neurol. Scand., Suppl. 14:39-41.

Ingvar, D. 1969. Correlation between cerebral function and cerebral blood flow and its disappearance following anoxia, pp. 66-69. In Pharmakologie: der lokalen Gehirndurchflutung, E. Betz and R. Wüllenweber (eds.) Werk-Verlag, Dr. E. Banaschewski, München-Gräfelfing.

Ingvar, D., and L. Gustafson. 1970. Regional cerebral blood flow in organic dementia with early onset. Acta. Neurol. Scand. Suppl. 43, 46:42-73.

Ingvar, D., and N. Lassen. 1973. Cerebral Complications following measurements of regional cerebral blood flow (rCBF) with the intra-arterial ^{133}Xenon injection method. Stroke 4:658-665.

Ingvar, D., and J. Risberg. 1967. Increase of regional cerebral blood flow during mental effort in normals and in patients with focal brain disorders. Exptl. Brain Res. 3:195-211.

Ingvar, D. and U. Soderberg. 1956. A new method for measuring cerebral blood flow in relation to the electroencephalogram. Electronenceph. Clinical Neurophysiol. 8:403-412.

Ingvar, D., and U. Soderberg. 1958. Cortical blood flow related to EEG patterns evoked by stimulation of the brain stem. Acta. Physiolog. Scand. 42:130-143.

Ingvar, D.H., and M.S. Schwartz. 1974. Blood flow patterns induced in the dominant hemisphere by speech and reading. Brain 97:273-288.

Innes, I., and M. Nickerson. 1970. Drugs acting on post-ganglionic adrenergic nerve endings and structures innervated by them (Sympathomimetic drugs) p. 478-523. In L. Goodman and A. Gilman (eds.) the pharmacological basis of therapeutics, 4th ed. The MacMillan Company, New York.

James, I., R. Miller, and M. Purves. 1969. Observations on the extrinsic neural control of cerebral blood flow in the baboon. Circulat. Res. 25:77-93.

Jasper, J., and T. Erickson. 1941. Cerebral blood flow and pH in excessive cortical discharge induced by metrazol and electrical stimulation. J. Neurophysiol. 4:333-347.

John, E., and K. Killam. 1959. Electrophysiological correlates of avoidance conditioning in the cat. J. Pharmacol. Exp. Ther. 125: 252-274.

Johnson, E., M. Roberts, A. Sobieszek, and D. Straughan. 1969. Noradrenaline sensitive cells in cat cerebral cortex. Int. J. Neuropharmac. 8:549-566.

Johnson, E., M. Roberts, and D. Straughan. 1969. The responses of cortical neurones to monoamines under differing anesthetic conditions. J. Physiol. 203:261-280.

Kanno, I., K. Uemura. 1975. Some experimental errors in calculating regional cerebral blood flow from the intracarotid ^{133}Xe clearance curve. Stroke 6:370-375.

Kanzow, E., D. Krause, and H. Kühnel. 1962. Die Vasometorik der Hirnrinde in den Phasen desynchronisierter EEG-Aktivität in natürlichen Schlaf der Katze. Pflügers Arch. Ger. Physiol. 274:593-607 (GER).

Keatinge, W., and J. Graham. 1974. Influence of papaverine and nitrite on the responses to norepinephrine of inner and outer smooth muscle of sheep carotid arteries. Blood Vessels 11:229-240.

Kellie, G. 1824 a. On account of the appearances observed in the dissection of two of three individuals presumed to have perished in the storm of the 3rd and whose bodies were discovered in the vicinty of Leith on the morning of the 4th, November 1821 with some reflections on the pathology of the brain. Trans. Edin. Med. Chirurg. Soc. 1:84-122.

Kellie, G. 1824 b. Reflections on the pathology of the brain. Trans. Edin. Med. Chirurg. Soc. 1:123-169.

Kety, S., and C. Schmidt. 1948. The nitrous oxide method for the quantitative determination of cerebral blood flow in man: Theory, procedure and normal values. J. Clin. Invest. 27:476-483.

Key, E.A.H., and G. Retzins. 1876. Studien in der Anatomie des Nervensystems und des Bindesgewebe. Samson and Wallin, Stockholm.

Killam, E. 1962. Drug action on the brain stem reticular formation. Pharmacol. Rev. 14:175-223.

Killam, E., and K. Killam. 1959. Phenothiazine-pharmacologic studies. Proc. Res. Nerv. Ment. Dis. 37:245-265.

Killam, K., and E. Killam. 1958. Drug action on pathways involving the reticular formation. p. 111-122. In Reticular Formation of the Brain. H. Jasper, L. Proctor, R. Knighton, W. Noshay, and R. Costello (eds.) Little, Brown and Co., Boston.

King, E. 1956. Differential action of anesthetics and interneuron depressants upon EEG arousal and recruitment responses. J. Pharmacol. 116:404-417.

Kontos, H.A. 1975. Mechanisms of regulation of the cerebral microcirculation. Current concepts of cerebrovascular disease, Stroke 10: 7-12.

Krupp, P. 1966. Cerebrale Durchblutung und elektrische Hirnaktivität. Schwabe, Basel.

Krupp, P., and A. Carpi. 1964. Die Beziehungen Zwischen dem Zerebrovaskularen und elektrographischen effect der Hyperkapnie unter der Einwirkung von Barbituraten. Helv. Physiol. Pharmac. Acta. 22: C78 - C80.

Kuhl, D.E., M. Reivich, A. Alavi, I. Nyary, and M. Staum. 1975. Local cerebral blood volume determined by three dimensional reconstruction of radionuclide scan data. Circ. Res. 36:610-619.

Lambertsen, D., S. Dwens, H. Wendel, M. Stroud, A. Lurie, W. Lochner, and G. Clark. 1959. Respiratory and cerebral circulation control during exercise at 0.21 and 2.0 atmosphere inspired PO_2. J. Appl. Physiol. 14:966-982.

Langendorff, E., and A. Seelig. 1886. Ueber die in Folge von Athinungs hindernissen eintretenden Storungen der Respiration. Pflügers Arch. Gs. Physiol. 39:223-237.

Langfitt, T., and N. Kassell. 1968. Cerebral vasodilation produced by brain - stem stimulation. Neurogenic control vs. autoregulation. Am. J. Physiol. 215:90-98.

Langfitt, T., J. Weinstein, and N. Kassell. 1965. Cerebral vasomotor paralysis produced by intracranial hypertension. Neurology 15: 622-641.

Lassen, N. 1966. The luxury-perfusion syndrome and its possible relation to acute metabolic acidosis localized within the brain. Lancet 2:1113-1115.

Lierse, W. 1963. Die Kapillardichte im Phinencephalon verschiedener Wirbeltiere und des Menschen. In Progress in Brain Research, vol. 3, W. Bargmann and J. Schade (eds.). Elsevier, Amsterdam.

Magendie. F. 1836. Experiences sur le liquide cephalo-rachidien. J. Hebd. progr. Sci. Inst. Med. 1:83-85.

Maynard, E., R. Schultz, and D. Pease. 1957. Electron microscopy of the vascular bed of the rat cerebral cortex. Am. J. Anat. 100:409-433.

Mchedlishvili, G., D. Ingvar, D. Baramidze, and R. Ekberg. 1970. Blood flow and vascular behavior in the cerebral cortex related to strychnine-induced spike activity. Experimental Neurol. 264:411-423.

Malzack, R., K.L. Casey. 1967. Localized temperature changes evoked in the brain by somatic stimulation. Exptl. Neurol. 17:276-292.

Meyer, J.S., and D. Denny - Brown. 1957. The cerebral collateral circulation I. factors influencing collateral blood flow. Neurology. 7:447-458.

Meyer, J., and A. Ericsson. 1971. Cerebral circulation and metabolism in neurological disorders pp. 253-296. In Pharmacology of the cerebral circulation. A. Carpi (ed.), Pergamon Press, Elmsford, New York.

Meyer, J.S., and F. Gotoh. 1961. Interaction of cerebral hemodynamics and metabolism. Neurology 11:46-65.

Meyer, J., K. Shimazu, Y. Fukuuchi, T. Ohuchi, S. Okamoto, A. Koto, and A. Ericsson. 1973. Cerebral dysautoregulation in central neurogenic orthostatic hypotension (Shy-Drager Syndrome). Neurology 23: 262-273.

Mitchell, G., D. Scriven, and C. Rosendorff. 1975. Adrenoceptors in intracerebral resistance vessels. Br. J. Pharmacol. 54:11-15.

Monro, A. 1783. Observations on the structure and functions of the nervous system, p. 2-7. W. Creech, Edinburgh.

Moody, R.A., P.B. Hoffer, R.E. Polcyn, H.J. Lowe, A. Gottschalk, and G.D. Dobben. 1971. K-shell fluorescence for the study of regional cerebral blood flow. J. Neurosurg. 35:181-184.

Moskalenko, Y.E., I.T. Demchenko, A.I. Krivchenko, and I.P. Fedulova. 1974. Dynamics and control mechanisms in maintenance of regional cerebral blood flow. Stroke 5:461-469.

McElligott, J.G., and R. Melzack. 1967. Localized thermal changes evoked in the brain by visual and auditory stimulation. Exptl. Neurol. 17:293-312.

McHenry,L., M. Jaffe, J. Kawamura, and H. Goldberg. 1970. Effect of papaverine on regional blood flow in focal vascular disease of the brain. New England J. Med. 282:1167-1170.

Nielsen, K., and C. Owman. 1970. Contractile response and amine receptor mechanisms in isolated pial arteries. Acta. Neurologica Scand. 46:626-627.

Nielsen, K., and C. Owman. 1971. Contractile response and amine receptor mechanisms in isolated middle cerebral artery of the cat. Brain Res. 27;33-42.

Obrist, W., H. Thompson, H. Wang, and W. Wilkinson. 1975. Regional cerebral blood flow estimated by ^{133}Xenon inhalation. Stroke 6:245-256.

Oldendorf, W.H. 1974. Blood-brain barrier permeability to drugs. Ann. Rev. Pharmacol. 14:239-248.

Owman, C., S. Aronson, G. Gennser, and N.-O Sjöberg. 1973. Histochemical and pharmacological evidence of amine mechanisms in human fetal vascular shunts. pp. 179-191. In Fetal Pharmacology, Boréus (ed.), Raven Press, New York.

Owman, C., L. Edvinsson, B. Falck, and K. Nielsen. 1974. Amine mechanisms in brain vessels, with particular regard to autonomic innervation and blood-brain barrier, p. 184-199. J. Cervós-Navarro (ed.) Pathology of cerebral microcirculation. Walter de Gryter & Co., Berlin.

Owman, C., L. Edvinsson, and K.C. Nielsen. 1974. Autonomic neuroreceptor mechanisms in brain vessels. Blood Vessels 11:2-31.

Phelps, M.E., R.L. Grubb, and M.M. Ter-pogossian. 1973. Correlation between Pa_{CO} and regional cerebral blood volume by X-ray fluorescence. J. Appl. Physiol. 35:274-280.

Phelps, M.E., R.L. Grubb, and M.M. Ter-Pogossian. 1973. In vivo regional cerebral blood volume by X-ray fluorescence: validation of method. J. Appl. Physiol. 35:741-747.

Phillis, J., and D. York. 1967. Strychnine block of neural and drug induced inhibition in the cerebral cortex. Nature 216:922-923.

Pohorecky, L., M. Zigmond, H. Karten, and R. Wurtman. 1969. Enzymatic conversion of norepinephrine to epinephrine by the brain. J. Pharmacol. Exp. Ther. 165:190-195.

Politoff, A., and F. Macri. 1966. Pharmacologic differences between isolated, perfused arteries of the choroid plexus and of the brain parenchyma. Int. J. Neuropharmacol. 5:155-162.

Puig, M., and S. Kirpekar. 1971. Inhibitory effect of low pH on norepinephrine release. J. Pharmacol. Exp. Ther. 176:134-138.

Purves, M.J. 1972. The physiology of the cerebral circulation. University Press, Cambridge, 420 p.

Purves, M. and I.M. James. 1969. Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb. Circulation Res. 25:651-667.

Raichle, M., B. Hartman, J. Eichling, and L. Sharpe. 1975. Central noradrenergic regulation of brain microcirculation. Neurosciences Abs. 1:413.

Rapela, C., and H. Green. 1964. Autoregulation of canine cerebral blood flow. Circulation Res. 15(Suppl.):205-212.

Risberg, J., D. Aneri, and D.H. Ingvar. 1969. Correlation between cerebral blood volume and cerebral blood flow in the cat. Exptl. Brain Res. 8:321-326.

Risberg, J., and D. Ingvar. 1973. Patterns of activation in the grey matter of the dominant hemisphere during memorizing and reasoning. Brain 96:737-756.

Roberts, E., and S. Matthysse. 1970. Neurochemistry: at the crossroads of neurobiology. Ann. Rev. Biochem. 39:777-820.

Rosenblum, W.I. 1971. Neurogenic control of cerebral circulation. Stroke 2:429-439.

Rosenblum, W.I. 1975. Cerebral microcirculation. Current Concepts of Cerebrovascular Disease, Stroke 10:1-6.

Rosendorff, C. 1972. The measurement of local cerebral blood flow and the effects of amines. p. 115-156. In Progress in Brain Research, vol. 35 Cerebral blood flow, J.S. Meyer, J.P. Schadé (eds.) Elsevier Publishing Co., New York.

Rothballer, A.B. 1959. The effects of catecholamines on the central nervous system. Pharmacol. Rev. 11:494-547.

Roy, C., and C. Sherrington. 1890. On the regulation of the blood-supply of the brain. J. Physiol. 11:85-108.

Russell, R.W.R. (ed.). 1971. Brain and blood flow. Fourth international symposium on the regulation of cerebral blood flow, Proceed. Pitman, London.

Saavedra, J., H. Grobecker, and J. Axelrod. 1976. Adrenaline-forming enzyme in brain stem: elevation in genetic and experimental hypertension. Science. 191:483-484.

Sako, Y. 1963. Hemodynamic changes during arteriography. J. Am. Med. Assoc. 83:253-256.

Schmidt, C.F., S. Kety, and H. Pennes. 1945. Gaseous metabolism of the brain of the monkey. Am. J. Physiol. 143:33-52.

Serota, H., and R. Gerard. 1938. Localized thermal changes in the cat's brain. J. Neurophysiol. 1:115-124.

Severinghaus, J. 1966. Blood gas calculator. J. Appl. Physiol. 21:1108-1116.

Seylaz, J., P. Aubineau, L. Edvinsson, H. Mamo, K. Nielsen, C. Owman, and R. Sercombe. Regional differences in beta-adrenergic effects on local cerebral blood flow and in sympathetic vascular innervation. Stroke 4:369-370.

Shalit, M., O. Reinmuth, S. Shimojyo, and P. Scheinberg, 1967. Carbon dioxide and cerebral circulatory control II. The intravascular effect. Arch. Neurol. 17:337-341.

Sharpless, S., and H. Jasper, 1956. Habituation of the arousal reaction. Brain 79:655-680.

Smith, A.L., G.R. Neufeld, A.J. Ominsky, and H. Wollman, 1971. Effect of arterial CO_2 tension on cerebral blood flow, mean transit time, and vascular volume, J. Appl. Physiol. 31:701-707.

Sokoloff, L. 1959. The action of drugs on the cerebral circulation. Pharmacol. Rev. 11:1-85.

Sokoloff, L. 1961. Local cerebral circulation at rest and during altered cerebral activity induced by anesthesia or visual stimulation p. 107-117. In Regional Neurochemistry, S.S. Kety, and J. Elkes (eds.) Pergamon Press, New York.

Soloman, A.K. 1974. Equations for tracer experiments. J. Clinical Investigation 28:1297-1307.

Stone, H., M. Raichle, and M. Hernandez. 1974. The effect of sympathetic denervation on cerebral CO_2 sensitivity. Stroke 5:13-18.

Stone, T. 1971. Nor-adrenaline excitations artefacts? Nature 234: 145-146.

Studer, R., and J. Potchen. 1971. The radioisotopic assessment of regional microvascular permeability to macromolecules. Microvascular Res. 3:35-48.

Takaori. S., Y. Nakai, M. Sasa, and K. Shimamoto. 1966. Central depressants and evoked click responses with special reference to the reticular formation in the cat. Jap. J. Pharmacol. 16:264-275.

Ter-Pogossian, M.M., M.E. Phelps, R.L. Grubb, and M. Gado. 1971/72. Measure of regional cerebral blood volume in vivo by means of excited fluorescent x-radiation,and factors affecting this parameter. Europ. Neurol. 6:218-223.

Tinney, J.B. 1971. In vivo fluorescence analysis-concepts and equipment. In: Semiconductors detectors in the future of nuclear medicine, P.B. Hoffer, R.N. Beck, and A. Gottschalk (eds.) New York, Society Nuclear Med. pp. 214-229.

Truex, R., and M. Carpenter. 1969. Human Neuroanatomy. 6th ed., Williams and Wilkins Co., Baltimore.

Uchida, E., D. Bohr, and S. Hoobler. 1967. A method for studying isolated resistance vessels from rabbit mesentery and brain and their responses to drugs. Circulat. Res. 21: 525-536.

Waltz, A. 1968. Effect of Pa_{CO_2} on blood flow and vessel size of ischemic cerebral cortex. Circulation 38:(Suppl. 6) 201.

Waltz, A.G., A.R. Wanck, and R.E. Anderson. 1972. Comparison of analytic methods for calculation of cerebral blood flow after intracarotid injection of ^{133}Xe. J. Nuc. Med. 13:66-72.

Weed, L. 1914. The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. J. Med. Res. 31:51-91.

Woldseth, R. 1973. All you ever wanted to know about X-ray energy spectrometry. Kevex Corporation, Burlingame, California.

Yoshida, K., J. Meyer, K. Sakamoto, and J. Handa. 1966. Autoregulation of cerebral blood flow. Electromagnetic flow measurement during acute hypertension in the monkey. Circ. Res. 19:726-738.