J. Biosci., Vol. 11, Numbers 1–4, March 1987, pp. 23–33. © Printed in India.

Metabolism of an isolated brain perfused with perfluoro blood substitute

B. MUKHERJI and H. A. SLOVITER*
Harrison Department of Surgical Research and Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 191 04, USA

Abstract. An unanesthetized, isolated, perfused rat brain, consisting of the skull and its contents with nearly all other tissues removed, has metabolic and electrical activity similar to that of the brain of the intact rat with its blood-brain barrier intact. Its use yielded results that are difficult or impossible to obtain from *in vitro* preparations or *in vivo.* With the perfused brain it was shown that, mannose can completely replace glucose as metabolic substrate, that insulin has no direct effect on the brain, in the absence of added substrate glutamate is metabolized to aspartate, the brain does not metabolize ethanol, and morphine probably inhibits mitochondrial oxidative activity. Since the use of a perfluoro blood substitute to perfuse the brain avoids the optical interference caused by haemoglobin, it was possible to measure changes in the oxidation-reduction state of NADH by surface fluorometry of the cerebral cortex.

Keywords. Brain metabolism; perfused brain; perfluoro chemicals; blood substitute.

Use of an isolated brain

When an animal is treated with a drug or other substance that causes some change in the function of the brain, the observed change may be due to any or all of 3 following actions: (i) a direct action of the substance on the brain, (ii) an action on the brain of a metabolite of the substance, and (iii) an effect on the brain which is secondary to the action of the substance or its metabolite on another organ. It is usually not possible to determine which of these actions is responsible for the observed effect. Further difficulties in the interpretation of *in vivo* experiments result from the artifacts due to the excitement or other stimuli caused by handling of the animal or by administration of a drug, or due to the use of an anesthetic agent.

Studies *in vitro* with brain tissue preparations (slices, homogenates, subcellular particles, etc.) provide useful information about the direct action of agents on tissue. These *in vitro* methods often permit the separation of the effects of a drug from those of its metabolites; however such experiments indicate those activities that may occur, but are not necessarily those that do occur, under physiological conditions. It is questionable whether measurements made with isolated pieces of brain tissue having no blood circulation, no blood-brain barrier, and sometimes little or no neural organization can be valid indicators of the functions of the intact brain. Furthermore, these tissue preparations usually contain injured cells and sometimes are incubated in an artificial and nonphysiological environment.

Many of the limitations and disadvantages of the *in vivo* and *in vitro* methods can be avoided by the use of an isolated brain maintained in viable condition by perfusion

*To whom all correspondance should be addressed.

Abbreviations used: EEG, Electroencephalogram; GABA, γ-aminobutyric acid; DMSO, dimethyl sulphoxide.

with a suitable fluid by means of a pump-oxygenator. The brain has been isolated from other tissues in varying degrees in different preparations. Various degrees of vascular isolation, necessary to avoid the influence of other organs, have been achieved in different preparations. Neural isolation has ranged from complete isolation, with removal of both cranial and spinal nerve input, to no isolation at all in different perfused preparations. In general, the perfused brain preparations resembled the brain of the intact animal in both its metabolic and electrical activities. However, the perfused brain preparation is not identical to the brain of the intact animal.

The perfused brain preparation permits control of variables, such as blood flow, temperature, and the composition of circulating blood, that are difficulty or impossible to control in the intact animal. For example, it was possible to study changes in an isolated brain when neither glucose nor any other metabolic substrate was present in the perfusion fluid (Mukherji *et al.,* 1971) whereas a blood glucose level of zero in the intact animal would rapidly have resulted in convulsions and death. Studies with radioactive isotopes are greatly facilitated by the isolated brain preparation, since the small volume of perfusion fluid and the absence of competition from other organs can markedly reduce the amount of radioactivity required for tracer studies.

Perfused brain preparations

The first perfused brain preparation was that of Heymans and Kochmann (1904) in which the decapitated head of a dog was perfused through its carotid arteries by anastomosis to the carotid arteries of a second dog. In a later modification of the preparation by Bouckaert and Jourdan (1956), the brain was perfused by a pump.

Another early perfused brain preparation was that of Schmidt (1928), in which the arterial circulation to the brain of a dog or cat was partially occluded and then perfusion fluid (blood) was pumped into the vertebral artery at a pressure higher than that in the aorta. This principle of circulatory isolation of the brain was also used by Moss (1964), to perfuse a calf brain *via* a carotid artery, and by Thompson *et al.* (1968), to perfuse the head of a rat *via* the aorta.

Geiger and Magnes (1947) developed a perfused cat brain preparation in which the arterial circulation to the brain was not isolated, but the venous outlets from the brain were occluded and a single venous outlet from a cerebral venous sinus was made. The central and peripheral nervous systems were kept intact, so they were able to observe a variety of neurological functions during their experiments.

White *et al.* (1964) prepared the most completely isolated of the perfused brain preparations when they removed all tissues except a small piece of the skull from the brain of a monkey. Circulatory isolation was complete, and neural isolation was almost complete, since all cranial (except the eighth) and spinal nervous connections were severed. They also used a preparation in which the isolated monkey brain was kept inside the skull and concluded that retention of the skull was desirable for protection and maintenance of the environment of the brain. Presumably, removal of the skull and dura was conducive to the development of cerebral edema.

We have used the isolated rat brain preparation developed by Andjus *et al.* (1967), which consisted of the skull and its contents with nearly all extracranial tissue removed. In contrast to all other perfused brain preparations, this one was prepared

without the use of a chemical anesthetic agent. The preparation was made from a rat that was adequately anesthetized for the surgical procedure by deep hypothermia (rectal temperature 16°C). The use of a chemical anesthetic agent is undesirable because it is known (Smith and Wollman, 1972) that the anesthetic drugs have marked effects on the metabolic activities of the brain. Even short-acting or volatile anesthetic agents may not be entirely eliminated from an isolated brain which has no liver or kidney associated with it, and the effects of the agent may persist for a considerable time after the agent is removed. In addition, the use of hypothermia during surgery diminishes bleeding, simplifies the surgical procedure, and reduces the hazard of the occurrence of shock. Furthermore, the decreased metabolic requirements of the brain during hypothermia reduce the hazard of damage to the brain by transient anoxia.

The isolated rat brain was perfused through catheters placed in the internal carotid arteries, with all other arterial channels occluded. The position of the catheters and the arrangement of the blood vessels are illustrated schematically in figure 1. Perfusion at a slow rate was started before the vertebral arteries were ligated so that at no time was there an interruption of circulation through the brain. As the extracranial tissues were removed, the perfusion rate was gradually increased. The vertebral column and spinal cord were cut and the completed preparation was mounted above the collecting funnel of the perfusion system as shown in figure 1. All venous channels were open and the venous drainage ran by gravity into the collecting funnel, then into the oxygenator-reservoir, from which it was pumped back to the brain. The entire surgical procedure was performed by a single operator without assistance in less than 1 h.

Perfusion with perfluoro blood substitute

The perfusion fluid used in our earlier experiments was a freshly prepared suspension (25% hematocrit) of washed dog erythrocytes in an 8% solution of purified bovine serum albumin in Krebs-Ringer bicarbonate buffer containing 11 mM glucose. In later experiments, Sloviter and Kamimoto (1967) found that the erythrocytes could be replaced advantageously by ultrasonically dispersed particles (*ca.* 0·2 μm diam) of fluorochemical which transported oxygen and carbon dioxide in the physically dissolved form. The fluorochemical was a commercial perfluoro compound (perfluorobutyltetrahydrofurane), which is a colorless, inert liquid. When equilibrated with oxygen, it contains about 10% more oxygen than does an equal volume of erythrocytes. A 15% (v/v) emulsion of this perfluoro compound in the same solution of bovine albumin that was used with erythrocytes was an entirely satisfactory perfusion fluid in maintaining the activities of the isolated brain. The spontaneous electrical activity of the isolated brain (electroencephalogram, EEG) is a very sensitive criterion of its functional condition. Cessation of perfusion for 30 s caused loss of the EEG activity, and when the isolated brain was perfused with bovine albumin solution alone, the EEG never persisted for more than 5 min. Figure 2 shows EEG recordings of an isolated brain perfused with the suspension of erythrocytes and those of another brain perfused with the emulsified perfluoro compound. The nature and duration of the EEG activity of the brain perfused with perfluoro compound were very similar to those of the brain perfused with erythrocytes. This indicates that the

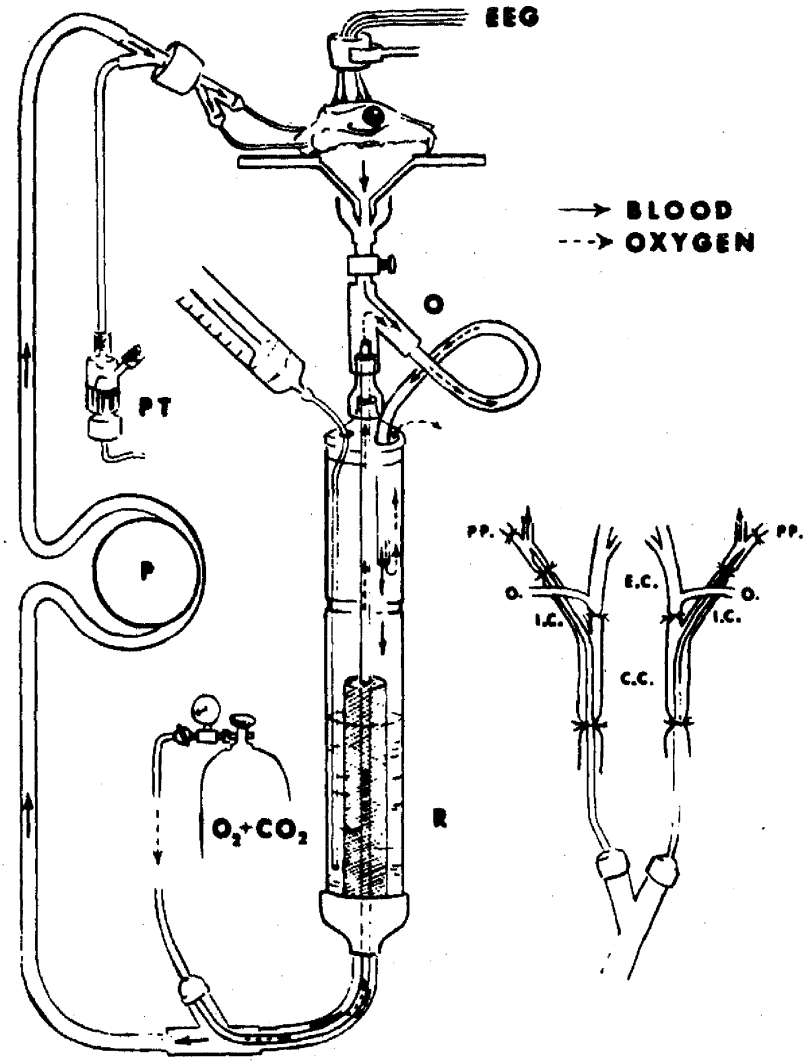

Figure 1. Schematic diagram of the system for perfusion of the isolated rat brain preparation. Venous efflux from the preparation is dispersed by the stream of gas (5% CO_2/95% O_2) into short segments and carried through the side branch of the oxygenator (O) into the reservoir (R). The roller pump (P) has a continuously variable speed. Perfusion pressure is monitored by a pressure transducer (PT). Reproduced with permission from (Andjus *et al.*, 1967).

perfluoro compound is adequately performing the gas transport functions of the erythrocytes. The use of the dispersed fluorochemical is advantageous because it is very easily prepared and thus eliminates the very tedious process of exhaustively washing erythrocytes. A further important advantage of the fluorochemical is that it is colorless and does not fluoresce and, therefore, permits spectrophotometric and fluorometric measurements which cannot be done in the presence of hemoglobin.

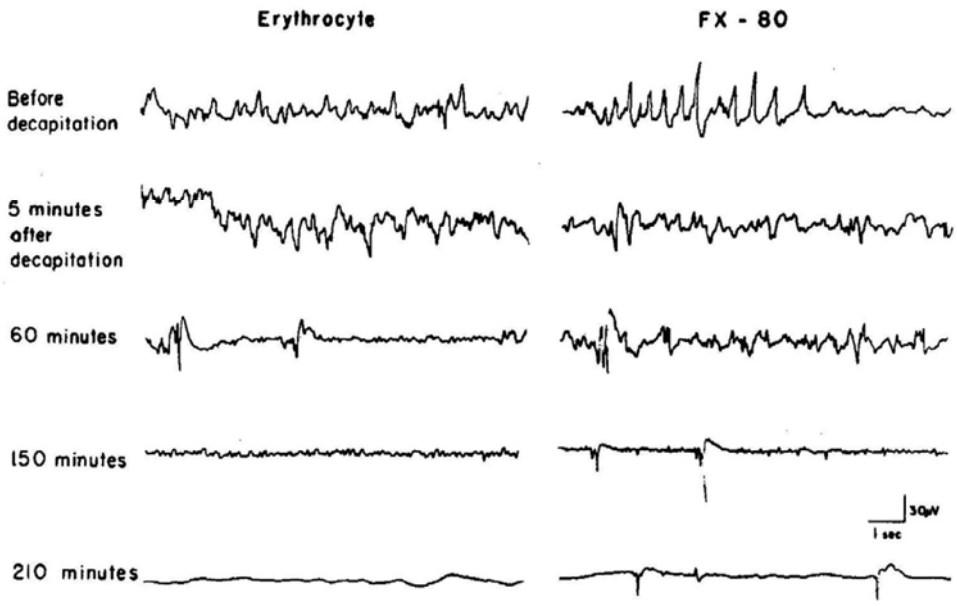

Figure 2. Spontaneous electroencephalographic activity of an isolated rat brain perfused with a suspension of erythrocytes (recordings on the left) and another perfused with a suspension of the dispersed fluorochemical FX-80 (recordings on the right) at various times. Reproduced with permission from Sloviter and Kamimoto (1967).

Blood-brain barrier in perfused brain

In the isolated, perfused rat brain preparation, the blood-brain barrier appears to be intact. This is indicated by the fact that pyruvate cannot replace glucose as a substrate for the maintenance of the electrical and metabolic activities of the brain. It is known (Elliott *et al.,* 1942) that brain slices or homogenates utilize pyruvate readily and that when both glucose and pyruvate are available, pyruvate is utilized preferentially by brain tissue *in vitro.* However, the brain of the intact animal is apparently unable to utilize pyruvate in the circulating blood at an appreciable rate (Maddock *et al.,* 1939). A further indication that the blood-brain barrier was intact in the isolated, perfused rat brain was the fact that the free amino acids of the brain tissue did not leak out during perfusion. After 2 h of perfusion of the isolated rat brain, no detectable amounts of glutamate, aspartate, N-acetylaspartate, or γ-aminobutyric acid (GABA), and only a trace of glutamine were present in the perfusion fluid (Andjus *et al.,* 1967). Furthermore, the addition of glutamate to the perfusion fluid did not cause an increase in the concentration of glutamate in the brain tissue. Therefore, it appears from these measurements that the blood-brain barrier functions in the perfused rat brain in the same way as in the brain of the intact rat.

Metabolic studies

The first dependable measurement of the rate of oxygen consumption by the intact brain was obtained by Chute and Smith (1939) with a perfused cat brain preparation.

The major problem in measuring the respiratory rate in the brain of the intact animal is the difficulty in obtaining reliable measurements of the rate of cerebral blood flow. This difficulty is avoided in the perfused brain because the rate of blood flow is easily controlled. Thus, these authors perfused the cat brain at 60–90 ml/min and observed arteriovenous differences in oxygen concentrations which gave values for oxygen consumed by the brain of 3˙3–5˙0 ml O_2/min per 100 g.

Geiger and Magnes (1947) and Geiger (1958) made a large number of studies on the metabolism of the perfused cat brain. Sacks (1961) evaluated the metabolic results obtained with this perfused cat brain preparation.

Our isolated rat brain preparation utilized glucose at an approximately linear rate for more than 3 h when perfused in a closed circuit with a small volume of perfusion fluid. From measurements of changes in glucose concentration and the known volume of the perfusion fluid, the rate of utilization of glucose by the brain was calculated. In experiments performed at 37°C, Sloviter and Yamada (1971) obtained values for glucose consumption by the perfused rat brain of 35 μmol/h/g. In the intact rat, Cremer (1970) obtained a value for glucose consumption by brain of 32 μmol/h/g. Lactate accumulated in the fluid perfusing the isolated rat brain at about 10 μmol/h/g. A considerable proportion of this lactate is produced by injured tissue (the spinal cord where it is ligated and cut) and by bone and remnants of muscle. In later experiments the contribution of extracranial and injured tissue was excluded by establishing a single channel of venous return from a catheter placed in the cerebral venous sinus, and the observed rate of lactate production by the perfused brain was much lower.

The intermediary metabolism of the perfused rat brain has been characterized by measurements of the concentrations of the intermediates of glycolysis in the perfused brain tissue (Ghosh *et al.,* 1976). These values and also the concentrations of creatine phosphate and the adenine nucleotides are listed in table 1. The effects of drugs or other agents or conditions on these values will give some indication of the nature of the alterations in metabolism. Changes in the levels of the glycolytic intermediates will indicate an effect on the regulation of glycolysis, while changes in the energy reserves will suggest an effect on mitochondrial function.

The isolated, perfused rat brain has also been used to study the ability of the brain to utilize substrates other than glucose. It was generally believed that the intact mammalian brain has an absolute requirement for glucose and that no other substance can entirely replace it. Experiments (Ghosh *et al.,* 1972) with the isolated, perfused rat brain have shown that its electrical and metabolic activity were maintained just as well with mannose as the metabolic substrate as with glucose. The glycolytic intermediates, the energy reserves, and the oxidative status of the brain tissue perfused with mannose were not materially different from those of the tissue perfused with glucose.

Amino acids

The concentrations of the free amino acids in the perfused brain tissue were also measured (table 2). When the rat brain was perfused with fluid containing no glucose or other metabolic substrate, there were marked reductions in the levels of glutamic acid and glutamine and an increase in the aspartic acid level, almost equal to the sum

Table 1. Concentrations of glycolytic intermediates and energy reserves in cerebral tissue of isolated, perfused rat brains.

	nmol/g wet wt ± SD
Glycogen (as glucose)	860 ± 100
Glucose-6-phosphate	73·0 ± 8·4
Fructose-6-phosphate	20·0 ± 4·0
Mannose-6-phosphate	228 ± 36
Fructose-1,6-diphosphate	93 ± 11
Triose phosphate	41·1 ± 4·6
3-Phosphoglycerate	39·8 ± 5·2
2-Phosphoglycerate	14·8 ± 1·9
Phosphoenolpyruvate	7·7 ± 1·2
Pyruvate	60·8 ± 6·4
Lacate	1,850 ± 400
Creatine phosphate	2,550 ± 230
ATP	1,990 ± 140
ADP	720 ± 50
AMP	240 ± 40
ATP + ADP + AMP	2,950 ± 130
Lactate/pyruvate	31 ± 7
ATP/ADP	2·93 ± 0·37

Table 2. Concentration of free amino acids in cerebral tissue of isolated, perfused rat brains.

	μmol/g wet wt ± SD
Glutamic acid	6·15 ± 0·56
Glutamine	7·39 ± 0·31
GABA	2·36 ± 0·08
Aspartic acid	2·24 ± 0·12
N-Acetylaspartic acid	6·94 ± 0·31

of the decreases in glutamic acid and glutamine. These results suggested that glutamate and glutamine had been converted to ketoglutarate, which was then oxidized *via* the tricarboxylic acid cycle to oxaloacetic acid. Since acetyl-coenzyme A was not available (owing to the lack of glucose) to react with the oxaloacetate, it was converted to aspartic acid. This partial oxidation of glutamate and glutamine to aspartate provided some energy for the brain, which was deprived of its normal source of metabolic energy.

The presence of a large amount of N-acetylaspartic acid (Tallan *et al.*, 1954) in the brain and its virtual absence from other tissue suggests that this free amino acid has some specialized function in the brain. However, no function has been found. When the isolated rat brain was perfused with fluid containing radioactive acetate (Mukherji and Sloviter, 1973), much of the radioactivity was incorporated into glutamate, glutamine, GABA, and aspartate, but very little into N-acetylaspartic. These results indicated that the N-acetylaspartic acid was metabolically inert and that it was not in equilibrium with free aspartic acid in the brain.

Pharmacologic studies

Ethanol

There are conflicting reports concerning the presence of alcohol dehydrogenase in mammalian brain and the ability of the brain to metabolize ethanol. Most of the early studies with brain tissue preparations in *vitro* concluded that brain tissues do not metabolize ethanol (Beer and Quastel, 1958). The question was opened anew when Raskin and Sokoloff (1974) using highly sensitive methods, detected small amounts of alcohol dehydrogenase activity in the soluble fraction of homogenates of whole rat brain and also reported that chronic ingestion of ethanol caused an increase in the alcohol dehydrogenase activity of cerebral tissue. These conflicting results from *in vitro* experiments did not answer the question of whether the intact mammalian brain can metabolize ethanol. It is difficult to obtain unambiguous results concerning this question from experiments in intact animals, because the metabolism of ethanol by the liver is so large and so rapid. In such experiments, one cannot exclude the possibility that acetaldehyde, or acetate produced in the liver from ethanol, was carried to the brain and further metabolized there. For this reason, the isolated, perfused brain was particularly well suited for such experiments, since it provided an intact brain with no interference from liver or other organs.

If [^{14}C]-ethanol were metabolized by the perfused brain, it would be converted to acetaldehyde and then to acetate. Perhaps the most sensitive, method to determine whether [^{14}C]-acetate was produced is to measure the incorporation of [^{14}C]-into amino acids in the brain. O'Neal and Koeppe (1966) found that when [^{14}C]-acetate was administered to rats, nearly all the radioactivity in the brain was in the free amino acids. Mukherji and Sloviter (1973) found that when the isolated rat brain was perfused with fluid containing [^{14}C]-acetate, there was considerable incorporation of radioactivity into the free amino acids of the cerebral tissue. Mukherji *et al.* (1975) performed experiments in which the isolated rat brain was perfused with fluid containing [^{14}C]-ethanol, [^{14}C]-acetaldehyde, or [^{14}C]-acetate. After perfusion, the cerebral tissue was rapidly frozen, extracts of the frozen tissue were prepared and the amino acids were separated and isolated by ion-exchange chromotography. The amount and the radioactivity of each amino acid were measured and specific activities were calculated. The results of these experiments are shown in table 3. After perfusion with either [^{14}C]-acetaldehyde or [^{14}C]-acetate, there was considerable incorporation of radioactivity into all the free amino acids of the cerebral tissue. The perfusion with [^{14}C]-acetate was done with 10 times as much radioactivity as was present in the

Table 3. Incorporation of radioactivity into amino acids of isolated perfused rat brain.

	Perfused with 10 μCi [1-^{14}C]-acetate (cpm/μmol)	Perfused with 1 μCi [1,2-^{14}C]-acetaldehyde (cpm/μmol)	Perfused with 5 μCi [1-^{14}C]-ethanol (cpm/μmol)
Glutamine	23,653	1,575	0
Glutamic acid	8,112	1,140	0
Aspartic acid	7,512	2,320	0
GABA	6,595	920	0

Perfusion with [^{14}C]-acetaldehyde. If the values for radioactivity incorporated from [^{14}C]-acetate are divided by 10, the values are of the same order of magnitude as those obtained with [^{14}C]-acetaldehyde.

The absence of incorporation of radioactivity from [^{14}C]-ethanol into the free amino acids of the brain showed that the perfused rat brain did not metabolize ethanol at a measurable rate. Since there was incorporation of [^{14}C]-acetaIdehyde and [^{14}C]-acetate into the free amino acids, it is apparent that the metabolism of ethanol did not occur because there was no measurable amount of alcohol dehydrogenase activity in the cerebral tissue. The fact that there was incorporation of radioactivity from acetaldehyde indicated that there is aldehyde dehydrogenase activity in cerebral tissue (Erwin and Dietrich, 1966). It does not exclude the possibility that the aldehyde dehydrogenase reaction is a rate-limiting one, since the permeability of the blood-brain barrier is probably greater to acetaldehyde than to acetate. Thus, if aldehyde dehydrogenase activity were rate limiting, the observed approximately equal incorporation might be due to a higher concentration of acetaldehyde than acetate in the cerebral tissue.

Insulin

It is generally believed that insulin does not have a direct effect on the metabolism of the brain; Quastel (1970) has reviewed the evidence for this belief. However, Rafaelsen (1967) has reviewed other experiments with results that have been interpreted as indicating that insulin does have a direct effect on the brain. The isolated perfused brain is well suited to determining whether insulin has a direct effect on the brain. It permits observations on the transport of substances from blood into the brain, which *in vitro* preparations do not. It also eliminates the difficulties of interpretation caused by secondary effects from other organs, which occur in intact animals. Sloviter and Yamada (1971) perfused the isolated rat brain with fluid containing insulin or perfused the brain of a rat previously injected with insulin. The spontaneous electrical activity of the brain, the rate of cerebral consumption of glucose and the rate of efflux of potassium from the brain were not affected by insulin. It was concluded that insulin does not act directly on the brain and that the effects on brain metabolism observed after administering insulin and glucose to the intact animal are probably secondary to the large stimulation of the metabolism of liver and other organs.

Dimethyl sulphoxide

Dimethyl sulphoxide (DMSO) has been shown to alter the permeability of cells and to enhance the transport of physiological substances and drugs across the blood-brain barrier (Hanig *et al.,* 1971). This property of DMSO makes it a potentially valuable agent for the study of psychopharmacologic agents. Ghosh *et al.* (1976) investigated the changes in metabolism of the isolated rat brain during perfusion with fluid containing DMSO. DMSO caused an increase in the rate of glycolysis, a slight decrease in the energy reserves (creatine phosphate and ATP) of the brain and a shift to a more reduced state (increase in lactate/pyruvate ratio) in the brain tissue. It was suggested that the observed effects were probably due to an inhibitory action of DMSO on mitochondrial oxidative activity.

Morphine and methadone

Variable results have been obtained in studies of the effects of the acute administration of morphine on the metabolism and energy reserves of brain tissue (Veech *et al.,* 1973). Mukherji *et al.* (1980) studied the effects of morphine and also of methadone on the cerbral cortex of the isolated, perfused rat brain. Perfusion of the isolated rat brain with fluid containing morphine (50 μg/ml) caused increases in both glucose consumption and lactate production; perfusion with methadone (50 μg/ml) caused an increase in glucose consumption but no increase in lactate production. Perfusion with morphine caused a marked decrease in the energy reserves of the brain tissue but perfusion with methadone caused only a slight decrease. The results suggested that morphine may inhibit mitochondrial oxidative activity.

Oxidation-reduction state

Since the perfluoro compound, used in place of red blood cells in the perfusion fluid, has no optical absorption or fluorescence, its use avoids the interference in optical measurements caused by haemoglobin. Thus, in the perfused brain preparation, which contained no haemoglobin, it was possible to determine the redox state of pyridine nucleotides (NADH) in cerebral cortex by fluorescence measurements using quartz fiber optics. It was found that increase in fluorescence from NADH caused by anoxia was about the same in the perfused brain as in the brain of the intact rat (Mayevsky *et al.,* 1981).

References

Andjus, R. K., Suhara, K. and Sloviter, H. A. (1967) *J. Appl. Physiol.,* **22,** 1033.
Beer, C. T. and Quastel, J. H. (1958) *Can. J. Biochem. Physiol.,* **36,** 531.
Bouckaert, A. J. and Jourdan, F. (1956) *Arch. Int. Pharmacodyn. Ther.,* **53,** 523.
Chute, A. L. and Smyth, D. H. (1939) *Q. J. Exp. Physiol.,* **29,** 379.
Cremer, J. E. (1970) *Biochem. J.,* **119,** 95.
Elliott, K. A. C., Scott, D. B. M. and Libet, B. (1942) *J. Biol. Chem.,* **146,** 251.
Erwin, V. G. and Dietrich, R. A. (1966) *J. Biol. Chem.,* **241,** 3533.
Geiger, A. (1958) *Physiol. Rev.,* **38,** 1.
Geiger, A. and Magnes, J. (1947) *Am. J. Physiol.,* **149,** 517.
Ghosh, A. K., Mukherji, B. and Sloviter, H. A. (1972) *J. Neurochem.,* **19,** 1279.
Ghosh, A. K., Ito, T., Ghosh, S. and Sloviter, H. A. (1976) *Biochem. Pharmacol.,* **25,** 1115.
Hanig, J. P., Morrison, J. M. and Krop, S. (1971) *J. Pharm. Pharmacol.,* **23,** 386.
Heymans, J. F. and Kochmann, M. (1904) *Arch. Int. Pharmacodyn. Ther.,* **13,** 379.
Maddock, S., Hawkins, J. E. and Holmes, E. (1939) *Am. J. Physiol.,* **125,** 551.
Mayevsky, A., Mizawa, I. and Sloviter, H. A. (1981) *Neurol. Res.,* **3,** 307.
Moss, G. (1964) *J. Surg. Res.,* **4,** 170.
Mukherji, B. and Sloviter, H. A. (1973) *J. Neurochem.,* **20,** 633.
Mukherji, B., Turinsky, J. and Sloviter, H. A. (1971) *J. Neurochem.,* **18,** 1783.
Mukherji, B., Kashiki, Y., Ohyanagi, H. and Sloviter, H. A. (1975) *J. Neurochem.,* **24,** 841.
Mukherji, B., Suemaru, K., Sakai, N., Ghosh, A. K. and Sloviter, H. A. (1980) *Biochem. Pharmacol.,* **29,** 1608.
O'Neal, R. M. and Koeppe, R. E. (1966) *J. Neurochem.,* **13,** 835.
Quastel, J. H. (1970) *Neurosci. Res.,* **3,** 1.
Rafaelsen, O. J. (1967) *Acta. Med. Scand.* (Suppl), **476,** 75.
Raskin, N. H. and Sokoloff, L. (1974) *J. Neurochem.,* **22,** 427.

Sacks, W. (1961) *Handbook Neurochem.,* **1,** 301.
Schmidt, C. F. (1928) *Am. J. Physiol.,* **84,** 202.
Sloviter, H. A. and Kamimoto, T. (1967) *Nature (London)* **216,** 458.
Sloviter, H. A. and Yamada, H. (1971) *J. Neurochem.,* **18,** 1269.
Smith, A. L. and Wollman, H. (1972) *Anesthesiology,* **36,** 378.
Tallan, H. H., Moore, S. and Stein, W. J. (1954) *J. Biol. Chem.,* **211,** 927.
Thompson, A. M., Robertson, R. C. and Bauer, T. A. (1968) *J. Appl. Physiol,* **24,** 407.
Veech, R. L., Harris, R. L., Veloso, D. and Veech, E. H. (1973) *J. Neurochem.,* **20,** 183.
White, R. J., Albin, M. S. and Verdura, J. (1964) *Nature (London),* **202,** 1082.