UTILIZATION OF GLUCOSE AND VOLATILE FATTY ACIDS BY CANINE AND CAPRINE BRAIN

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BY CANINE AND CAPRINE BRAIN

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CHAPTER I

INTRODUCTION

It is generally accepted that under normal physiological conditions glucose is the major energy source for brain metabolism in mammals. Previous work has shown that energy metabolism in ruminant animals differs somewhat from energy metabolism of non-ruminant animals. In non-ruminant animals, glucose is the major end-product of carbohydrate digestion and its absorption occurs primarily from the small intestine. In ruminant animals, most of the dietary carbohydrates are converted by rumen microorganisms to volatile fatty acids (primarily acetic, propionic, and n-butyric). These volatile fatty acids are absorbed directly from the rumen and pass into the portal circulation. Only small amounts of glucose escape this action and are absorbed from the small intestine. For this reason, the additional glucose requirements of ruminant animals must be met through gluconeogenesis.

The concentration of glucose found in ruminant blood is low (40-60 mg/100 ml blood) when compared to non-ruminant blood glucose levels (90-110 mg/100 ml blood). Despite this, the ruminant animal lives a very normal life at these levels which in the non-ruminant animal would be considered a severe hypoglycemia and would cause central nervous system disturbances. Because of the marked species difference in blood glucose concentration, it is probable that a difference in brain tissue metabolism in ruminant and non-ruminant animals exists. This difference

could be that there is a more efficient system for moving glucose from blood to brain tissue in ruminant animals or that metabolites other than glucose may become important sources of energy for brain tissue metabolism in these animals.

Large quantities of volatile fatty acids are formed by rumen microorganisms and subsequently absorbed into the blood. It is well established that these acids are an important source of energy in the ruminant animal. The possibility exists that these acids may constitute an important energy source for ruminant brain tissue and thus overcome the apparent ruminant hypoglycemia.

The advent of electronic blood-flowmeters has made possible the assessment of relative blood flow to the brain. By measuring blood flow to the brain and simultaneously determining the concentration of glucose and volatile fatty acids (acetic, propionic, and butyric) in both arterial blood and venous blood supplying the brain, a quantitative evaluation of the metabolites used per weight of brain tissue per unit time can be determined.

The in vivo study reported herein was designed to compare the role of glucose and volatile fatty acids as energy sources for brain tissue in ruminant and non-ruminant animals.

CHAPTER II

REVIEW OF LITERATURE

Carbohydrate Metabolism

There is general agreement that glucose is the main substrate used for oxidative metabolism by the mammalian brain under normal physiological conditions (1). Only glucose is taken up by the brain from the blood in sufficient amounts to satisfy brain metabolic requirements (24). Since the oxidative metabolism of the mammalian brain depends primarily on glucose as a substrate, there is a relative lack of versatility which, in turn, renders the central nervous system peculiarly vulnerable to a variety of agents or conditions which can at one point or another upset its delicate metabolic balance (66).

Geiger et al. (24) suggested that glucose is the carbon source for all the main metabolic processes in the brain including the production, by exchange or by synthesis, of most amino acids, proteins, and lipids. Their experiments indicated that in addition to glucose, comparatively large amounts of other substances are constantly oxidized to CO_2, and that carbon derived from ^{14}C-glucose is incorporated into amino acids and other compounds in the brain in the resting state. This agrees with the work of Sacks (59) who found that in hypoglycemia in humans, glucose consumption by brain tissue is reduced more than oxygen utilization, indicating oxidation of other substrates. This further points to the possibility that ruminant animals, living in an apparent hypoglycemic

state, may, indeed, use other metabolites for energy.

In experiments conducted by Bergman (8), it was shown that there is a lower rate of glucose oxidation and metabolism in ruminant animals than in non-ruminant animals. It is well known that ruminant animals absorb little or no glucose from their digestive tracts inasmuch as rumen microorganisms rapidly convert dietary carbohydrates to volatile fatty acids. It has been observed that glucose oxidation and its contribution to CO_2 is lower in sheep than in non-ruminant animals (8). These findings support the concept of Baxter et al. (7) that glucose metabolism is relatively lower in ruminants than in non-ruminants. One cannot say that it is less important in ruminants, however, since an optimal rate of glucose production and utilization is definitely required (8).

Numerous experiments have shown that only about one-fourth of the glucose taken up by the brain is oxidized to CO_2, which, in turn, accounts for approximately 20% of the O_2 consumed. Most of the remaining glucose is converted to lactic acid. Using ^{14}C-labeled glucose, evidence has been obtained suggesting that the greater part of the remaining O_2, which is used by the brain is used for oxidation of non-glucose endogenous substances to CO_2 (1,18). These results agree with those of Geiger (23), who demonstrated that only 30-35% of the glucose taken up by the cat brain was directly oxidized during resting conditions. Another 20-30% was rapidly transformed into acid-soluble components, among which amino acids figured prominently. Quantitatively, next to carbohydrates, endogenous proteins seemed to be the most important substrates for cat brain metabolism.

Gainer et al. (20) demonstrated that metabolic CO_2 produced by the anaesthetized intact dog brain was derived almost exclusively from

glucose. When convulsions were induced by electroshock, alterations in brain metabolism suggested that some of the metabolic CO_2 produced during the first 3 minutes of the convulsive episode was derived from non-glucose sources.

Gilboe (27), working with the isolated dog brain, found that glucose consumption was between 3.65-4.90 mg per 100 grams of brain tissue per minute. Anaerobic glucose metabolism alone did not appear to account for a substantial amount of the glucose uptake. The increased glycolysis observed by Geiger et al. (22) with isolated cat brain was probably due to the use of "simplified" blood. These findings suggest that a metabolic inhibitor is contained in normal blood.

The glucose that is utilized directly in the mammalian brain is metabolized almost entirely by the Embden-Meyerhof pathway and under normal conditions the direct oxidative pathway (hexosemonophosphate shunt) is of little importance (18). The hexosemonophosphate shunt has been implicated in the synthetic routes as providing reduced nicotinamide adenine dinucleotide phosphate (NADP) for lipid synthesis and as a carbon source for the de novo generation of nucleotides and the nucleic acids in newborn pups. Although the enzymes and cofactors necessary for the operation of the pentose phosphate pathway are present, most authors conclude that this pathway is normally inoperative in adult dog brain (49). Glucose-6-phosphate dehydrogenase, the enzyme leading into the hexosemonophosphate shunt, has been shown by Jones (34) to be more active in caprine brain than in canine brain.

Glucose metabolism by the Embden-Meyerhof pathway involves a series of non-oxidative reactions and one oxidative step leading to the production of pyruvic acid. Under anaerobic conditions, the oxidative step is

balanced by reduction of pyruvic acid to lactic acid. Under aerobic contitions, most of the pyruvic acid is oxidized through the tricarboxylic acid cycle to carbon dioxide and water. In glycolysis, 54 kilocalories are released or two units of high energy phosphate are produced per mole of glucose. In complete oxidation, about 690 kilocalories are released or about 38 units of high energy phosphate produced per mole of glucose (18).

In general, ruminant animals have lower arterio-venous glucose differences than non-ruminant animals, even though the general metabolic rates are comparable (8). The lower blood glucose levels in adult ruminant animals (40-60 mg per 100 ml blood) compared with the higher glucose levels in non-ruminants (90-110 mg per 100 ml blood) may be due to the ruminant animal's peculiar digestive system or it may reflect a lesser importance of glucose as a metabolite. In either case, it appears that gluconeogenesis from certain amino acids and fatty acids makes a major contribution to the glucose requirements of ruminant animals.

Volatile Fatty Acid Metabolism

The production in the rumen of large amounts of acetic acid, propionic acid, and butyric acid is well established. These are absorbed from the rumen, omasum, and small intestine and enter portal circulation. Under normal feeding conditions, propionate and butyrate are largely removed by the liver, but substantial quantities of acetate appear in peripheral blood (3).

Ruminant animals are known to derive as much as 70-80% of their total energy requirements from the metabolism of volatile fatty acids (9,33,42,48,52,62). The possible glucogenicity of acetate and butyrate

in sheep has been studied with liver slices (40), and no evidence was obtained for a net synthesis of glucose or glycogen from these substrates. Annison et al. (3) using intact sheep, confirmed these results that the incorporation of acetate carbon and butyrate carbon into glucose in sheep involves no net synthesis of carbohydrate. They further found that the incorporation of ^{14}C from ^{14}C-1 or ^{14}C-2-propionate into glucose was consistent with the conversion of propionate into a symmetrical C_4 compound before conversion into phosphoenolpyruvate, and was in agreement with the established pathway, namely, propionate → methylmalonate → succinate → phosphoenolpyruvate → glucose.

Studies by Weil (69) and by Paul et al. (51) suggest that increased utilization of free fatty acids by dog skeletal muscle tissue influences the metabolic rate of glucose and results in higher concentrations of tissue and, subsequently, of blood pyruvate and lactate.

The level of volatile fatty acids in the peripheral blood of sheep is considerably higher than in non-ruminant animals, arterial levels reaching 10 mg per 100 ml blood (as acetic acid) or higher. Small amounts of propionic and butyric and trace amounts of at least one other acid are also present. There was no evidence that acids other than acetic were removed by peripheral tissues in significant quantities (56).

Baxter et al. (7) suggested that the lower blood glucose levels in the cow may reflect a decreased quantitative importance of glucose as a metabolite. This latter view is supported by the observation that glucose may be partially replaced by acetate for energy metabolism in most ruminant tissues.

Ash et al. (5) suggested that in sheep the carbohydrate requirements

are met largely by gluconeogenesis. It can be predicted, from established metabolic reactions, that propionate can be converted to carbohydrate in the animal body. It is much less certain whether acetate or butyrate, the other two main acids produced in the rumen, can give rise to a net synthesis of carbohydrate in animal tissues. Weinman et al. (70) have concluded that such does not occur. It was shown that ^{14}C-labelled fatty acids were incorporated into glucose and that, in mammals, the major pathway by which such incorporation occurs is via acetyl-CoA entering the Krebs' cycle, decarboxylation of oxalacetate with the formation of phosphopyruvate, and the modified reversal of the Embden-Meyerhof scheme of glycolysis. It should be emphasized that the mere demonstration of incorporation of isotope into glucose from the labelled fatty acid does not necessarily mean that net synthesis of glucose has occurred in the animal body. Since two carbons (as acetyl-CoA) enter the Krebs cycle and two carbons are evolved as CO_2 with each turn of the cycle, no net carbons are made available via this cycle for synthetic reactions.

Extra-hepatic tissues (adipose, muscle, and heart) accounted for most of the acetate oxidation in sheep in a study conducted by Mayfied et al. (43). The decreasing order of acetate utilization by sheep tissues was adipose tissue, kidney, muscle, heart, lung, liver, and brain, when expressed as acetate incorporation per milligram of protein. This gave support to studies by Armstrong (4), who found that many sheep organs, including the heart and resting skeletal muscle, derive energy from oxidizing fatty acids or ketone bodies, but that nervous tissue had a primary requirement for glucose as a source of energy.

The metabolism of acetate, propionate, and butyrate by sheep-liver

slices was studied by Leng and Annison (4) using ^{14}C-labelled substrates. The production of labelled CO_2 and incorporation of radioactivity into glucose were determined. Propionate and butyrate were more readily oxidized than acetate, and propionate was incorporated into glucose to a greater extent than the other two substrates. They concluded that propionate could act as a source of glucose, but the incorporation of acetate and butyrate was consistent with entry into glucose as acetyl-CoA through the TCA cycle. Increased ketone-body production by liver slices was observed in the presence of acetate and butyrate.

Blaxter (10) has calculated that acetic acid is used as an energy source with less efficiency than glucose, but efficiency increases with the increasing chain length of fatty acids. For example, propionic acid is used more efficiently than acetic acid while butyric acid is used more efficiently than propionic acid.

Acetate may contribute as much as 50% of the ruminant's total energy supply (43). Biochemists have long recognized the central role of acetate in the energy metabolism of most forms of life. But, whereas for most organisms, the supply of acetate, or rather its active form, acetyl-CoA, is almost entirely endogenous, ruminant animals have a large exogenous supply of acetate, since it is produced in the rumen by microbial fermentation of ingested foodstuffs (58).

Holdsworth et al. (31) conducted experiments using labelled acetate and found it rapidly removed from the blood of sheep. Most of the activity recovered 6 minutes after injection was in the form of acid-soluble compounds. Specific activities in liver and heart following steady infusion and labelling was consistent with metabolism of much of the acetate via the TCA cycle.

Arterio-venous difference studies on the brain of sheep by McClymont and Setchell (44) failed to show a utilization of acetate. The mean glucose uptake by the brain (6.2 $\pm$ 0.7 mg per 100 ml of blood) was sufficient, assuming complete oxidation of the glucose, to account for the mean oxygen utilization of 4.8 $\pm$ 0.2 volumes per 100 ml of blood. These results imply that the sheep is similar to non-ruminant animals in that acetate is not utilized by the brain, and that the mean glucose uptake, assuming complete oxidation of the glucose, is sufficient to account for the mean oxygen uptake.

Stith (65), in an _in vitro_ study, found that acetic and propionic acids, when presented together as substrates and, to a lesser extent, propionic acid when presented by itself, were taken up by homogenates of caprine brain, but not to a significant degree. There was no evidence of fatty acid utilization by homogenates of canine brain.

O'Neal _et al._ (48) using ^{14}C-labelled acetate, propionate, and butyrate, concluded that the short-chain fatty acids enter the brain without prior metabolism by other tissues and are metabolized in the brain via the TCA cycle. In experiments with ^{14}C-labelled propionate, it was strongly suggested that brain tissue metabolized propionate via succinate and that this metabolic route may be a limited but nevertheless important source of dicarboxylic acids to the brain.

The major portion of ^{14}C-labelled carbon from butyrate injected into the omasum of calves appeared in the blood as beta-hydroxybutyrate in close association with blood lactate in experiments conducted by Joyner (35). Ramsey and Davis (54,55), working with ^{14}C-labelled butyrate, suggested that beta-hydroxybutyrate is a normal intermediate in the metabolism n-butyrate absorbed from the rumen, and that the rumen epithelium

is a major site of the conversion.

Experiments have been conducted with cows and goats to determine the normal concentrations of plasma free fatty acids and their relationship to blood sugars and blood ketones (53). It was suggested that blood sugar is a controlling factor in ketogenesis, under both fed and fasting conditions, but that plasma free fatty acids are a primary source of ketones only under fasting conditions.

Blood-Brain Barrier

When certain substances, e.g. thiocyanate, iodide, sucrose, etc., are injected into the blood stream, and their concentration in this fluid is maintained at a steady level, equilibrium with nervous tissue requires many hours or even days, whereas the equilibrium of the same substances with the extracellular space of muscle is attained in a matter of seconds or minutes. It is this phenomenon that has given rise to the term "blood-brain barrier", and has been attributed to the presence of exceptionally impermeable capillaries in nervous tissue, this permeability being either a characteristic of the capillary endothelium or due to the presence of some other cellular layer, e.g. one composed of the processes of glial cells (15). Earlier workers assumed that the transfer of glucose between blood and the cerebrospinal fluid (CSF) is dependent upon simple diffusion.

Geiger (22) found that perfusion of the cat brain with a semi-synthetic "simplified" blood caused brain tissue to lose its ability to take up glucose from the perfusing medium. It was further suggested that the uptake of glucose by the brain is an active process which is dependent on the presence of certain activators. However, Williams (71)

concluded that glucose diffuses directly into the spinal subarachnoid at a rapid rate in the dog, caused perhaps by a difference in anatomy of this species.

The mechanism of transfer of glucose from blood to brain tissue was studied in anaesthetized dogs by Crone (11,12). Using the "indicator diffusion" technique, it was found that the fraction of glucose which passed into the cerebral tissue decreased with increasing concentrations of glucose in the concentration range of 25-240 mg/100 ml blood. The extraction at low concentrations of glucose was almost 50% and that at high glucose concentrations was about 10%. This change in extraction rates suggests a carrier-mediated transport mechanism which facilitates the passage of glucose across the blood-brain barrier. The transport probably takes place both by diffusion and by a special transport mechanism which appeared to be saturated at concentration of about 70 mg/100 ml of blood. The capacity of this process was found to be 20-22 mg of glucose removed/100 ml of blood. These findings are supported by Fishman (19), who found in dogs that the glucose concentration in CSF approached a maximum, despite increasing intravenous loads of glucose, which suggested saturation kinetics.

Crone (11) suggested that the permeability of the blood-brain barrier reflects the permeability of the endothelial cells proper and the slow exchange of material in the brain is due to a very low permeability of the cerebral capillaries. The chorioid plexus has a concentrating mechanism for sugars and this mechanism is inhibited by anoxia, by metabolic poisons, by the lack of sodium, and by cardioactive steroids (13).

Blood Flow

Kety (38) found evidence for increased blood flow in those regions

of the brain that have experienced an increase in functional activity. There is a well-known ability of carbon dioxide to dilate cerebral vessels and decrease cerebrovascular resistance more profoundly than any other known substance. Neurogenic influences may also be present which affect cerebral blood flow.

Baldwin and Bell (6), in studying the blood flow to the brain in the sheep and calf, found that there is no vessel suitable for the insertion of a connection to a flowmeter to measure cerebral blood flow directly and exclusively in these animals due to anatomical differences in their blood-vascular system.

No method of flow measurement is free from technical error, and most, if not all, involve some degree of physiologic error. The electromagnetic flowmeter is currently the most widely used device for measuring blood flow in surgically exposed vessels. The principle of operation is Faraday's law: an electrically conductive material moving through a magnetic field induces an electric potential perpendicular to both the magnetic field and the direction of motion of the conductor. The electromagnetic transducers, or probes, are constructed of fine coils of wire embedded in plastic and produce a uniform magnetic field. Located on opposite points of the probe surrounding the vessel are electrodes that register the induced voltage, which may then be recorded as either pulsatile or mean flow (46,57).

It is well-known that cuff flowmeters are liable to considerable error in both base line zero and sensitivity (72). Dobson et al. (16) using chronic implantation of probes in sheep found that repeated calibration was possible with accurate zero flow. The sensitivity in vivo was 67-93% of that in vitro in accord with existing theory. However,

Sellers and Dobson (61) later found appreciable error, both in sensitivity and in zero setting. To obtain a zero setting, it is necessary to completely stop blood flow in the vessel under study. Occluding the vessel some distance from the probe, with the fair certainty of missing a side branch artery results in a zero error. On the other hand, occluding the vessel close to the probe alters the geometry of the vessel and also results in a zero error.

Kayser (36) compared the accuracy, baseline stability, noise, operation, and probe characteristics between a sine-wave and a square-wave excitation instrument during a six-week period and found no significant difference in their accuracy. O'Rourke (50) demonstrated that magnetic field non-uniformity within the probe of the sine-wave instrument did not alter the accuracy in recording steady or oscillatory flow.

Meyer et al. (45) measured the cerebral blood flow in the monkey by the nitrous oxide method and found flow to be 61 ml blood per 100 gm brain tissue per minute. Cerebral glucose consumption was 3.52 mg/100 gm brain per minute. Dumke et al. (17) reported values of 55.4 ml/100 gm brain per minute calculated from bubble flowmeter measurements of internal carotid artery flow in monkeys. These values are comparable to those found in man (37).

Baldwin and Bell (6) measured the cerebral blood flow in the sheep and the calf using the density flowmeter. It was found that there was considerable variation both between and within species. The average rate of blood flow in the carotid artery of the sheep was 214 ml/min, and that of the vertebral artery was 30 ml/min. In the calf, the average rate of flow was 191 ml/min in the carotid artery and 54 ml/min in the vertebral artery.

CHAPTER III

MATERIALS AND METHODS

Treatment of Animals

Adult dogs and goats of both sexes were chosen as the experimental animals. The dogs were placed in outdoor runs with adequate housing and were fed a commercially prepared dry dog food. The goats were kept in outdoor pens and were fed hay, a commercially prepared concentrate supplement, and, in season, had free access to bermuda pasture.

Preparation of Animals

Both dogs and goats were anaesthetized with freshly prepared pentabarbital solution containing 6.5 gm pentabarbital sodium dissolved in 30 ml ethyl alcohol and distilled water q.s. 100 ml. The concentration of this solution was 1 grain sodium pentabarbital per ml. The anaesthetic was given intravenously to effect in all animals. Body weights were recorded at the beginning of the experiment.

The animals were clipped along the lower neck, head, and the inside areas of both rear legs. Surgical isolation of the trachea permitted insertion of a tracheal cannula which prevented the possibility of saliva or regurgitated material entering the lungs during the experiment.

The right carotid and vertebral arteries were isolated and dissected relatively free of connective tissue. Blood-flow transducers were placed around each artery to permit the continuous measurement of blood flow in

these vessels throughout the experiment. The femoral artery and vein were dissected free from associated structures and polyethylene tubing inserted several inches into each to permit the injection of solutions into the vein and removal of blood from the artery. A constant flow infusion pump was attached to the cannula going to the femoral vein for the injection of solutions of volatile fatty acids. Arterial blood sampling was necessary for the determination of blood glucose and blood volatile fatty acid concentrations.

After making a midline incision along the frontal portion of the head, tissue was cleared away from the skull by blunt dissection. A small hole was drilled through the midline of the skull and into the saggital venous sinus of the brain using a Foredom dental drill[1] with a 0.1 mm burr. Small diameter polyethylene tubing was fitted tightly into the saggital sinus opening to permit the withdrawal of venous blood.

A solution of heparin in normal saline was injected through each of the cannulas to prevent the coagulation of blood and blockage of the cannulas.

Calibration of Blood Flowmeter and Transducers

All transducers were calibrated before use. Calibration of the transducers consisted of an *in vitro* system which permitted blood to flow through the lumen of a blood vessel at various known rates and then simultaneously measuring the recorder deflections caused by those different rates. Calibration curves were then plotted on linear graph paper.

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[1]Foredom Electric Co., Inc. Bethel, Connecticut

A gravity system was used for calibration procedures (Figure 1). The reservoir was placed 2 meters above the transducer level. A large rubber connecting tube from the reservoir to the central cannula was used to minimize resistance. The central cannula was made of glass and was as large as practical for connection with the strip of blood vessel which had been cleaned of all surrounding tissues. (Non-conductive plastics such as polyvinyl chloride tubing cannot be used in place of a blood vessel to calibrate a transducer. An acceptable substitute for a blood vessel has not been found.) The strip of blood vessel used was of a diameter large enough to give a good transducer fit. A good fit supported the transducer snugly but was not so tight as to restrict flow significantly. The distal portion of the blood vessel was connected to the distal cannula. The distal cannula was then attached to rubber tubing which was fitted with a screw clamp to regulate flow. A spout was connected to the rubber tubing. This was used to direct the blood into a graduated cylinder for measuring the actual blood flow.

The segment of blood vessel with the attached transducer was immersed in a large non-metallic container of saline, making sure that the transducer ground ring was also in the saline.

The settings on the blood flowmeter were calibrated for zero flow and were not changed from these settings. Readings from the blood flowmeter were then plotted versus ml flow per minute and kept as a permanent record. The calibration curves for all transducers which were used in this study are shown (Appendix).

Collecting Samples

Blood samples were taken simultaneously from the femoral artery and

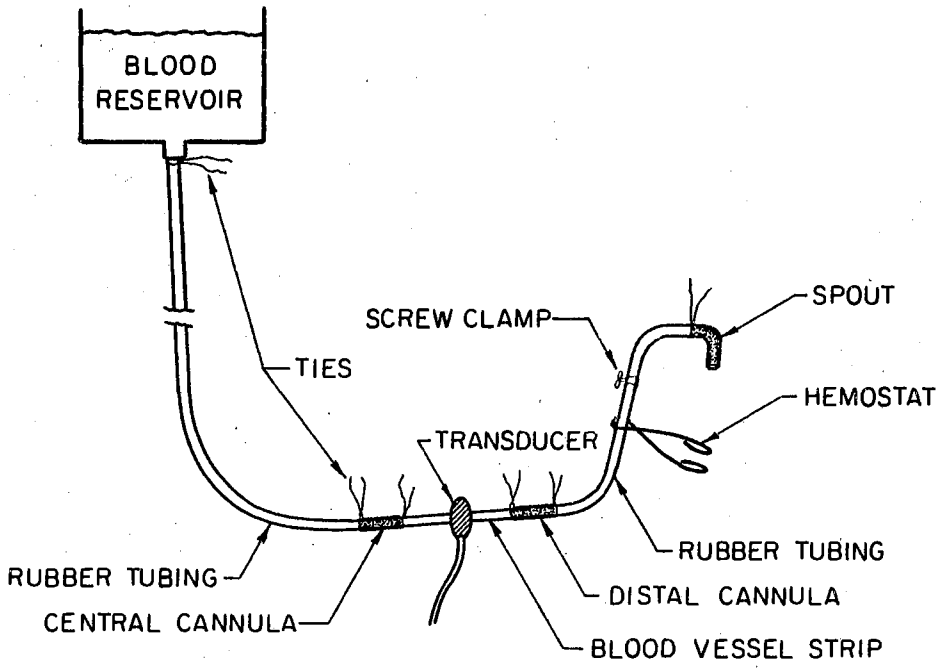

Figure 1. Schematic of Gravity System for Calibration of Blood Flowmeter Transducers

the saggital sinus at 5 minute intervals until 10 samples were taken. During the entire experiment, volatile fatty acids (1.0 M solutions of sodium acetate, sodium, propionate, and sodium butyrate) were mixed in equal volumes and injected into the femoral vein at the rate of 0.975 ml/minute with a Harvard infusion pump[1]. Blood flow in the right carotid and vertebral arteries was recorded continuously throughout the blood collection period using a Pulsed-Logic Electromagnetic Blood Flowmeter, Model BL-610[2]. After 10 blood samples were collected, the animal was euthanized using an overdose of pentabarbital sodium solution. The brain was removed immediately posterior to the cerebellum and weighed.

Preparation of Samples

A protein-free filtrate of each blood sample collected was prepared as follows: One volume of blood was first mixed with 5 volumes of water. Two volumes of 1.8% barium hydroxide was added to the blood-water mixture and allowed to stand for approximately 1 hour. Two volumes of 2.0% zinc sulfate was then added to this mixture. After a few minutes, the mixture was centrifuged and the supernatant fluid was filtered through Whatman #40 filter paper.

For the determination of the volatile fatty acids, a protein-free filtrate was prepared from 10 ml of blood by the above procedure and the entire filtrate was concentrated as follows: The filtrate was first made basic by the addition of 10% NaOH to bring the pH to approximately 9.0. The basic filtrate was then heated in a boiling water bath

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[1]Harvard Apparatus Co., Inc., Dover, Mass. Model No. 975

[2]Biotronex Laboratories, Inc., Silver Spring, Md.

to evaporate off the water until a final volume of less than 2 ml was reached. The samples were then capped and frozen until the final volatile fatty acid analysis was made.

Glucose Determination

One ml of blood was used to prepare a protein-free filtrate by the method described above and used for the determination of glucose by the Nelson (47) and Somogyi (63) method.

Volatile Fatty Acid Determination

The concentrated, basic protein-free filtrate was made acidic (pH approximately 3) with the addition of 10% phosphoric acid and the final volume adjusted exactly to 2.0 ml with glass distilled water. The acetic, propionic, and butyric acids were separated on a 62 inch stainless steel column (1/8" diameter) packed with 20% Carbowax 20M (terephthalic acid) on Chromasorb W (AW-DMCS) 80/100 mesh[1]. A Perkin-Elmer model 900 Gas Chromatograph[2] equipped with a hydrogen flame ionization detector was used for gas chromatographic analysis of the volatile acids. Helium was used as the carrier gas. The oven temperature was isothermally maintained at 280°C. The peak areas formed on the gas chromatogram by the acetic, propionic, and butyric acid peaks were determined by triangulation. The concentrations of the three volatile fatty acids were determined by comparing the areas of peaks formed by the injection of the 1 µl of a standard solution containing acetic acid (100 mg/100 ml), propionic acid (100 mg/100 ml), and butyric acid (100 mg/ml).

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[1]Applied Science Laboratories, Inc., State College, Pennsylvania

[2]Perkin-Elmer Corporation, Norwalk, Connecticut

Analysis of Data

All data were analyzed statistically using Student's t-test (64) for the comparisons. The following comparisons were made: (1) a between species comparison for blood flow per minute per 100 gm of brain tissue, (2) a within species comparison between arterial and venous concentrations of blood glucose, acetate, propionate, and butyrate, (3) a between species comparison of arterio-venous differences for glucose, for acetate, for propionate, and for butyrate, and (4) a between species comparison of the rate of substrate disappearance (glucose, acetate, propionate, and butyrate) per 100 gm of brain tissue.

CHAPTER IV

RESULTS

The objective of this study was to compare the rates of utilization of glucose and volatile fatty acids in brain tissue of ruminant and non-ruminant animals. This was accomplished by measuring the *in vivo* disappearance of these metabolites from the blood as they circulated to the brain.

Blood Flow

A summary of blood flow and brain weight is shown in Table I. Blood flow, when expressed as ml per minute per 100 gm of brain tissue, was significantly greater ($P<0.05$) in the dog than in the goat. The mean blood flow per 100 gm of brain tissue per minute was 56.66 ml in dogs and 41.40 ml in goats.

Metabolite Levels in Arterial and Venous Blood

Glucose, acetate, propionate, and butyrate levels in arterial and venous blood are shown in Tables II, III, IV, and V. Even though metabolite levels were consistently higher in arterial blood than in venous blood, when arterial and venous blood levels were compared statistically, it was found that no significant differences in arterial and venous blood levels existed for any of the metabolites in either dogs or goats.

TABLE I

SUMMARY OF BLOOD FLOW AND BRAIN WEIGHT IN DOGS AND GOATS

Dog				Goat			
No.	Blood flow ml./min.	Brain wt. gm.	Flow/ 100 gm./min.	No.	Blood flow ml./min.	Brain wt. gm.	Flow/ 100 gm./min.
1	48.8	90.0	54.2	1	65.2	106.3	61.3
2	53.0	73.8	71.8	2	67.9	100.5	67.6
3	29.2	84.4	34.6	3	38.0	123.2	30.9
4	58.8	74.0	79.5	4	52.1	114.7	45.4
5	33.0	76.6	43.1	5	43.0	113.0	38.1
6	36.5	83.6	43.7	6	38.5	115.1	33.4
7	41.0	90.9	45.1	7	42.5	111.1	38.3
8	48.9	83.9	58.3	8	22.5	88.5	25.4
9	64.1	77.9	82.3	9	56.2	107.1	52.5
10	42.1	78.0	54.0	10	21.1	100.0	21.1
Mean	45.5	81.3	*56.7	Mean	44.7	108.0	*41.4
$\pm$ S.D.	11.24	6.18	16.32	$\pm$ S.D.	15.94	9.80	15.22

*($P<0.05$)

TABLE II

BLOOD GLUCOSE LEVELS (mg/100 ml BLOOD) IN DOGS AND GOATS

Dog			Goat		
No.	Arterial	Venous	No.	Arterial	Venous
1	102	98	1	59	47
2	85	78	2	57	55
3	86	73	3	95	83
4	122	113	4	100	87
5	64	52	5	94	85
6	78	64	6	72	69
7	109	96	7	84	75
8	79	76	8	100	88
9	106	93	9	68	58
10	97	85	10	79	72
Mean	92.8	82.8	Mean	80.8	71.9
$\pm$ S.D.	17.44	17.93	$\pm$ S.D.	16.36	14.53

TABLE III

BLOOD ACETATE LEVELS (mg/100 ml BLOOD) IN DOGS AND GOATS

Dog			Goat		
No.	Arterial	Venous	No.	Arterial	Venous
1	9.44	8.88	1	36.40	31.30
2	9.77	9.77	2	33.20	30.30
3	13.53	11.49	3	15.62	15.32
4	9.81	8.98	4	20.40	17.05
5	4.80	4.69	5	21.58	19.44
6	12.06	10.46	6	13.93	13.52
7	12.52	11.18	7	20.90	16.59
8	9.49	9.04	8	20.85	18.38
9	11.62	10.33	9	12.38	11.02
10	13.92	13.48	10	27.00	25.95
Mean	10.70	9.83	Mean	22.23	19.89
$\pm$ S. D.	2.66	2.29	$\pm$ S.D.	7.89	6.97

TABLE IV

BLOOD PROPIONATE LEVELS (mg/100 ml BLOOD) IN DOGS AND GOATS

Dog			Goat		
No.	Arterial	Venous	No.	Arterial	Venous
1	4.74	3.56	1	1.15	0.82
2	2.78	2.62	2	1.65	1.51
3	2.59	1.96	3	1.34	1.02
4	2.61	2.20	4	1.03	0.75
5	1.38	0.97	5	1.74	1.20
6	2.25	1.69	6	1.02	0.87
7	3.40	2.80	7	1.15	0.76
8	2.78	2.70	8	2.61	1.91
9	2.92	2.13	9	0.63	0.42
10	2.22	1.90	10	1.53	1.22
Mean	2.77	2.53	Mean	1.39	1.05
$\pm$ S.D.	0.87	0.71	$\pm$ S.D.	0.54	0.43

TABLE V

BLOOD BUTYRATE LEVELS (mg/100 ml BLOOD) IN DOGS AND GOATS

Dog			Goat		
No.	Arterial	Venous	No.	Arterial	Venous
1	2.21	2.00	1	1.84	1.60
2	3.56	3.36	2	0.41	0.39
3	3.04	2.31	3	1.50	1.21
4	2.75	2.58	4	0.88	0.63
5	1.31	0.91	5	1.90	1.32
6	2.40	1.84	6	1.20	1.11
7	3.40	2.69	7	0.91	0.68
8	2.65	2.54	8	0.93	0.89
9	3.05	2.29	9	0.86	0.39
10	2.51	2.12	10	1.59	1.22
Mean $\pm$ S.D.	2.69 $\pm$ 0.65	2.26 $\pm$ 0.64	Mean $\pm$ S.D.	1.20 $\pm$ 0.49	0.94 $\pm$ 0.41

Arterio-venous Differences and Metabolite Disappearance

Arterio-venous (A-V) differences for glucose, acetate, propionate, and butyrate were determined by subtracting the venous concentration of each metabolite from the arterial concentration, the arterial and venous blood samples having been drawn simultaneously. The A-V difference for glucose, acetate, propionate, and butyrate are shown in Tables VI, VII, VIII, IX respectively. A dog-goat comparison of the A-V difference for each metabolite is depicted graphically in Figure 2. In addition, Tables VI, VII, VIII, IX and Figure 3 show the disappearance rate for each metabolite. The disappearance rate is defined as the mg of metabolite (e.g., glucose) which disappeared from the blood supplying the brain per 100 gm of brain tissue per minute.

Table VI shows the A-V differences and disappearance rates of glucose in dogs and goats. When goat and dog A-V differences were compared statistically, they were not significantly different. Similarly, there was no significant difference in the disappearance rates of glucose between dogs and goats. The mean A-V differences were 10.00 mg per 100 ml blood (mg%) in dogs and 8.90 mg% in goats. The mean rate of disappearance of glucose in dogs was 5.49 mg per 100 gm of brain tissue per minute and 3.55 mg per 100 gm of brain tissue per minute in goats.

A-V differences and disappearance rates for acetate are shown in Table VII. The A-V differences were significantly higher ($P<0.05$) in goats than in dogs. The mean A-V differences were 0.868 mg% in dogs and 2.330 mg% in goats. There was no significant difference in the acetate disappearance rate when the two species were compared. The mean acetate disappearance rate was 0.46 mg per 100 gm of brain tissue per minute in dogs and 1.09 mg per 100 gm of brain tissue per minute in goats.

TABLE VI

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES FOR GLUCOSE IN DOGS AND GOATS

Glucose A-V (mg%)			Glucose mg/100 gm/min		
Animal No.	Dog	Goat	Animal No.	Dog	Goat
1	4	12	1	2.17	7.36
2	7	2	2	5.03	1.35
3	13	12	3	4.50	3.71
4	9	13	4	7.16	5.45
5	12	9	5	5.17	3.43
6	14	3	6	6.12	1.00
7	13	9	7	5.86	3.45
8	3	12	8	1.75	3.05
9	13	10	9	10.70	5.25
10	12	7	10	6.48	1.48
Mean	10.0	8.9	Mean	5.49	3.55
$\pm$ S.D.	4.3	3.8	$\pm$ S.D.	2.53	2.03

TABLE VII

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES FOR ACETATE IN DOGS AND GOATS

Acetate A-V (mg%)			Acetate mg/100 gm/min		
Animal No.	Dog	Goat	Animal No.	Dog	Goat
1	0.56	5.10	1	0.304	3.130
2	0.00	2.90	2	0.000	1.960
3	2.04	0.30	3	0.706	0.090
4	0.85	3.35	4	0.676	1.520
5	0.11	2.14	5	0.047	0.820
6	1.60	0.41	6	0.699	0.137
7	1.34	4.31	7	0.604	1.650
8	0.45	2.47	8	0.262	0.627
9	1.29	1.36	9	1.062	0.714
10	0.44	1.05	10	0.238	0.222
Mean	*0.868	*2.339	Mean	0.460	1.090
$\pm$ S.D.	0.674	1.612	$\pm$ S.D.	0.340	0.970

*($P<0.05$)

TABLE VIII

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES FOR PROPIONATE IN DOGS AND GOATS

Propionate A-V (mg%)			Propionate mg/100 gm/min		
Animal No.	Dog	Goat	Animal No.	Dog	Goat
1	1.18	0.33	1	0.640	0.202
2	0.16	0.14	2	0.115	0.095
3	0.63	0.32	3	0.218	0.099
4	0.41	0.28	4	0.326	0.127
5	0.41	0.54	5	0.177	0.206
6	0.56	0.15	6	0.245	0.050
7	0.40	0.39	7	0.180	0.149
8	0.08	0.70	8	0.047	0.178
9	0.79	0.21	9	0.652	0.110
10	0.32	0.31	10	0.173	0.065
Mean	0.494	0.337	Mean	*0.280	*0.130
$\pm$ S.D.	0.319	0.030	$\pm$ S.D.	0.210	0.050

*($P<0.05$)

TABLE IX

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES FOR BUTYRATE IN DOGS AND GOATS

Butyrate A-V (mg%)			Butyrate mg/100 gm/min		
Animal No.	Dog	Goat	Animal No.	Dog	Goat
1	0.21	0.24	1	0.114	0.147
2	0.20	0.02	2	0.144	0.014
3	0.73	0.29	3	0.253	0.090
4	0.17	0.25	4	0.135	0.114
5	0.40	0.58	5	0.172	0.221
6	0.56	0.09	6	0.804	0.030
7	0.71	0.22	7	0.320	0.084
8	0.11	0.04	8	0.064	0.010
9	0.76	0.47	9	0.625	0.247
10	0.39	0.37	10	0.211	0.078
Mean	0.422	0.257	Mean	*0.280	*0.100
$\pm$ S.D.	0.252	0.181	$\pm$ S.D.	0.240	0.080

*($P<0.05$)

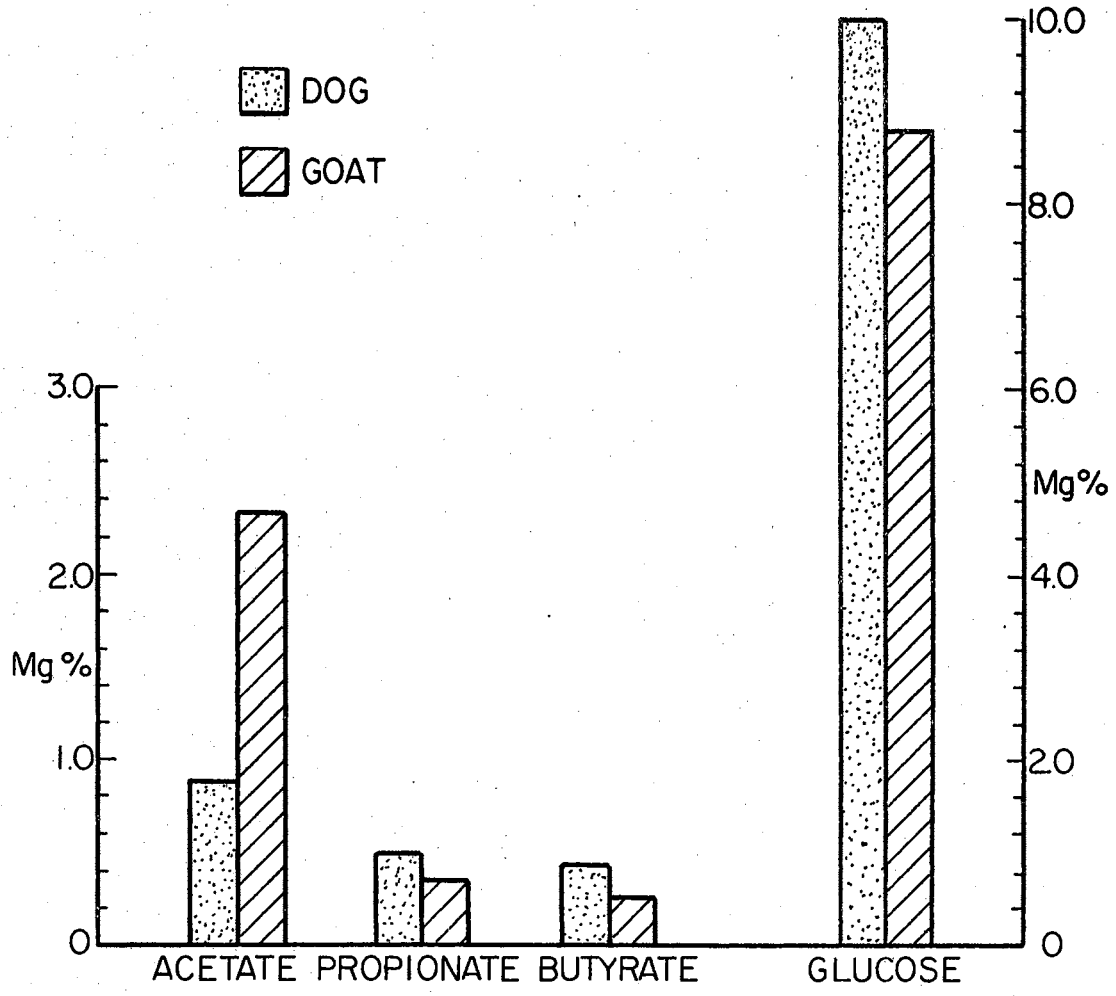

Figure 2. Arterio-venous Differences of Metabolites in Dogs and Goats

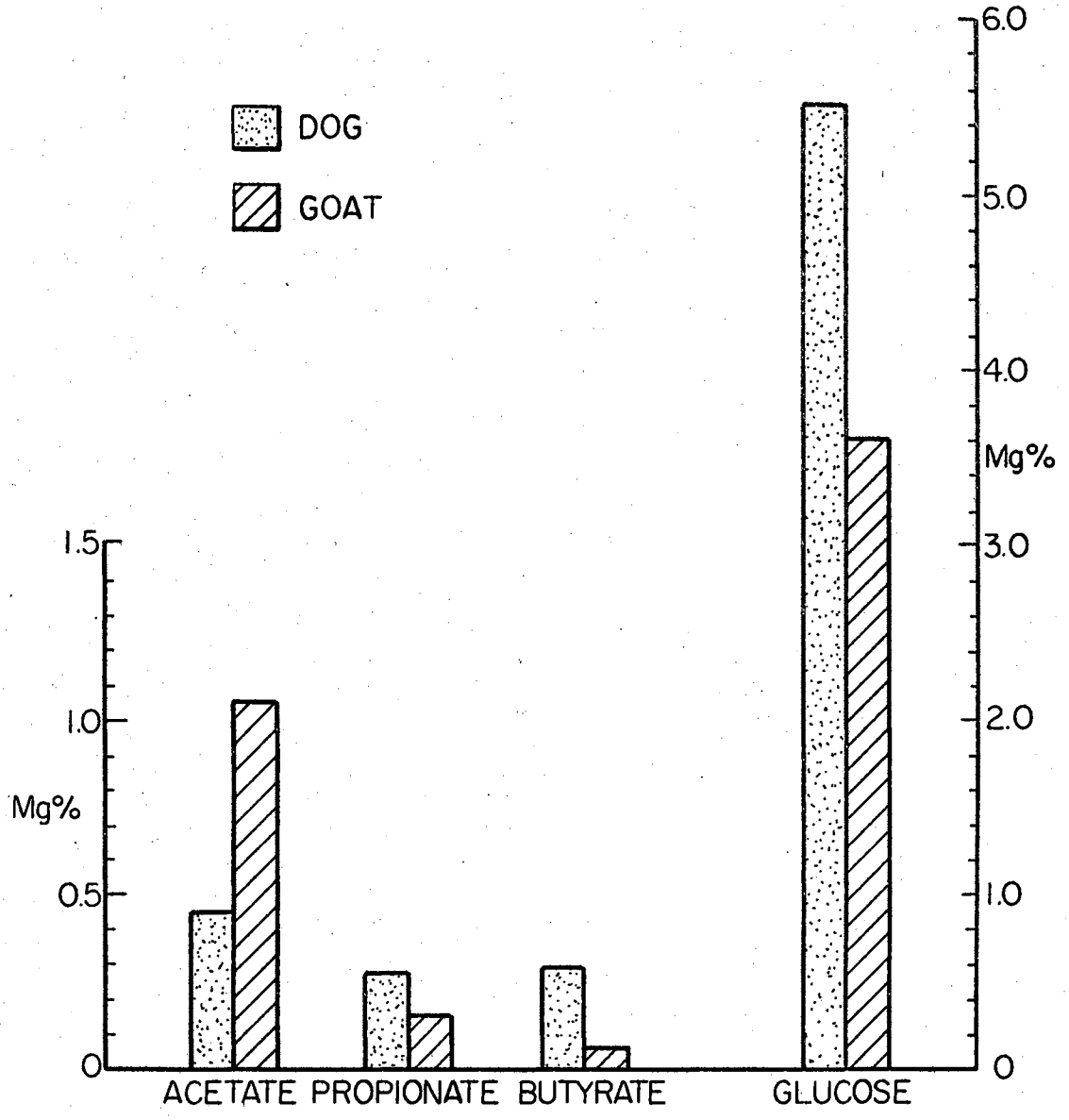

Figure 3. Disappearance Rates of Metabolites per 100 gm Brain per Minute in Dogs and Goats

A-V differences for propionate were not significantly different in dogs and goats (Table VIII). Mean A-V differences were 0.494 mg% in dogs and 0.337 mg% in goats. Disappearance rates were significantly greater ($P<0.05$) in dogs (0.28 mg per 100 gm of brain tissue per minute) than in goats (0.13 mg per 100 gm of brain tissue per minute).

Table IX shows that butyrate A-V differences were not significantly different in dogs and goats. Mean A-V differences were 0.422 mg% in dogs and 0.257 mg% in goats. Dogs showed a significantly greater butyrate disappearance rate ($P<0.05$) than goats. The mean disappearance rates of butyrate were 0.28 mg per 100 gm of brain tissue per minute in dogs and 0.10 mg per 100 gm of brain tissue per minute in goats.

CHAPTER V

DISCUSSION

There is general agreement that glucose is the main substrate used for oxidative metabolism by the mammalian brain under normal physiological conditions (1). Only glucose is taken up by the brain from the blood in sufficient amounts to completely satisfy brain metabolic requirements (24).

Glucose consumption was found to be 5.494 mg/100 gm brain tissue/min in dogs and 3.553 mg/100 gm brain tissue/min in goats (Figure 3). This compares favorably with the results of Gilboe (27), who, working with the isolated dog brain, found that glucose consumption was between 3.65-4.90 mg/100 gm of brain tissue/minute. Similarly, Meyer et al. (45) found in monkeys that cerebral glucose consumption was 3.52 mg/100 gm brain tissue per minute.

Bergman (8) found that, in general, ruminant animals have lower arterio-venous glucose differences than non-ruminant animals even though the general metabolic rates, based on metabolic size, are comparable. This work substantiates the findings reported herein.

The production of large amounts of acetic acid, propionic acid, and butyric acid by rumen bacteria is well established. These volatile acids are absorbed by the rumeno-reticulum, omasum and small intestine and enter the portal system. Under normal feeding conditions, propionate and butyrate are largely removed by the liver, but substantial

quantities of acetate appear in peripheral blood (3). In the current study, acetate levels in arterial blood were much higher in the goat (Table III), whereas propionate (Table IV) and butyrate (Table V) levels in the arterial blood were higher in the dog. It should be pointed out that a 1.0M solution of sodium acetate, propionate and butyrate was constantly infused into both dogs and goats during the experiment. The higher acetate levels in goat blood is not surprising since normal goat blood contains 8-10 mgs % acetate (56), whereas dog blood normally contains only trace amounts of acetate. In the case of the goat, the infused acetate would merely add on to acetate levels already present.

The greater disappearance rates for propionate (Table VIII) and butyrate (Table IX) in dogs is probably a result of the abnormally high levels available due to the constant infusion.

Reid (56), working with fasted and fed sheep and dogs, found no evidence that acids other than acetic are removed by peripheral tissues in significant quantities.

Baxter et al. (7) suggested that the low blood glucose levels in the cow may reflect the lesser quantitative importance of glucose as a metabolite in this species. This latter view is supported by the observation that glucose may be partially replaced by acetate for energy metabolism in most ruminant tissues.

The significantly higher ($P<0.05$) arterio-venous differences for blood acetate (Table VII) and the greater disappearance rates of this acid in the goat suggest that the ruminant brain, like most ruminant tissues, utilizes this metabolite as an energy source.

Blaxter (10) has calculated that acetic acid is used as an energy source with less efficiency than glucose in sheep, but that efficiency

increases with the increasing chain length of fatty acids.

Acetate may contribute as much as 50% of the ruminant's total energy supply (43). Biochemists have long recognized the central role of acetate in the energy metabolism of most forms of life. But, whereas for most organisms, the supply of acetate, or rather its active form, acetyl-Co-A, is almost entirely endogenous, ruminant animals have a large exogenous supply of acetate since it is produced in the rumen by microbial fermentation of ingested foodstuffs (58).

Results in this study are in contrast to arterio-venous difference studies on the brain of sheep by McClymont and Setchell (44) who failed to show any utilization of acetate. In their studies the mean glucose uptake by the brain (6.2 $\pm$ 0.7 mg per 100 ml of blood) was sufficient, assuming complete oxidation of the glucose, to account for the mean oxygen utilization of 4.8 $\pm$ 0.2 volumes per 100 ml of blood. Their results imply that the sheep is similar to non-ruminant animals in that acetate is not utilized by the brain.

Stith (65) in an _in vitro_ study, found that acetic and propionic acids, when presented together as substrates and, to a lesser extent, propionic acid when presented by itself, were taken up by homogenates of caprine brain, but not to a significant degree. There was no evidence of fatty acid utilization by homogenates of canine brain.

Arterio-venous glucose differences (Table VI) are slightly greater in dogs than in goats. When this small A-V difference between dogs and goats is compared to the large difference in the blood glucose levels in these species (Table II), some transport mechanism for glucose, and not simple diffusion is suggested.

The mechanism of transfer of glucose from blood into brain tissue

was studied in anesthetized dogs by Crone (11,12). It was found that the fraction of glucose which passed into the cerebral tissue decreased with increasing concentrations of glucose in the concentration range of 25-240 mg/100 ml blood. The extraction at low concentrations of glucose was almost 50% and that at high glucose concentrations was about 10%. This change in extraction rates suggests a carrier-mediated transport mechanism which facilitates the passage of glucose across the blood-brain barrier. The capacity of this process was found to be 20-22 mg of glucose removed per 100 ml blood. These findings are supported by Fishman (19), who found in dogs that the glucose concentration in CSF approached a maximum, despite increasing intravenous loads of glucose. These findings also suggested saturation kinetics.

In the current study, blood flow was found to be 56.66 ml/100 gm brain tissue/minute in the dog and 41.40 ml/100 gm brain tissue/minute in the goat. This compares favorably with the work by Meyer et al. (45) who measured the cerebral blood flow in the monkey by the nitrous oxide method and found flow to be 61 ml blood per 100 gm brain tissue per minute, and by Dumke et al. (17) who reported values of 55.4 ml/100 gm brain tissue per minute from measurements of internal carotid artery flow in the monkey. These values are comparable to those found in man by Kety and Schmidt (37).

CHAPTER VI

SUMMARY AND CONCLUSIONS

There is general agreement that glucose is the main substrate used for oxidative metabolism by the mammalian brain under normal physiological conditions. However, previous work has shown that energy metabolism in ruminant animals differs somewhat from energy metabolism of non-ruminant animals. Since large quantities of volatile fatty acids are formed by rumen microorganisms and subsequently absorbed into the blood, and since glucose levels in the blood of ruminants is somewhat lower than the levels found in non-ruminants, the possibility exists that these volatile fatty acids may constitute an important energy source for ruminant brain tissue.

An in vivo study was designed to compare the role of glucose and volatile fatty acids as energy sources for brain tissue in ruminant and non-ruminant animals by measuring the disappearance of these metabolites from the blood as they circulated to the brain. Volatile fatty acids were infused into the blood stream during the experiment to raise the circulating levels above those which are considered physiological.

It was found that the rate of blood flow to the brain per 100 gm brain tissue was significantly greater ($P<0.05$) in dogs than in goats. There was no significant difference in dogs and goats when brain A-V differences or disappearance rates of glucose were compared. The A-V difference of acetate was significantly greater ($P<0.05$) in goats than

in dogs. The disappearance rate for blood acetate as it circulated to the brain appeared to be higher in goats than in dogs; however, this difference was not significant. Propionate and butyrate brain A-V differences were not significantly different between dogs and goats, but the rates of disappearance of both of these metabolites were significantly greater ($P<0.05$) in dogs than in goats.

From the findings of this study, it may be concluded that glucose is, indeed, the main energy source for brain tissue in ruminant as well as in non-ruminant animals. Acetate appears to be utilized to a greater extent by caprine brain tissue. Both propionate and butyrate, when available in higher than physiological blood levels, are utilized to a greater extent by canine brain tissue.

SELECTED BIBLIOGRAPHY

1. Allweis, C. and J. Magnes. "The Uptake and Oxidation of Glucose by the Perfused Cat Brain." J. Neurochem. 2:326-336, 1958.

2. Altszuler, N., R. Steele, I. Rathgeb, and R. C. De Bodo. "Glucose Metabolism and Plasma Insulin Level During Epinephrine Infusion in the Dog." Am. J. Physiol. 212(3):677-682, 1967.

3. Annison, E. F., R. A. Leng, D. B. Lindsay, and R. R. White. "The Metabolism of Acetic Acid, Propionic Acid and Butyric Acid in Sheep." Biochem. J. 88:248-251, 1963.

4. Armstrong, D. G. "Carbohydrate Metabolism in Ruminants and Energy Supply." Physiology of Digestion in the Ruminant. R. W. Dougherty, Ed. Washington: Butterworths, 1965.

5. Ash, R. W., R. J. Pennington, and R. S. Reid. "The Effect of Short-Chain Fatty Acids on Blood Glucose Concentration in Sheep." Biochem. J. 90:353-360, 1964.

6. Baldwin, B. A., and F. R. Bell. "Blood Flow in the Carotid and Vertebral Arteries of the Sheep and Calf." J. Physiol. 167:448-462, 1963.

7. Baxter, C. F., M. Kleiber, and A. L. Black. "Glucose Metabolism in the Lactating Dairy Cow." Biochim. Biophys. Acta 17:354-361, 1955.

8. Bergman, E.N. "Quantitative Aspects of Glucose Metabolism in Pregnant and Non-pregnant Sheep." Am. J. Physiol. 204:147-152, 1963.

9. Bergman, E. N., R. S. Reid, M. G. Murray, J. M. Brockway, and F. G. Whitelow. "Interconversions and Production of Volatile Fatty Acids in the Sheep Rumen." Biochem. J. 97:53-58, 1965.

10. Blaxter, K. L. The Energy Metabolism of Ruminants. Springfield, Illinois: Charles C. Thomas (Publisher), 1962.

11. Crone, C. "The Permeability of Brain Capillaries to Non-Electrolytes." Acta Physiol. (Scand.) 64:407-417, 1965.

12. Crone, C. "Facilitated Transfer of Glucose from Blood into Brain Tissue." J. Physiol. (London) 181:103-113, 1965.

13. Csaky, T. Z. and B. M. Rigor, Sr. "A Concentrative Mechanism for Sugars in the Chorioid Plexus." Life Sci. 3:931-936, 1964.

14. Davis, L. E., B. A. Westfall, and H. E. Dale. "Effects of Ruminal Insufflation on Cerebral Circulation and Metabolism in the Goat." Amer. J. Vet. Res. 26:1403-1412, 1965.

15. Davson, H. and E. Spaziani. "The Blood-Brain Barrier and the Extracellular Space of Brain." J. Physiol. 149:135-143, 1959.

16. Dobson, A., A. F. Sellers, and F. D. McLeod. "Performance of a Cuff-Type Blood Flowmeter in vivo." J. Appl. Physiol. 21(5): 1642-1648, 1966.

17. Dumke, P. R. and C. F. Schmidt. "Quantitative Measurements of Cerebral Blood Flow in the Macaque Monkey." Am. J. Physiol. 138:421-431, 1943.

18. Elliott, K. A. C. and L. S. Wolfe. "Brain Tissue Respiration and Glycolysis." Neurochemistry. Edited by Elliot, K. A. C., I. H. Page, and J. H. Quastel. Charles C. Thomas, Springfield, 1962.

19. Fishman, R. A. "Carrier Transport of Glucose Between Blood and Cerebrospinal Fluid." Am. J. Physiol. 206:836-844, 1964.

20. Gainer, H., C. L. Allweis, and I. L. Chaikoff. "Precursors of Metabolic CO_2 Produced by the Brain of the Anaesthetized, Intact Dog: The Effect of Electrical Stimulation." J. Neurochem. 10:903-908, 1963.

21. Gehrke, C. W. and W. M. Lamkin. "Quantitative Determination of Steam-Volatile Fatty Acids by Gas-Liquid Chromatography." Ag. and Food Chem. 9:85-88, 1961.

22. Geiger, A., J. Magnes, R. M. Taylor, and M. Veralli. "Effect of Blood Constituents on Uptake of Glucose and on Metabolic Rate of the Brain in Perfusion Experiments." Am. J. Physiol. 177:138-149, 1954.

23. Geiger, Alexander. "Correlation of Brain Metabolism and Function by the Use of a Brain Perfusion Method In Situ." Physiol. Rev. 88:1-20, 1958.

24. Geiger, A., Y. Kawakita, and S. S. Barkulis. "Major Pathways of Glucose Utilization in the Brain in Perfusion Experiments In Vivo and In Situ." J. Neurochem. 5:323-338, 1960.

25. Geiger, A. "Metabolism and Function in the Brain." Neurochemistry. Edited by Elliot, K. A. C., I. H. Page, and J. H. Quastel. Charles C. Thomas, Springfield, 1962.

26. Gessner, U. and D. H. Bergel. "Frequency Response of Electromagnetic Flowmeters." J. Appl. Physiol. 19:1209-1211, 1964.

27. Gilboe, D. D., W. W. Cotanch, and M. B. Glover. "Extracorporeal Perfusion of the Isolated Head of a Dog." Nature (London) 202:399-400, 1964.

28. Gilboe, D. D., M. B. Glover, and W. W. Cotanch. "Blood Filtration and Its Effect on Glucose Metabolism by the Isolated Dog Brain." Am. J. Physiol. 213(1):11-15, 1967.

29. Goldfien, A., K. S. Gullixson, and G. Hargrove. "Evidence for Centers in the Central Nervous System that Regulate Fat Mobilization in Dogs." J. Lipid Res. 7:357-367, 1966.

30. Hegedus, S. A. and R. R. Shackelford. "A Comparative-Anatomical Study of the Cranio-Cervical Venous Systems in Mammals, with Special Reference to the Dog: Relationship of Anatomy to Measurements of Cerebral Blood Flow." Am. J. Anat. 116:375-386, 1965.

31. Holdsworth, E. S., E. Neville, and C. Nader. "The Metabolism of Acetate in the Sheep." Biochim. Biophys. Acta 86:240-249, 1964.

32. Issekutz, B. Jr., H. I. Miller, and K. Rodahl. "Lipid and Carbohydrate Metabolism During Exercise." Fed. Proc. 25:1415-1420, 1966.

33. Johnson, R. B. "The Relative Rates of Absorption of the Volatile Acids from the Rumen and Their Relationship to Ketosis." Cornell Vet. 41:115-121, 1951.

34. Jones, K. "Enzyme Activity and Glucose Studies in Caprine and Canine Brain." (Ph. D. thesis, Oklahoma State University, 1969).

35. Joyner, A. E. Jr., E. M. Kesler, and J. B. Holter. "Absorption From the Omasum and Subsequent Metabolism of Butyrate and Acetate." J. Dairy Sci. 46:1108-1113, 1963.

36. Kayser, K. L. "Performance Characteristics of Two Commercial Blood Flowmeters." Am. J. Med. Electronics 4:113-116, 1965.

37. Kety, S. S. and C. F. Schmidt. "Determination of Cerebral Blood Flow in Man by the Use of Nitrous Oxide in Low Concentration." Am. J. Physiol. 143:53-66, 1945.

38. Kety, Seymore S. "The Control of the Cerebral Circulation." Circulation 30(4):481-483, 1964.

39. Kolin, A. "Discussion of 'Some Applications and Limitations of Electromagnetic Blood Flow Measurements in Chronic Animal Preparations'." Gastroenterology 52(2):379-380, 1967.

40. Leng, R. A. and E. F. Annison. "Metabolism of Acetate, Propionate and Butyrate by Sheep-Liver Slices." Biochem. J. 86:319-327, 1963.

41. Mager, M. and G. Farese. "What is 'True' Blood Glucose?" Am. J. Clin. Path. 44(1):104-108, 1965.

42. Marston, H. R. "Energy Transactions in the Sheep. I. The Basal Heat Production and Heat Increment." Aust. J. Sci. Res. 1:93-129, 1948.

43. Mayfield, E. D., A. Bensadoun, and B. C. Johnson. "Acetate Metabolism in Ruminant Tissues." J. Nutr. 89:189-196, 1966.

44. McClymont, G. L. and B. P. Setchell. "Non-Utilization of Acetate and Utilization of Glucose by the Brain of the Sheep." Aust. J. Biol. Sci. 9:184-187, 1956.

45. Meyer, J. S., F. Gotoh, M. Akayama, and S. Yoshitake. "Monitoring Cerebral Blood Flow and Oxygen, Glucose, Lactate and Ammonia Metabolism." Circ. Res. 21:649-660, 1967.

46. Moran, J. M. "Medical Intelligence." New Engl. J. Med. 276:225-228, 1967.

47. Nelson, Norton. "A Photometric Adaptation of the Somogyi Method for the Determination of Glucose." J. Biol. Chem. 153:375-380, 1944.

48. O'Neal, R. M., R. E. Koeppe, and E. I. Williams. "Utilization in vivo of Glucose and Volatile Fatty Acids by Sheep Brain for the Synthesis of Acidic Amino Acids." Biochem. J. 101:591-597, 1966.

49. O'Neill, J. J. and T. E. Duffy. "Alternate Metabolic Pathways in Newborn Brain." Life Sci. 5:1849-1857, 1966.

50. O'Rourke, M. F. "Dynamic Accuracy of the Electromagnetic Flowmeter." J. Appl. Physiol. 20(1):142-147, 1965.

51. Paul, P., B. Issekutz, Jr., and H. I. Miller. "Interrelationship of Free Fatty Acids and Glucose Metabolism in the Dog." Am. J. Physiol. 211(6):1313-1320, 1966.

52. Phillipson, A. T. "Fermentation in the Alimentary Tract and the Metabolism of the Derived Fatty Acids." Nutr. Abstr. Rev. 17:12-18, 1947.

53. Radloff, H. D., L. H. Schultz, and W. G. Hoekstra. "Relationship of Plasma Free Fatty Acids to Other Blood Components in Ruminants Under Various Physiological Conditions." J. Dairy Sci. 49:179-182, 1966.

54. Ramsey, H. A. and C. L. Davis. "Metabolism of Butyrate in the Adult Goat." J. Dairy Sci. 48:381-390, 1965.

55. Ramsey, H. A. and C. L. Davis. "Metabolism of n-Butyric Acid by the Adult Goat." J. Dairy Sci. 47:698, 1964.

56. Reid, R. L. "Studies on the Carbohydrate Metabolism of Sheep. II. The Uptake by the Tissues of Glucose and Acetic Acid from the Peripheral Circulation." Aust. J. Agri. Res. 1:338-354, 1950.

57. Rose, G. A. "Magnetic Field Measurement for Electromagnetic Flowmeters." Phys. Med. Biol. 11(1):109-112, 1966.

58. Sabine, J. R. and B. C. Johnson. "Acetate Metabolism in the Ruminant." J. Biol. Chem. 239:89-93, 1964.

59. Sacks, W. "Cerebral Metabolism of Butyrate-1-^{14}C in Normal Human Subjects." Fed. Proc. 16:240-247, 1957.

60. Samson, F. E. Jr. "Energy Flow in Brain." Progr. Brain Res. 16:216-228, 1965.

61. Sellers, A. F. and A. Dobson. "Some Applications and Limitations of Electromagnetic Blood Flow Measurements in Chronic Animal Preparations." Gastroenterology 52(2):374-379, 1967.

62. Shaw, J. C. "Metabolic Disturbances Associated with Lactation." Milk: The Mammary Gland and Its Secretion. Vol. II Edited by Kon, S. K. and A. T. Cowie. Academic Press, New York and London, 1961.

63. Somogyi, M. "Notes on Sugar Determination." J. Biol. Chem. 195: 19-23, 1952.

64. Steel, R. G. and J. H. Torrie. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York, 1960.

65. Stith, R. D. "An in vitro Study of the Oxidative Metabolism of Glucose, Acetic Acid, Propionic Acid, and Butyric Acid in Brain Tissue of Ruminant and Non-Ruminant Animals." (M. S. thesis, Oklahoma State University, 1966).

66. Tower, D. B. "Some Neurochemical Factors Affecting Cerebral Function and Activity." Southern Med. J. 50:1453-1466, 1957.

67. Tucek, S. "The Synthesis of Acetyl-Coenzyme A and Acetylcholine from Citrate and Acetate in the Nerve Endings of Mammalian Brain." Biochim. Biophys. Acta 117:278-280, 1966.

68. Waelsch, H. S., S. Berl, C. A. Rossi, D. D. Clarke, and D. P. Purpura. "Quantitative Aspects of CO_2 Fixation in Mammalian Brain in vivo." J. Neurochem. 11:717-728, 1964.

69. Weil, R., P. Ho, and N. Altszuler. "Effect of Free Fatty Acids on Metabolism of Pyruvic and Lactic Acids." Am. J. Physiol. 208(5):887-891, 1965.

70. Weinman, E. O., E. H. Strisower, and I. L. Chaikoff. "Conversion of Fatty Acids to Carbohydrate: Application of Isotopes to this Problem and Role of the Krebs Cycle as a Synthetic Pathway." Physiol. Rev. 37:252-272, 1957.

71. Williams, R. D. B. and A. McCord. "Diffusion of Glucose Through Blood-Cerebrospinal Fluid Barrier in Normal Dogs." Am. J. Physiol. 203(3):453-456, 1962.

72. Wyatt, D. G. "Electromagnetic Flowmeter for Use with Intact Vessels." J. Physiol. (London) 173:8P, 1964.

APPENDIX

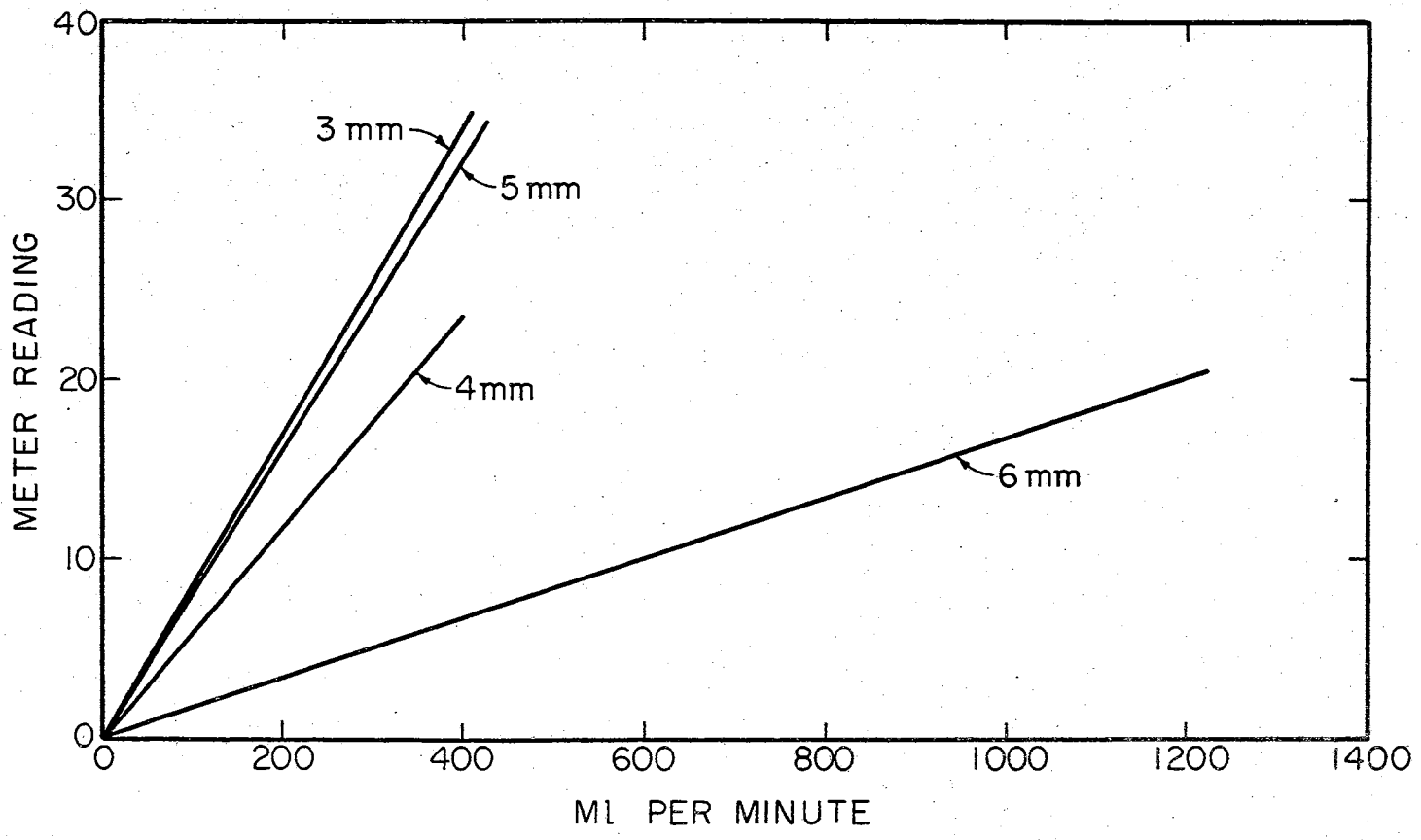

Figure 4. Calibration Curves for Blood Flowmeter Transducers

TABLE X

BLOOD FLOW, BODY AND BRAIN WEIGHTS, AND BLOOD FLOW PER 100 GM BRAIN PER MINUTE IN DOGS AND GOATS

	Carotid Flow (ml/min)	Vertebral Flow (ml/min)	Total Flow (ml/min)	Body Weight (Kg)	Brain Weight (gm)	Flow/100 gm brain/min (ml)
Dog						
1	30.5	18.3	48.8	25.45	90.0	54.2
2	36.0	17.0	53.0	14.09	73.8	71.8
3	17.0	12.2	29.2	21.13	84.4	34.6
4	38.0	20.8	58.8	26.63	74.0	79.5
5	20.8	12.2	33.0	26.81	76.6	43.1
6	24.0	12.5	36.5	20.45	83.6	43.7
7	24.0	17.0	41.0	19.54	90.9	45.1
8	34.3	14.6	48.9	21.36	83.9	58.3
9	39.8	24.3	64.1	19.45	77.9	82.3
10	27.4	14.7	42.1	22.27	78.0	54.0
Goat						
1	41.2	22.0	65.2	62.72	106.3	61.3
2	41.2	26.7	67.9	60.90	100.5	67.6
3	27.0	11.0	38.0	52.72	123.2	30.9
4	27.8	24.3	52.1	45.45	114.7	45.4
5	31.0	12.0	43.0	28.63	113.0	38.1
6	23.8	14.7	38.5	47.72	115.1	33.4
7	27.8	14.7	42.5	39.54	111.1	38.3
8	15.0	7.5	22.5	25.00	88.5	25.4
9	35.4	20.8	56.2	53.63	107.1	52.5
10	13.6	7.5	21.1	25.90	100.0	21.1

TABLE XI

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES OF GLUCOSE AS BLOOD PASSES THROUGH THE BRAIN IN DOGS AND GOATS

	Arterial (mg%)	Venous (mg%)	A-V Difference (mg%)	Disappearance Rate (mg/100 gm brain/min)
Dog				
1	102	98	4	2.17
2	85	78	7	5.03
3	86	73	13	4.50
4	122	113	9	7.16
5	64	52	12	5.17
6	78	64	14	6.12
7	109	96	13	5.86
8	79	76	3	1.75
9	106	93	13	10.70
10	97	85	12	6.48
Goat				
1	59	47	12	7.36
2	57	55	2	1.35
3	95	83	12	3.71
4	100	87	13	5.45
5	94	85	9	3.43
6	72	69	3	1.00
7	84	75	9	3.45
8	100	88	12	3.05
9	68	58	10	5.25
10	79	72	7	1.48

TABLE XII

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES OF ACETATE AS BLOOD PASSES THROUGH THE BRAIN IN DOGS AND GOATS

	Arterial (mg%)	Venous (mg%)	A-V Difference (mg%)	Disappearance Rate (mg/100 gm brain/min)
Dog				
1	9.44	8.88	0.56	0.304
2	9.77	9.77	0.00	0.000
3	13.53	11.49	2.04	0.706
4	9.81	8.96	0.85	0.676
5	4.80	4.69	0.11	0.047
6	12.06	10.46	1.60	0.699
7	12.52	11.18	1.34	0.604
8	9.49	9.04	0.45	0.262
9	11.62	10.33	1.29	1.062
10	13.92	13.48	0.44	0.238
Goat				
1	36.40	31.30	5.10	3.130
2	33.20	30.30	2.90	1.960
3	15.62	15.32	0.30	0.090
4	20.40	17.05	3.35	1.520
5	21.58	19.44	2.14	0.820
6	13.93	13.52	0.41	0.137
7	20.90	16.59	4.31	1.650
8	20.85	18.38	2.47	0.627
9	12.38	11.02	1.36	0.714
10	27.00	25.95	1.05	0.222

TABLE XIII

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES OF PROPIONATE AS BLOOD PASSES THROUGH THE BRAIN IN DOGS AND GOATS

	Arterial (mg%)	Venous (mg%)	A-V Difference (mg%)	Disappearance Rate (mg/100 gm brain/min)
Dog				
1	4.74	3.56	1.18	0.640
2	2.78	2.62	0.16	0.115
3	2.59	1.96	0.63	0.218
4	2.51	2.20	0.41	0.326
5	1.38	0.97	0.41	0.177
6	2.25	1.69	0.56	0.245
7	3.40	2.80	0.40	0.180
8	2.78	2.70	0.08	0.047
9	2.92	2.13	0.79	0.652
10	2.22	1.90	0.32	0.173
Goat				
1	1.15	0.82	0.33	0.202
2	1.65	1.51	0.14	0.095
3	1.34	1.02	0.32	0.099
4	1.03	0.35	0.28	0.127
5	1.74	1.20	0.54	0.206
6	1.02	0.87	0.15	0.050
7	1.15	0.76	0.39	0.150
8	2.61	1.91	0.70	0.178
9	0.63	0.42	0.21	0.110
10	1.53	1.22	0.31	0.065

TABLE XIV

ARTERIO-VENOUS DIFFERENCES AND DISAPPEARANCE RATES OF BUTYRATE AS BLOOD PASSES THROUGH THE BRAIN IN DOGS AND GOATS

	Arterial (mg%)	Venous (mg%)	A-V Difference (mg%)	Disappearance Rate (mg/100 gm brain/min)
Dog				
1	2.21	2.00	0.21	0.114
2	3.56	3.36	0.20	0.144
3	3.04	2.31	0.73	0.253
4	2.75	2.58	0.17	0.135
5	1.31	0.91	0.40	0.172
6	2.40	1.84	0.56	0.804
7	3.40	2.69	0.71	0.320
8	2.65	2.54	0.11	0.064
9	3.05	2.29	0.76	0.625
10	2.51	2.12	0.39	0.211
Goat				
1	1.84	1.60	0.24	0.147
2	0.41	0.39	0.02	0.014
3	1.50	1.21	0.29	0.090
4	0.88	0.63	0.25	0.114
5	1.90	1.32	0.58	0.221
6	1.20	1.11	0.09	0.030
7	0.91	0.68	0.22	0.084
8	0.93	0.89	0.04	0.010
9	0.86	0.39	0.47	0.247
10	1.59	1.22	0.37	0.078

TABLE XV

ARTERIO-VENOUS DIFFERENCES(mg%) OF METABOLITES AT 5 MINUTE INTERVALS IN GOATS AND DOGS

	Glucose		Acetate		Propionate		Butyrate	
Time	Goat	Dog	Goat	Dog	Goat	Dog	Goat	Dog
0	8.4	10.6	3.24	0.88	0.51	0.85	0.33	0.51
5	9.1	9.1	1.58	0.85	0.34	0.33	0.30	0.31
10	6.6	11.9	2.32	0.61	0.40	0.40	0.26	0.23
15	7.6	7.6	3.43	0.57	0.31	0.29	0.32	0.51
20	9.0	8.9	1.07	0.97	0.16	0.42	0.21	0.42
25	8.2	10.4	1.41	1.08	0.15	0.54	0.15	0.40
30	10.5	8.6	3.21	0.90	0.27	0.66	0.35	0.49
35	9.7	10.3	3.40	0.99	0.58	0.70	0.19	0.45
40	11.3	11.0	2.26	0.66	0.32	0.27	0.34	0.35
45	8.6	11.6	1.47	1.17	0.33	0.48	0.12	0.55
Mean	8.90	10.00	2.339	0.868	0.337	0.494	0.257	0.422

VITA

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Doctor of Philosophy

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