IN VITRO ENZYMATIC ACTIVITY AND ^{14}C-GLUCOSE METABOLIC STUDIES WITH CAPRINE AND CANINE BRAIN HOMOGENATES

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CHAPTER I

INTRODUCTION

During the past decade there has been much controversy concerning the so-called "blood brain barrier" in domestic animals. The main point of contention has dealt with the possibility of the existence of a physical barrier between blood and brain tissues. Several experiments have demonstrated that the passage of glucose from blood to brain tissue is not dependent upon simple diffusion (20,21,22,24,29,35,39,41,91). For example, the studies of Crone (21) have demonstrated that glucose passes from blood to brain tissue in greater quantities than either fructose or glycerol, two substances which are chemically similar to glucose. This has raised the question of how glucose can pass to the brain in sufficient quantities to adequately supply the energy requirements of the brain. It is known that blood glucose concentrations in the ruminant animal are normally 40 to 60 mg per 100 ml of blood (mg %), whereas in non-ruminant animals, blood glucose values are normally 100 to 120 mg %. In the non-ruminant animal, if blood glucose concentrations are lowered to the 40 to 60 mg % range, symptoms of central nervous system dysfunctions will occur. This has raised the question of how the ruminant animal is able to live normally at a blood glucose level which would constitute a hypoglycemia in the non-ruminant animal.

In the ruminant animal, most dietary carbohydrates are fermented by ruminal microorganisms, resulting in the formation of acetic, propionic,

and butyric acids (7,12,38). Also, it has been established that acetate, propionate, and butyrate serve as major sources of energy to the ruminant animal (7,52). Ford (30) has suggested that the utilization of these three volatile fatty acids by ruminant tissues may prevent a potential caloric deficiency which could occur due to low blood glucose levels. The studies of Stith (80) have demonstrated that canine brain homogenates have greater rates of oxygen uptake than caprine brain homogenates. In addition, it was demonstrated that the brain homogenates from goats have a significantly greater uptake of glucose from the incubation medium when compared to the glucose uptake in the canine brain homogenates (80). Although these studies have provided little information with respect to the precise metabolic pathways for glucose in neural tissue, they do suggest that a species difference may exist between ruminant and non-ruminant animals with regard to glucose metabolism in brain tissue.

The studies of Moss (55) have demonstrated that in a semi-isolated calf brain, the pentose phosphate pathway was the major pathway for glucose metabolism in the brain. Additional evidence has been gathered which supports the existence of the pentose phosphate pathway in brain tissue (16,17,46,59,66). On the other hand, Gilboe, et al. (41) have demonstrated that anaerobic glucose metabolism alone does not account for the amount of glucose taken up by the perfused canine brain. It is now generally accepted that all or a portion of glucose ultimately is metabolized via the tricarboxylic acid (TCA) cycle.

Since there is some evidence which suggests that a species difference may exist between ruminant and non-ruminant animals with regard to the metabolism of glucose by brain tissue, an experiment was designed to compare glucose metabolism in canine and caprine brain homogenates. The

specific aims of this study were: (1) to compare the activities of several key enzymes of the glycolytic pathway, the pentose phosphate pathway, and the TCA cycle in canine and caprine brain homogenates, and (2) to compare the metabolic fate of uniformly labelled ^{14}C-glucose in canine and caprine brain homogenates.

CHAPTER II

REVIEW OF LITERATURE

It is now generally accepted that glucose serves as the major source of energy for the metabolic processes occurring in the brain, and that the glycolytic pathway and the TCA cycle are the major metabolic pathways for glucose catabolism in neural tissue. During the past decade, several investigators have dealt with the problem of how glucose passes from the blood to the brain in sufficient quantities to supply the energy requirements of the brain. Not all authors have been in complete agreement with the concept that the "blood-brain barrier" is a structural barrier. More recently, terms such as "carrier transport", "carrier mediated transport", and "facilitated diffusion" have appeared in the literature to describe the mechanism for the exchange of glucose between blood and brain tissues (14,20,21,22,24,29,90).

Previous work has shown that metabolism in the ruminant animal differs somewhat from that of the monogastric animal. An interesting aspect in the metabolism of glucose in the ruminant animal is that the newborn ruminant animal has a blood glucose concentration of approximately 100 mg per 100 ml of blood (mg %) (53). The non-ruminant animal likewise has a blood glucose concentration of approximately 100 mg %. However, the studies of Reid (64) have shown that the blood glucose level in the newborn ruminant animal gradually decreases from 100 mg % to a level of 40 to 60 mg % at approximately 6 to 9 weeks after birth.

Previous work has shown there is a change in the utilization of substrates for energy purposes, i.e., the major source of energy in the adult ruminant animal is derived from acetic, propionic, and butyric acids. As the ruminant animal matures, the rumen gradually becomes functional and dietary carbohydrates ingested by the ruminant animal are fermented in the rumen by microorganisms resulting in the formation of volatile fatty acids. The studies of Goetsch (44) have shown there is a decrease in the activity of the liver glycolytic enzymes during the development of the rumen in calves. This decrease in the activity of the liver glycolytic enzymes may be attributed to the gradual decrease in glucose absorption during the development of the rumen since dietary carbohydrates are fermented to volatile fatty acids. This reduction in glucose catabolism in the maturing ruminant animal is supported by other investigators (47). Since the blood glucose concentration in the ruminant animal is considerably lower than the blood glucose level in the non-ruminant animal, the natural question which follows is how the adult ruminant animal can live normally at a blood glucose concentration which would constitute a hypoglycemia in the non-ruminant animal. Oyler (60) has demonstrated that glucose is utilized in caprine and canine brain, *in vivo*, at equal rates. Also, propionic and butyric acids are utilized at a significantly higher rate in the canine brain, whereas there was no significant species difference in the utilization of acetate by caprine or canine brain.

The studies of Jarret and Potter (48) have shown that young lambs are more sensitive to insulin injections than adult sheep, and that large doses of insulin administered to adult sheep did not cause convulsions even when blood glucose levels were low prior to the insulin

injections. Baxter, et al. (9) have shown that in the lactating dairy cow that acetate has a more significant role than glucose as an energy source. Although it has been demonstrated that there is a lower rate of glucose oxidation and metabolism in ruminant animals, this does not indicate that the ruminant animal lives in the absence of glucose since optimal rates of glucose utilization are definitely required.

It is generally accepted that glucose serves as the major source of energy in neural tissue. Elliot and Wolfe (27) have demonstrated that brain slices will metabolize fructose and mannose, but that in vivo, the brain exclusively utilizes glucose for energy metabolism and other sugars are not exchanged between blood and brain tissues. The studies of Setchell (70) have shown that fructose, which is chemically similar to glucose, is not taken up in sufficient quantity by the brain of a newborn lamb to supply the energy demands of the newborn ovine brain. However, glucose is utilized by the brain of a newborn lamb in sufficient quantity to supply the brain's energy requirements (70). Setchell (70) also has demonstrated that the brain of the newborn lamb utilizes fructose in vitro but not in vivo, thus illustrating the differences in the passage of these sugars through the "blood-brain barrier". Himwich (45) has shown that glucose is the only substance which is exclusively taken up by the brain in sufficient amounts to adequately supply the energy requirements of neural tissue.

Previous work has demonstrated that substances other than glucose can be used for energy metabolism by the brain. Geiger, et al. (38) have shown that the brain can be maintained in perfusion experiments for up to two hours with the complete absence of glucose in the brain perfusion medium. Until the study of Geiger, et al. (38) it was assumed

that the brain utilized glucose almost exclusively as the major source of energy. More recent investigations have provided evidence that protein, amino acids, and lipids are also metabolized by the brain (5, 37,38). Geiger, et al. (36) have suggested that substances other than glucose are oxidized during increased neural activity to support the increased rate of metabolism. It has been shown that during convulsions there is an increased rate in metabolic activity which is supported by an increased metabolic rate of non-carbohydrate substances (37).

Prior to the past decade it was assumed that fatty acid oxidation was essentially a hepatic function. However, evidence has been gathered establishing the fact that tissues other than the liver have the capability of fatty acid oxidation even in the presence of an adequate supply of glucose (4,13,40,51,83,88). Several investigators have provided evidence that the brain has the enzymatic capacity for fatty acid oxidation (40,61,83,87). It is well recognized that the ruminant animal has a greater resistance to hypoglycemia than a non-ruminant animal. Further, it has been suggested by some workers that possibly the ruminant animal utilizes substances other than glucose as a source for metabolic energy and that this phenomena could account for the ruminant animal's ability to live at a lower blood glucose concentration. The studies of Setchell (68) have shown that electrical stimulation of brain slices from sheep resulted in an increased oxygen uptake even without added glucose as a substrate. Gainer, et al. (31) have shown that convulsions produced in the dog by electroshock result in alterations in canine brain metabolism which indicated that part of the metabolic CO_2 produced was derived from non-glucose sources. In contrast to this, Gainer, et al. (31) have demonstrated that metabolic CO_2 produced by the brain in the intact

anesthetized dog was derived almost exclusively from blood glucose. Alweis and Magnes (5) have shown that CO_2 produced in the perfused brain of the cat was not derived from the radioactive labelled glucose source which was added to the perfusion medium. In addition, it was demonstrated that glucose uptake by the perfused brain of the cat continued at a rapid rate in spite of a low glucose content produced by perfusion with a "simplified blood". Apparently, the perfusion experiments did not disrupt the permeability of the brain to glucose. Stith, et al. (81) have clearly demonstrated that brain tissue homogenates from dogs have higher rates of oxygen uptake than homogenates prepared from caprine brains, whereas there was a significantly greater uptake of glucose from the incubating medium in brain tissue homogenates prepared from the goats when compared to homogenates of canine brain. McClymont and Setchell (54) have reported that ruminant brain tissue is similar to non-ruminant brain tissue in that glucose is the major source of energy for both.

It is generally accepted that the Embden-Meyerhof pathway and the tricarboxylic acid cycle are the major pathways for glucose utilization in neural tissue. It is known that carbon 1 of the glucose molecule is lost as CO_2 during the metabolism of glucose via the pentose phosphate pathway, whereas carbon 1 of glucose is incorporated into the formation of trioses during the metabolism of glucose via the glycolytic pathway. Further, these triose glycolytic intermediates enter the tricarboxylic acid cycle and are oxidized to CO_2. When glucose is oxidized to pyruvate via the glycolytic pathway, carbons 1 and 6 of glucose become carbon 3 of pyruvate. If pyruvate is then oxidized to CO_2 via the tricarboxylic acid cylce, equal molar quantities of CO_2 would arise from

carbons 1 and 6 of glucose. The studies of Bloom (10) have demonstrated that radioactive yields of $^{14}CO_2$ from 6-^{14}C-glucose and 1-^{14}C-glucose were identical in rabbit brain slices indicating that glucose is metabolized via the Embden-Meyerhof pathway. Sacks (67) has shown that equivalent yields of $^{14}CO_2$ were obtained from 1-^{14}C-glucose and 6-^{14}C-glucose in the perfused brain of human beings indicating that glucose is oxidized via the glycolytic pathway. Sutherland, et al. (82) have demonstrated that the production of lactic acid from 1-^{14}C-glucose by cerebral cortex slices prepared from the brain of human subjects had the necessary incorporation and quantity of anticipated radioactivity to indicate exclusive operation of the Embden-Meyerhof pathway. Wenner and Weinhouse (89) have provided evidence that brain slices of rats oxidized glucose via the glycolytic pathway. DiPietro and Weinhouse (25) have clearly demonstrated that equal radioactive yields of labelled $^{14}CO_2$ and trioses occurred in brain slices of rats indicating exclusive operation of the glycolytic pathway, and that only a small amount of glucose is metabolized via the pentose phosphate pathway. In summary, most evidence indicates that glucose and oxidized metabolites ultimately pass through the tricarboxylic acid cycle (10,27,55,67).

In most tissues, glycolytic enzyme systems are exclusively confined to the soluble portion of the cell, whereas mitochondria are largely involved in aerobic metabolism of the cell. The studies of DuBuy and Hasselback (26) have shown that mitochondrial preparations from the brain of rats have the capability to completely oxidize glucose to CO_2. Also, Gallagher, et al. (34) have shown in their experiments that brain mitochondria of rats were capable of oxidizing glucose and other glycolytic intermediates, whereas liver, kidney, and heart mitochondrial

preparations did not possess these properties. In contrast to these studies, Abood, et al. (2) and Brody and Bain (11) failed to demonstrate glycolytic activity in mitochondrial preparations from rat brain and therefore was unable to account for the complete oxidation of glucose.

Although it is generally accepted that the Embden-Meyerhof scheme and the tricarboxylic acid cycle are the major metabolic pathways for glucose metabolism in neural tissue, evidence for the existence of the pentose phosphate pathway in neural tissue has been demonstrated by several investigators (6,16,17,25,46,55,58,66). DiPietro and Weinhouse (25) have shown in rats that glucose is oxidized by neural tissue largely via the Embden-Meyerhof pathway, and that only a small amount of glucose is catabolized via the pentose phosphate pathway. Angeletti, et al. (6) have demonstrated, in vitro, that the addition of nerve growth factor (NGF), a hormonal agent, to nerve tissue resulted in a greater yield of labelled $^{14}CO_2$ from 1-^{14}C-glucose than that produced from 6-^{14}C-glucose. These findings suggested that the pentose phosphate pathway is operative under the influence of nerve growth factor. It is shown that during the operation of the pentose phosphate pathway the major end products are reduced nicotinamide adenine dinucleotide phosphate (NADPH), CO_2, and ribose. The ribose molecule may be used in nucleic acid synthesis and NADPH may be used in fatty acid and steroid synthesis. Several investigators have demonstrated an increased rate of oxidation of carbon 1 of glucose in neural tissue in the presence of nicotinamide adenine dinucleotide phosphate (NADP) (17,25,46). The studies of Robinson and Phillips (66) have shown that the activity for glucose-6-phosphate dehydrogenase, the first enzyme of the pentose phosphate pathway, is greater in white matter than its activity in gray matter of neural

tissues. O'Neill and Duffy (58) have concluded from their studies that a portion of glucose is metabolized via the pentose phosphate pathway in the canine neonatal cortex. Moss (55) has demonstrated in the semi-isolated calf brain that the major pathway for glucose catabolism is the pentose monophosphate pathway.

During the past decade evidence has accumulated suggesting that glucose can be converted into amino acids in the brain. More recent investigations have provided evidence that when ^{14}C-glucose is metabolized by neural tissue, part of the glucose carbon appears as labelled $^{14}CO_2$ and a considerable amount of the glucose carbon is incorporated into amino acids, mainly aspartic and glutamic acids (56,57,84). The studies of Vrba (84) have demonstrated that 2 minutes after the injection of U-^{14}C-glucose into the tail vein of rats about 40% of the glucose carbon retained in the brain was in the form of protein, lipids, and amino acids. Both aspartic and glutamic acids were highly labelled.

O'Neal _et al_. (57), using ^{14}C-glucose in their experiments, have demonstrated that glucose is by far the best precursor of the free amino acids in sheep brain. Most of the radioactivity incorporated into amino acids was found to be in aspartic and glutamic acids (57). O'Neal _et al_. (57) have concluded from their experiments that the labelling patterns of glutamic acid from the incorporation of the ^{14}C-glucose carbons indicated that pyruvate is metabolized via acetyl-CoA in ovine brain. In addition, it was shown that the ovine brain metabolizes the volatile fatty acids via the tricarboxylic acid cycle and that the volatile fatty acids enter the brain in sheep without prior metabolism by other tissues. Gaitonde, _et al_. (32) have shown that within 2.5 minutes after the subcutaneous injection of U-^{14}C-glucose into rats and cats, about 24-36% of

the total radioactivity in the brain was incorporated into the free amino acids and that glutamic acid was the more highly labelled amino acid. Vrba, et al. (86) have demonstrated that after the subcutaneous injection of U-^{14}C-glucose into the rat, glutamic and aspartic acids accounted for about 62-69% of the incorporated activity in the brain protein fraction. In addition, it was shown that after the subcutaneous injection of U-^{14}C-glucose in rats, the proportion of incorporated radioactivity into aspartic and glutamic acids was higher in the brain than in any other organ. Geiger (35) has shown that endogenous proteins are highly important substrates for metabolic energy in neural tissue. Also, Geiger, et al. (38) have shown that convulsions produced either by electric shock or by pentylenetetrazol treatment in the perfused brain of the cat resulted in an increased rate of metabolic activity in the brain tissue which is supported by the oxidation of substances other than glucose. Alweis and Magnes (5) have demonstrated that pentylenetetrazol induced convulsions produced in cats, when brains from these cats were perfused with labelled glucose, resulted in an increased production of non-labelled CO_2 and a decrease in the incorporation of radioactivity into the CO_2. Klein and Olsen (49) have demonstrated that after the intravenous injection of glutamate, lactate, or succinate into rats the concentration of these substances in the brain was less than the concentration of glucose required to maintain central nervous system function. It was concluded from these experiments that these substances would not serve as a substitute for glucose to relieve hypoglycemic symptoms. Vrba (84) has suggested that since the carbons of glucose are incorporated into various substances and retained in the brain for considerable periods of time, that the CO_2 produced by the brain is due

in part to the metabolism of amino acids, proteins, lipids, and other non-carbohydrate substances.

Previous work dealing with the activities of enzymes involved in the utilization of glucose in neural tissue has established that a possible species difference does exist with regard to glucose metabolism in the ruminant brain and the non-ruminant brain. Raggi and Kronfeld (62) have demonstrated that the enzyme hexokinase of ovine brain has a greater affinity for glucose than does the hexokinase in the brain of rats. Oyler (60) has shown that caprine and canine brains utilize glucose as a substrate at comparable rates. Since the normal blood glucose concentrations in the ruminant animal are considerably lower than that of the non-ruminant animal the above results have indicated that, considering blood glucose concentrations, a proportionately larger amount of blood glucose may be taken up by the ruminant brain as compared to that which is taken up by the non-ruminant brain. The studies of Setchell (68) have shown that the hexokinase activity in ovine brain was 150 micromoles of glucose phosphorylated/g fresh wt./hr. at 30°, whereas the hexokinase activity in the brain of the guinea pig was 220 micromoles of glucose phosphorylated/g fresh wt./hr. at 30°. In addition, Setchell (69) has shown that there was no difference in hexokinase activity between homogenates from the brains of insulin-treated sheep and normal sheep. Kronfeld and Raggi (50) have shown that ovine brain glucokinase activity is not decreased during pregnancy toxemia even though glucose utilization by the ovine brain may be inhibited. The studies of Raggi, _et al._ (63) have clearly demonstrated that the enzymatic activities for soluble glucokinase in the duodenum, brain, kidney, liver, muscle, and adipose tissues of the rat are 2.3 to

6.3 times higher than that in the corresponding bovine tissues. Abraham, _et al_. (3) have shown that washed mitochondrial preparations prepared from brains of rats accounted for 55% of the glucokinase activity present in the brain. Crane and Sols (18) have shown that 70-90% of the total activity for brain glucokinase of the rat was present in the mitochondrial fraction. These studies have demonstrated that brain mitochondria, in contrast to those of other tissues, are involved in glycolysis. It has been demonstrated in rat brain homogenates over 75% of the glycolytic oxidation-reduction activity is found in the supernatant fraction (non-particulate) prepared from the gray or white matter (2). In 1959, the studies of Abood, _et al_. (1) showed that glycolytic enzymes are present in brain mitochondria. Previous work has shown that the brain is capable of oxidizing glucose via the pentose phosphate pathway. The studies of Glock and McLean (42,43) showed that glucose-6-phosphate dehydrogenase, the enzyme leading into the pentose phosphate pathway, and glucose-6-phosphogluconate are present in the brain of the rat in sufficient quantity to account for the operation of the pentose phosphate pathway. It is well established that NADPH is produced via the pentose phosphate pathway. It is known that NADPH is used in the metabolic processes involved in fatty acid and steroid synthesis. Glock and McLean (42,43) have demonstrated that the levels of NADP and NADPH are low in the brain of the rat, suggesting the possibility that the brain of the rat has the necessary enzymatic capacity to oxidize glucose via the pentose phosphate pathway; however, since the levels of NADP and NADPH present in the brain are low, probably only a small amount of glucose is metabolized via this process.

Since it has been demonstrated that a species difference exists

with regard to glucose metabolism in neural tissue between the ruminant and non-ruminant animals, there is a need for a comparative study of glucose metabolism in brain tissue of the ruminant and non-ruminant animal. Earlier work has shown that glucose is metabolized in neural tissue via the glycolytic pathway, the pentose phosphate pathway, and the TCA cycle. It is not known if there is a species difference between the ruminant brain and the non-ruminant brain as to the major metabolic routes for glucose utilization. Also, there has been some disagreement with regard to the activities of several key enzymes in brain tissue of ruminant and non-ruminant animals. Since there have been conflicting reports appearing in the literature concerning glucose metabolism in brain tissue between the ruminant and non-ruminant animals, it was felt that an additional investigation was needed to compare the activities of several key enzymes of the glycolytic pathway, the pentose phosphate pathway, the electron transport chain, and the TCA cycle. In addition, it was felt that a comparative study of the fate of uniformly labelled ^{14}C-glucose incubated in brain tissues of the ruminant and non-ruminant animals would be of value. The enzymic activity portion of this study should point to any species differences which may exist with respect to routes and rates of substrate utilization by brain tissue. The labelled glucose portion of this study should furnish additional evidence concerning any species differences which may exist in the pathway of glucose utilization between the ruminant and non-ruminant brain.

CHAPTER III

MATERIALS AND METHODS

Twenty mature goats and twenty dogs of mixed sex and breed were used for this experiment. The goats were kept in exposed pens and their diet consisted of prairie hay *ad libitum* and a daily feeding of a commercially prepared grain mixture. The dogs were kept in exposed pens and their diet consisted of a single daily feeding of a commercially prepared dog food. Ten dogs and goats were used for the enzymic activity experiments and a like number were used for the U-^{14}C-glucose experiments.

The goats and dogs were sacrificed by electrocution and entrance was made into the cranial cavity within one minute after death for the removal of a portion of tissue form the ectolateral gyrus of the brain. The brain tissue samples were weighed and homogenized in a sufficient quantity of cold, isotonic KCl (1.15%) to make a 5.0% and a 10.0% homogenate. The brain tissue was homogenized with a motor-driven resin-coated pestle[1]. The brain tissue homogenates were stored at 0° until assayed for enzymatic activity. The activity for cytochrome oxidase, lactic dehydrogenase, malic dehydrogenase, and succinic dehydrogenase were measured within one hour after the collection of the brain tissue.

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[1]Potter-Elvehjem homogenizer, Arthur H. Thomas Co., Philadelphia, Pa.

Glucose-6-phosphate dehydrogenase and phosphoglycerate kinase activities were measured within three hours after the collection of the brain tissue. Aldolase, isocitric dehydrogenase, and phosphohexose isomerase activities were determined within eight hours after the collection of the brain tissue. The protein content of each brain homogenate was determined by the Biuret method as described in Clark (15), so that the activity of all enzymes could be expressed on the basis of the rate of activity per unit weight of brain protein.

Enzymatic Activity Procedures

Aldolase (EC No. 4.1.2.7) activity was measured by the method described in Sigma Technical Bulletin No. 750 (72), which is a modification of the method first described by Sibley and Leniger (71). The procedure was further modified for tissue homogenates by using 0.2 ml of the 10.0% homogenate diluted 1 to 10 parts with cold distilled water to make a 1% homogenate dilution instead of using 0.2 ml of serum. A trizma buffer (pH 8.6) was added to the 1% brain homogenate (72). The reproducibility of this procedure was estimated ± 10% (72). The aldolase activity was expressed as the millimoles of fructose-1,6-diphosphate hydrolyzed per hour per gram of brain protein.

Cytochrome oxidase (EC No. 1.9.3.1) activity was determined by measuring the oxygen uptake of brain homogenates on the Gilson Differential Respirometer[2]. The respirometer flasks were prepared by adding 1.4 ml of 0.2 M phosphate buffer (pH 7.4), 0.6 ml of 2.4×10^{-4} M cytochrome C, 0.3 ml of 4×10^{-3} M $AlCl_3$, 0.3 ml of 0.114 M Na ascorbate

[2]Gilson Medical Electronics, Middleton, Wisconsin.

(pH 7.4), 0.2 ml of distilled water, and 0.2 ml of the 10.0% brain homogenate to the flask, and adding 0.2 ml of a 25% aqueous KOH solution to the center well of the flask. After 10 minutes of equilibration of the reaction mixture at 37°, oxygen uptake was measured at 10 minute intervals for a period of 30 minutes. The final pH of the reaction mixture was 7.4. The enzymatic activity was linerally dependent upon the amount of homogenate added in the range of 0.2-0.5 ml of brain homogenate. The results for cytochrome oxidase activity were expressed as the ml of oxygen uptake per hour per gram of brain protein.

The 10.0% brain homogenate was centrifuged at 10,000 x gravity for 30 minutes at 4° and the resulting supernatant was utilized for measuring glucose-6-phosphate dehydrogenase (EC No. 1.1.1.49) activity. The following materials were incubated in a quartz cuvette (1.0 cm x 0.5 cm x 4.0 cm) for 10 minutes at room temperature: 100 µl of the centrifuged 10.0% brain homogenate, 100 µl of 0.1 M tris (hydroxymethyl aminomethane) buffer (pH 7.4), 200 µl of 0.05 M $MgCl_2$ (pH 7.4), 100 µl of 0.005 M reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 200 µl of 0.01 M nicotinamide adenine dinucleotide phosphate (NADP). After a 10 minute equilibration period, 200 µl of 0.25 M glucose-6-phosphate was added to the reaction mixture and the change in optical density was measured at 340 mµ for 10 minutes on a Carey Model 15 Recording Spectrophotometer[3]. The enzymic activity was linerally dependent upon the amount of homogenate added in the range of 50 µl to 200 µl of the centrifuged brain homogenate. The glucose-6-phosphate dehydrogenase activity was expressed as the micromoles of NADP reduced

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[3]Applied Physics Corporation, 2724 S. Peck Rd., Monrovia, Calif.

to NADPH per hour per gram of brain protein.

The enzymic activity of isocitric dehydrogenase (EC No. 1.1.1.42) was determined by the method described in Sigma Technical Bulletin No. 175 (73). The method was modified by using 0.2 ml of the 10.0% brain homogenate which was diluted to a 1% homogenate with cold distilled water in place of using 0.2 ml of serum. The final reaction mixture was buffered at pH 7.5. The isocitric dehydrogenase activity units were obtained by converting optical density units to Sigma units of isocitric dehydrogenase per ml of homogenate as described in Sigma Technical Bulletin No. 175 (73). A calibration curve for this conversion was determined by the method described in Sigma Technical Bulletin No. 175 for a Model 14 Universal Spectrophotometer[4]. The isocitric dehydrogenase activity was expressed as the micromoles of NADP reduced to NADPH per hour per gram of brain protein.

The lactic dehydrogenase (EC No. 1.1.1.27) activity was determined by the method described in Sigma Technical Bulletin No. 500 (74). The method was modified by using 0.1 ml of the 5.0% brain homogenate in place of 0.1 ml of diluted (1:6) serum. The final reaction mixture was buffered at pH 7.5. The lactic dehydrogenase activity was obtained by converting the optical density units determined in the enzymic assay to lactic dehydrogenase units per ml of homogenate using a prepared calibration curve on a Model 14 Universal Spectrophotometer as described in Sigma Technical Bulletin No. 500 (74). The lactic dehydrogenase activity was expressed as the micromoles of pyruvate reduced to lactic acid per hour per gram of brain protein.

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[4]Coleman Instruments, Inc., Maywood, Ill.

Malic dehydrogenase (EC No. 1.1.1.40) activity was determined by measuring the oxygen uptake by the brain homogenates on the Gilson Differential Respirometer. The complete enzymic assay system contained: 0.3 ml of 0.1 M nicotinamide (pH 7.4), 0.3 ml of 0.5 M glutamate (pH 7.4), 0.3 ml of 0.5 M malate (pH 7.4), 0.3 ml of 4×10^{-4} M cytochrome C, 1.0 ml of 0.2 M phosphate buffer (pH 7.4), 0.5 ml of the 10.0% brain homogenate to each of the flasks. The final reaction mixture was at pH 7.4. Two-tenths ml of an aqueous 25% KOH solution was added to the center well and 0.3 ml of 5% nicotinamide adenine dinucleotide (NAD) was pipetted into the sidearm. The flasks were allowed to equilibrate in the respirometer water bath (37°) for 10 minutes. After the 10 minute equilibration period, the NAD in the sidearm was added to the reaction mixture and oxygen uptake was measured at 10 minute intervals for 30 minutes. The enzymic activity was linerally dependent upon the amount of homogenate added in the range of 0.2 ml to 0.5 ml of the 10% brain homogenate. Malic dehydrogenase activity was expressed as the ml of oxygen uptake per hour per gram of brain protein.

The 10.0% brain homogenate was centrifuged at 10,000 x gravity for 30 minutes at 4° and the resulting supernatant was diluted with distilled water to make a 2.0% solution which was used for measuring phosphoglycerate kinase (EC No. 2.7.2.3) activity. The following materials were incubated in a quartz cuvette (1.0 cm x 0.5 cm x 4.0 cm) for 10 minutes at room temperature: 100 µl of the 2.0% centrifuged homogenate, 100 µl of 0.1 M tris buffer (pH 7.4), 50 µl of 1.0% hydrazine HCl (pH 7.4), 50 µl of 3.3×10^{-4}M glycine, 100 µl of 1.0×10^{-3} M $MgSO_4$, 100 µl of 0.05 M adenosine triphosphate (ATP), 100 µl of 0.035 M NADH, 100 µl of 0.2 M cysteine (pH 7.4), and 200 µl of 0.01% glyceraldehyde

phosphate dehydrogenase. After 10 minutes of incubation of the reaction mixture, 100 μl of 0.1 M phosphoglyceric acid was added to the reaction mixture and the change in optical density was measured at 340 mμ for 10 minutes on a Carey Model 15 Recording Spectophotometer. The final enzymic assay system was at pH 7.4. The phosphoglycerate kinase activity was lineral1y dependent upon the amount of centrifuged 2.0% brain homogenate added in the range of 50 μl to 200 μl of the homogenate. The phosphoglycerate kinase activity was expressed as the millimoles of NADH oxidized to NAD per hour per gram of brain protein.

The phosphohexose isomerase (EC No. 5.3.1.9) activity was determined by the method described in Sigma Technical Bulletin No. 650 (75). The procedure was modified by using 0.1 ml of the 10.0% brain homogenate diluted with distilled water to make a 1% brain homogenate in place of using 0.1 ml of blood serum. The final enzymic assay system was at a pH 7.4. The phosphohexose isomerase activity was obtained by converting the optical density units determined in the enzymic assay to phosphohexose isomerase units per ml of homogenate using a prepared calibration curve on a Model 14 Universal Spectrophotometer as described in Sigma Technical Bulletin No. 650 (75). The phosphohexose isomerase activity was expressed as the milligrams of glucose-6-phosphate converted to fructose-6-phosphate per hour per gram of brain protein.

The succinic dehydrogenase (EC No. 1.3.99.1) activity was determined by measuring the oxygen uptake by brain homogenates on the Gilson Differential Respirometer. The following materials were added to the respirometer flasks: 0.2 ml of the 10.0% brain homogenate, 1.9 ml of 0.2 M phosphate buffer (pH 7.4), 0.3 ml of 0.01 M methylene blue, 0.3 ml of 0.1 M KCN, and 0.3 ml of 0.4 M Na succinate. Two-tenths ml of an

aqueous 25% KOH solution was added to the center well of the flask. After a 10 minute equilibration period at 37°, oxygen uptake by the reaction mixture was measured at 10 minute intervals for a period of 30 minutes. The final enzymic assay system was at pH 7.4. The succinic dehydrogenase activity was linerally dependent upon the amount of 10% brain homogenate added in the range of 0.2-0.4 ml of homogenate. Succinic dehydrogenase activity was expressed as the ml of oxygen uptake per hour per gram of brain protein.

^{14}C-Glucose Procedures

The following materials were incubated for 18 hours in a 50 ml Erlenmeyer flask in a shaking water bath (37°): 5.0 ml of a 5.0% brain homogenate, 4.9 ml of 0.55 M glucose, 5.0 ml of 0.2 M phosphate buffer (pH 7.4), and 0.1 ml of D-U-^{14}C-glucose[5] (radioactive concentration of 1.0 microcurie per 0.1 ml of solution). Following the 18 hour incubation period, the carbohydrate, cholesterol, glycerolphospholipid, sphingolipid, protein, and nucleic acid fractions were fractionated from the brain homogenates. The $^{14}CO_2$ produced during the 18 hour incubation period by the brain homogenate was collected in 1.0 ml of hydroxide of Hyamine 10-X[6] which was pipetted into a suspended glass center well of each flask according to the method described by Cuppy and Crevase (23).

The following procedure was used for fractionating the brain homogenates into the carbohydrate, cholesterol, glycerolphospholipid, sphingolipid, protein, and nucleic acid fractions. Ten ml of cold (4°)

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[5] Amersham/Searle Corporation, Des Plaines, Ill.

[6] Packard Instrument Co., Inc., Downers Grove, Ill.

15% trichloroacetic acid (TCA) was added to the incubation mixture, and the solution was allowed to stand for 15 minutes in an ice bath with occasional mixing. The TCA-homogenate mixture was centrifuged at 1,000 x gravity, and the supernatant solution from the above centrifugation was saved and labelled as the carbohydrate fraction. The above procedure including centrifugation was repeated two times using 5.0 ml of a 5.0% TCA solution and 2.0 ml of a 1.0% TCA solution in place of the 15.0% TCA solution. The supernates from the preceeding TCA wash were added to the carbohydrate fraction. The precipitates from the above TCA steps were combined and used for further fractionation procedures.

The lipid fractions were fractionated from the TCA precipitates according to a combination of methods described by Clark (15), Sripati, _et al_. (78), and Steele (79). The residues were treated with 8.0 ml of dioxane and the resulting solution was centrifuged at 1,000 x gravity. Sripati, _et al_. (78) have demonstrated that dioxane prevents the loss of ribonucleic acid (RNA) to lipid solvents. Five ml of acetone were added to the precipitate from the above dioxane step and the solution was allowed to stand for 10 minutes in an ice bath with occasional mixing. The mixture was centrifuged at 1,000 x gravity for 5 minutes at 4°, and the resulting supernate was reserved and labelled as the cholesterol fraction. The preceeding acetone procedure was repeated 2 times, and the resulting acetone supernatant solutions were added to the cholesterol fraction. The precipitates from the preceeding acetone steps were combined and used for the isolation of the glycerolphospholipid fraction. Ten ml of a diethyl-ether (3:1) solution were added to the acetone precipitates from the above acetone steps, and the mixture was allowed to stand in an ice bath for 10 minutes with occasional mixing. The mixture

was centrifuged at 1,000 x gravity, and the resulting supernatant was labelled the glycerolphospholipid fraction. The precipitate from the above glycerolphospholipid centrifugation was suspended in 10.0 ml of 95% ethanol. The ethanol mixture was placed in a 80° water bath for two minutes. The mixture was cooled to room temperature and centrifuged at 1,000 x gravity for 15 minutes. The resulting supernatant from the above centrifugation was labelled as the sphingolipid fraction. The precipitate from the sphingolipid centrifugation was suspended in 2.0 ml of cold 10.0% TCA, and the mixture was allowed to stand in an ice bath for 20 minutes with occasional mixing. The mixture was centrifuged at 1,000 x gravity at 4° and the resulting supernatant solution from the above centrifugation was labelled the nucleic acid fraction. The above procedure was repeated using 5.0 ml of 5.0% TCA in place of the 10.0% TCA, and the mixture was heated for 10 minutes in an 80° water bath. The mixture was cooled to room temperature and centrifuged at 10,000 x gravity for 30 minutes. The resulting supernatant solution from the above centrifugation step was added to the nucleic acid fraction. The remaining precipitates from the final two steps were suspended in 5.0 ml of water and labelled as the protein fraction.

A 0.2 ml aliquot was removed from each fraction isolated above and was used in liquid scintillation counting. A Packard Series 314 E Tri-Carb Liquid Scintillation Spectrometer[7] was used for liquid scintillation counting. A liquid scintillation solution containing 7 g/l PPO[8], 0.3 g/l dimethyl POPOP[8], and 100 g/l naphthalene in dioxane (reagent

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[7]Packard Instrument Co., Inc., La Grange, Ill.

[8]Packard Instrument Co., Inc., Downers Grove, Ill.

grade) was used in counting the 0.2 ml aliquots from each fraction. Background counts were subtracted from the ^{14}C activity counts for each sample. Optimal window settings were made for quenching corrections and a machine counting efficiency of 79% was determined for the spectrometer.

CHAPTER IV

RESULTS

The specific aims of this investigation were: (1) to compare the activities of several key enzymes of the glycolytic pathway, the pentose phosphate pathway, the electron transport chain, and the TCA cycle in canine and caprine brain homogenates, and (2) to compare the fate of uniformly labelled ^{14}C-glucose incubated with brain homogenates prepared from canine and caprine brain homogenates. The first part of this study was designed to point to any species differences which may exist with respect to routes and rates of substrate utilization by brain tissue. The intent of the second part of this study was to furnish additional evidence concerning any species differences in glucose utilization which may exist between canine and caprine brains.

Enzymatic Activities

The four glycolytic enzymes studied in this experiment were aldolase, lactic dehydrogenase, phosphoglycerate kinase, and phosphohexose isomerase. The activities for these glycolytic enzymes are shown in Table I. There was a very highly significant species difference ($p < 0.001$) in the activities for aldolase, lactic dehydrogenase, and phosphohexose isomerase when comparing caprine and canine brain homogenates. The average activity for aldolase was 1.67 $\pm$ 0.05 millimoles of fructose-1,6-diphosphate hydrolyzed per hour per gram of canine

TABLE I

THE ACTIVITY FOR THE FOUR GLYCOLYTIC ENZYMES STUDIED IN CANINE AND CAPRINE BRAIN HOMOGENATES[1]

Enzyme	Activity Units[2]	
	Dog	Goat
Aldolase	1.67 ± 0.05	1.31 ± 0.03
Lactic Dehydrogenase	978.0 ± 24.6	872.3 ± 31.0
Phosphoglycerate Kinase	4.44 ± 0.40	3.63 ± 0.31
Phosphohexose Isomerase	78.8 ± 2.1	67.8 ± 1.7

[1]Each value represents the mean of 10 animals ± standard error of the mean.

[2]Activity units are explained in the text for each individual enzyme.

brain protein, whereas in the caprine brain homogenates the average activity for aldolase was 1.31 ± 0.03 millimoles of fructose-1,6-diphosphate hydrolyzed per hour per gram of brain protein. The average activity for canine lactic dehydrogenase was 978.0 ± 24.6 micromoles of pyruvate reduced to lactic acid per hour per gram of brain protein as compared to an activity of 872.3 ± 31.0 in the caprine brain. The average activity for phosphohexose isomerase, expressed in milligrams of glucose-6-phosphate converted to fructose-6-phosphate per hour per gram of brain protein, was 78.8 ± 2.1 in the canine brain homogenates as compared to a value of 67.8 ± 1.7 for that of the caprine brain. Also, there was a highly significant species difference ($p < 0.01$) between the caprine and canine brain homogenates in the activity for phosphoglycerate kinase. The average activity for phosphoglycerate kinase in the canine

brain homogenates was 4.44 ± 0.40 millimoles of NADH oxidized to NAD per hour per gram of brain protein when compared to an average activity of 3.63 ± 0.31 in the caprine brain.

Table II summarizes the results for the activity of glucose-6-phosphate dehydrogenase in the canine and caprine brain homogenates. There was a very highly significant species difference ($p < 0.001$) between the caprine and canine brain homogenates in the activity for glucose-6-phosphate dehydrogenase. The average activity for glucose-6-phosphate dehydrogenase in the caprine brain was 717.0 ± 32.4 micromoles of NADP reduced to NADPH per hour per gram of brain protein compared to an average activity of 572.4 ± 25.9 in the canine brain homogenates.

TABLE II

THE ACTIVITY FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN CANINE AND CAPRINE BRAIN HOMOGENATES[1]

Enzyme	Activity Units[2]	
	Dog	Goat
Glucose-6-phosphate dehydrogenase	572.4 ± 25.9	717.0 ± 32.4

[1]Each value represents the mean of 10 animals ± standard error of the mean.

[2]Glucose-6-phosphate dehydrogenase is expressed as the micromoles of NADP reduced to NADPH per hour per gram of brain protein.

There were no significant species differences in the activities of

cytochrome oxidase, isocitric dehydrogenase, malic dehydrogenase, and succinic dehydrogenase between the canine and caprine brain homogenates. Tables III and IV summarize the results for the activities of these enzymes which are closely associated with the electron transport chain and the TCA cycle. The average activity of the canine brain cytochrome oxidase was 60.0 $\pm$ 5.0 ml of oxygen uptake per hour per gram of brain protein compared to an average activity of 65.2 $\pm$ 1.8 for the caprine brain homogenates. The average activity of canine brain isocitric dehydrogenase was 168.2 $\pm$ 8.1 micromoles of NADP reduced to NADPH per hour per gram of brain protein as compared to an average activity of 155.1 $\pm$ 7.3 in the caprine brain homogenates. The average malic dehydrogenase activity of the canine brain homogenates was found to be 42.7 $\pm$ 2.1 ml of oxygen uptake per hour per gram of brain protein compared to the caprine brain malic dehydrogenase average activity of 41.1 $\pm$ 2.0. The average activity of succinic dehydrogenase of the canine brain homogenates was 6.5 $\pm$ 0.4 ml of oxygen uptake per hour per gram of brain protein compared to an average activity of 6.4 $\pm$ 0.5 for the caprine brain succinic dehydrogenase.

^{14}C-Glucose Incorporation

The results of the studies on the percent of incorporation of U-^{14}C-glucose carbon atoms into CO_2, carbohydrate, lipid, protein, and nucleic acid fractions isolated from canine and caprine brain homogenates following 18 hours of incubation are summarized in Tables V and VI. In order to determine the percent of incorporation of U-^{14}C-glucose into each fraction the ^{14}C activity in each fraction was divided by the total ^{14}C activity present in the brain homogenate prior to

TABLE III

THE ACTIVITY FOR THE THREE TCA CYCLE ENZYMES STUDIED IN CANINE AND CAPRINE BRAIN HOMOGENATES[1]

Enzyme	Activity Units[2]	
	Dog	Goat
Isocitric Dehydrogenase	168.2 ± 8.1	155.1 ± 7.3
Malic Dehydrogenase	42.7 ± 2.1	41.1 ± 2.0
Succinic Dehydrogenase	6.5 ± 0.4	6.4 ± 0.5

[1]Each value represents the mean of 10 animals ± standard error of the mean.

[2]Activity units are explained in the text for each individual enzyme.

TABLE IV

THE ACTIVITY FOR CYTOCHROME OXIDASE IN CANINE AND CAPRINE BRAIN HOMOGENATES[1]

Enzyme	Activity Units[2]	
	Dog	Goat
Cytochrome Oxidase	60.0 ± 5.0	65.2 ± 1.8

[1]Each value represents the mean of 10 animals ± standard error of the mean.

[2]Cytochrome oxidase activity is expressed as the ml of oxygen uptake per hour per gram of brain protein.

TABLE V

THE AVERAGE PERCENT OF INCORPORATION OF RADIOACTIVITY FROM U-^{14}C-GLUCOSE INTO CO_2, CARBOHYDRATE, LIPID, PROTEIN, AND NUCLEIC ACID FRACTIONS IN CANINE AND CAPRINE BRAIN HOMOGENATES[1]

Fraction	Percent Incorporation	
	Dog	Goat
Carbohydrate	96.4%	94.9%
CO_2	2.4%	3.7%
Cholesterol	0.3%	0.4%
Glycerolphospholipid	0.2%	0.2%
Sphingolipid	0.2%	0.2%
Protein	0.5%	0.4%
Nucleic Acid	0.1%	0.1%

TABLE VI

THE AVERAGE TOTAL RADIOACTIVITY FOUND IN THE CO_2, CARBOHYDRATE, LIPID, PROTEIN, AND NUCLEIC ACID FRACTIONS IN CANINE AND CAPRINE BRAINE HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS[1]

Fraction	Radioactivity[2]	
	Dog	Goat
Carbohydrate	15,673 ± 1,171	15,032 ± 1,462
CO_2	383.5 ± 39.7	590.9 ± 79.5
Cholesterol	56.4 ± 6.4	62.7 ± 9.0
Glycerolphospholipid	25.0 ± 3.0	32.9 ± 5.8
Sphingolipid	31.6 ± 3.2	32.7 ± 3.3
Protein	68.2 ± 16.2	83.7 ± 26.1
Nucleic Acid	17.6 ± 1.5	18.5 ± 2.3

[1]Each value represents the mean of 10 animals ± standard error of the mean.

[2]Radioactivity is expressed as the counts per minute per milligram of brain protein.

fractionation (Table V). There was a highly significant species difference ($p < 0.01$) between the incorporation of radioactivity into $^{14}CO_2$ between the canine and caprine brain homogenates (Table VI). The average incorporation of radioactivity into the $^{14}CO_2$ fraction by canine brain was 383.5 ± 39.7 counts per minute per milligram of brain protein compared to a value of 590.9 ± 79.5 for the caprine brain homogenates. There was no signficant species difference in the incorporation of radioactivity from U-^{14}C-glucose into the carbohydrate, lipid, protein, or nucleic acid fractions.

CHAPTER V

DISCUSSION

It has been well established that glucose is the primary source of energy for the metabolic requirements in neural tissue. During the past decade there has been much controversy among investigators with regard to the passage of glucose across the so-called "blood-brain barrier". The main controversy is concerned with the possible existence of a physical barrier between blood and brain tissues. Since it has been demonstrated that the blood glucose concentration of the ruminant animal is considerably lower than that of the non-ruminant animal, the question which has been asked is how the ruminant animal is able to live normally at a blood glucose concentration which would constitute a hypoglycemia in a non-ruminant animal. Since it has been demonstrated that metabolism in the ruminant animal differs somewhat from that of the non-ruminant animal, the question has arisen concerning glucose metabolism in the ruminant brain versus glucose metabolism in the non-ruminant brain. The studies of Oyler (60) have shown that glucose is taken up at comparable rates by the caprine and canine brains. The studies of Stith (80) have demonstrated that there is a greater uptake of glucose from the incubating medium by caprine brain homogenates compared to that taken up by canine brain homogenates. The above results have shown that a proportionately greater amount of glucose is taken up from the blood by the caprine brain compared to that taken up by the canine brain since the

caprine blood glucose levels are roughly one-half canine blood glucose levels. The obvious question is how can glucose be taken up at comparable rates by caprine and canine brains in view of the lower blood glucose levels in goat than that in the dog. These experiments have further emphasized that a unique relationship exists between blood and brain tissues regarding the passage of glucose. The studies of Raggi and Kronfeld (62) have demonstrated that ovine brain hexokinase has a greater affinity for glucose than rat brain hexokinase. If glucose is rapidly utilized by ovine brain tissue, one would expect an increase in the concentration gradient for glucose between blood and brain tissues. This in turn could offer a partial explanation for glucose movement from blood to brain tissue of sheep despite low blood glucose levels in this species.

It is generally believed that the glycolytic pathway and the TCA cycle are the major metabolic routes for glucose utilization in neural tissue; however, the existence of the pentose phosphate pathway and other metabolic routes have been demonstrated in brain tissue. Also, it has been demonstrated that glucose carbon atoms are incorporated into protein, amino acids, and other non-carbohydrate substances by neural tissue. Earlier work has demonstrated that a species difference exists with regard to the activities of enzymes involved in the utilization of glucose by brain tissue in the ruminant and non-ruminant animals (62). The work of Raggi and Kronfeld (62) already noted above demonstrated that ovine brain hexokinase has a greater affinity for glucose than rat brain hexokinase. The studies of Setchell (68) have shown that ovine brain hexokinase has an activity of 150 micromoles of glucose phosphorylated per gram of fresh tissue per hour as compared to a value

of 220 in the brain of the guinea pig. In addition, Raggi, _et al._ (63) have demonstrated that soluble glucokinase in the brain tissue of rats has an activity approximately 6 times greater than that of the bovine brain. The studies of Glock and McLean (42,43) have demonstrated that glucose-6-phosphate dehydrogenase and glucose-6-phosphogluconate are present in neural tissue of the rat in sufficient quantities to account for the operation of the pentose phosphate pathway, but because of low levels of NADP and NADPH in rat brain tissue it is probably that only a small amount of glucose is metabolized by this process. The current investigation has demonstrated that a species difference does exist between caprine and canine brain homogenates with regard to the activity for aldolase, lactic dehydrogenase, phosphoglycerate kinase, and phosphohexose isomerase. The activities for these four glycolytic enzymes were highly significantly greater ($p < 0.01$) in the canine brain homogenates than in the caprine brain homogenates. On the other hand, the activity for glucose-6-phosphate dehydrogenase was very highly significantly greater ($p < 0.001$) in the caprine brain homogenates than in the canine brain homogenates. Since glucose-6-phosphate dehydrogenase activity was significantly greater in the caprine brain than in the canine brain, it is suggested that a species difference does exist with regard to the operation of the pentose phosphate pathway. There were no significant species differences for the three TCA cycle enzymes and the electron transport chain enzyme studied. Also, the current investigation has demonstrated that the radioactive yields of $^{14}CO_2$ from U-^{14}C-glucose were highly significantly greater ($p < 0.01$) in the caprine brain homogenates compared to yields in the canine brain homogenates.

The current investigation has made no attempt to compare the

activities of different enzymes within a species. It would be meaningless to compare results obtained by different methods using different units of measure without a careful study of the differences between each method. It is generally accepted that the glycolytic pathway and the TCA cycle are the major routes for glucose catabolism in neural tissue, and the existence of the pentose phosphate pathway has been demonstrated by other workers. The studies of DiPietro and Weinhouse (25) have clearly shown that the glycolytic pathway is the major route for glucose utilization in the brain of rats, and that only a small amount of glucose is oxidized via the pentose phosphate pathway. Evidence of the pentose phosphate pathway in neural tissue has been demonstrated by several investigators (6,16,17,25,46,55,58,66).

During the past decade experiments dealing with the fate of ^{14}C-glucose carbons have helped to establish the metabolic pathways for glucose catabolism in neural tissue. More recent investigations have demonstrated that when ^{14}C-glucose is metabolized in brain tissue, a portion of the glucose carbons are incorporated into carbon dioxide, protein, amino acids, and other non-carbohydrate substances (56,57,84). The studies of O'Neal, _et al_. (57) have demonstrated that most of the radioactivity incorporated into amino acids in ovine brain from labelled glucose appears mainly in glutamic and aspartic acids. Vrba, _et al_. (86) have shown that after the subcutaneous injection of U-^{14}C-glucose into rats that glutamic and aspartic acids accounted for 62-69% of the incorporated activity which appeared in the protein fraction. The current investigation has demonstrated that when U-^{14}C-glucose is incubated with caprine brain and canine brain homogenates, there is incorporation of the labelled glucose carbons into the carbon dioxide,

carbohydrate, lipid, protein, and nucleic acid fractions. The caprine brain homogenate yield of $^{14}CO_2$ following incubation with U-^{14}C-glucose was highly significantly greater ($p < 0.01$) than that of the canine brain homogenate. There was no significant species difference in the incorporation of activity into the carbohydrate, lipid, protein, and nucleic acid fractions. The protein fraction was hydrolyzed in 6 N HCl and the constituent amino acids were separated by paper chromatography. Analysis of the amino acids for the percent of radioactivity incorporated into the amino acids from U-^{14}C-glucose revealed that several amino acids including glutamic and aspartic acids were labelled.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The metabolism of glucose in ruminant and non-ruminant brain tissues has been studied by several investigators. Since the results of these studies have failed to demonstrate the precise metabolic pathways by which glucose is utilized by neural tissues of ruminant and non-ruminant animals, a comparative study was designed to study further the utilization of glucose by ruminant and non-ruminant brain tissue. The specific objectives of the current investigation were: (1) to compare the activities of several key enzymes of the glycolytic pathway, the pentose phosphate pathway, the electron transport chain, and the TCA cycle in canine and caprine brain homogenates, and (2) to compare the fate of U-^{14}C-glucose in canine and caprine brain homogenates.

The activities for aldolase, lactic dehydrogenase, and phosphohexose isomerase were very highly significantly greater ($p < 0.001$) in the canine brain homogenates when compared to their activities in caprine brain homogenates. Also, the activity for phosphoglycerate kinase was highly significantly greater ($p < 0.01$) in the canine brain homogenates when compared to that of the caprine brain homogenates. On the other hand, the activity for glucose-6-phosphate dehydrogenase was very highly significantly greater ($p < 0.001$) in the caprine brain homogenates when compared to that of the canine brain homogenates. In addition, there was a highly significantly greater yield ($p < 0.01$) of

$^{14}CO_2$ when U-^{14}C-glucose was incubated with caprine brain homogenates than when U-^{14}C-glucose was incubated with canine brain homogenates. There were no significant species differences in the activities for the three TCA cycle enzymes and the electron transport enzyme studied. Also, there were no significant species differences in the incorporation of the U-^{14}C-glucose carbons into the carbohydrate, lipid, protein, and nucleic acid fractions of the brain homogenates.

The results of this study have demonstrated that a species difference does exist in the activities of several key enzymes involved in the glycolytic and pentose phosphate pathways in canine brain and caprine brain homogenates. The activities for the four glycolytic enzymes studied were highly significantly greater in canine brain homogenates than in caprine brain homogenates, whereas the activity for the pentose phosphate pathway enzyme studied (glucose-6-phosphate dehydrogenase) was very highly significantly greater in caprine brain homogenates than in canine brain homogenates. Since the activity for glucose-6-phosphate dehydrogenase was significantly greater in the caprine brain homogenates than in the canine brain homogenates, it is postulated that the pentose phosphate pathway is more active in the caprine brain than in the canine brain. Also, the current investigation has demonstrated that the four glycolytic enzymes studied were more active in the canine brain than in the caprine brain homogenates.

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APPENDIX A

TABLE VII

ALDOLASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	1.43	1	1.16
2	1.39	2	1.27
3	1.87	3	1.46
4	1.91	4	1.28
5	1.63	5	1.39
6	1.82	6	1.33
7	1.65	7	1.37
8	1.65	8	1.42
9	1.71	9	1.27
10	1.63	10	1.18
Mean $\pm$ $S_{\bar{x}}$ = 1.67 $\pm$ 0.05		Mean $\pm$ $S_{\bar{x}}$ = 1.31 $\pm$ 0.03	

[1]Aldolase activity is expressed as the millimoles of fructose-1,6-diphosphate hydrolyzed per hour per gram of brain protein.

TABLE VIII

CYTOCHROME OXIDASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	32.4	1	74.5
2	34.7	2	72.8
3	75.2	3	66.4
4	49.5	4	59.0
5	74.0	5	66.5
6	61.1	6	64.2
7	68.3	7	63.2
8	63.9	8	56.5
9	71.5	9	65.8
10	69.7	10	62.9
Mean $\pm S_{\bar{x}}$ = 60.0 $\pm$ 5.0		Mean $\pm X_{\bar{x}}$ = 65.2 $\pm$ 1.8	

[1]Cytochrome oxidase activity is expressed as the ml of oxygen uptake per hour per gram of brain protein.

TABLE IX

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	480	1	546
2	702	2	720
3	468	3	684
4	582	4	930
5	660	5	810
6	456	6	750
7	588	7	708
8	576	8	678
9	594	9	714
10	618	10	630
Mean $\pm S_{\bar{x}}$ = 572 $\pm$ 25.9		Mean $\pm S_{\bar{x}}$ = 717 $\pm$ 32.4	

[1]Glucose-6-phosphate dehydrogenase activity is expressed as the micromoles of NADP reduced to NADPH per hour per gram of brain protein.

TABLE X

ISOCITRIC DEHYDROGENASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	141.1	1	132.2
2	135.5	2	207.7
3	206.6	3	148.8
4	196.6	4	176.1
5	178.9	5	149.3
6	153.8	6	149.5
7	168.9	7	131.6
8	135.8	8	149.3
9	180.0	9	166.7
10	185.2	10	140.2
Mean $\pm S_{\bar{x}}$ = 168.2 ± 8.1		Mean $\pm S_{\bar{x}}$ = 155.1 ± 7.3	

[1]Isocitric dehydrogenase activity is expressed as the micromoles of NADP reduced to NADPH per hour per gram of brain protein.

TABLE XI

LACTIC DEHYDROGENASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	1074.2	1	887.0
2	982.1	2	1028.2
3	1126.1	3	843.8
4	872.6	4	987.8
5	982.1	5	861.1
6	964.8	6	887.0
7	947.5	7	694.1
8	901.4	8	780.5
9	915.8	9	936.0
10	1013.8	10	817.9
Mean $\pm S_{\bar{x}}$ = 978.0 $\pm$ 24.6		Mean $\pm S_{\bar{x}}$ = 872.3 $\pm$ 31.0	

[1]Lactic dehydrogenase activity is expressed as the micromoles of pyruvate reduced to lactic acid per hour per gram of brain protein.

TABLE XII

MALIC DEHYDROGENASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	33.1	1	46.3
2	29.2	2	52.8
3	46.4	3	39.9
4	42.8	4	41.0
5	51.5	5	39.1
6	45.1	6	40.4
7	43.4	7	34.5
8	42.7	8	33.1
9	46.0	9	47.9
10	47.2	10	36.1
Mean $\pm S_{\bar{x}}$ = 42.7 $\pm$ 2.1		Mean $\pm S_{\bar{x}}$ = 41.1 $\pm$ 2.0	

[1]Malic dehydrogenase activity is expressed as the ml of oxygen uptake per hour per gram of brain protein.

TABLE XIII

PHOSPHOGLYCERATE KINASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	5.39	1	4.69
2	5.84	2	4.52
3	2.92	3	3.76
4	4.09	4	4.66
5	3.61	5	3.26
6	3.49	6	4.82
7	6.72	7	3.21
8	3.38	8	2.47
9	5.38	9	2.48
10	3.61	10	2.41
Mean $\pm S_{\bar{x}}$ = 4.44 $\pm$ 0.41		Mean $\pm S_{\bar{x}}$ = 3.63 $\pm$ 0.31	

[1]Phosphoglycerate kinase activity is expressed as the millimoles of NADH oxidized to NAD per hour per gram of brain protein.

TABLE XIV

PHOSPHOHEXOSE ISOMERASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	71.0	1	73.7
2	73.3	2	61.4
3	87.9	3	68.7
4	85.1	4	65.6
5	72.5	5	66.5
6	73.0	6	67.3
7	73.0	7	59.8
8	86.4	8	67.2
9	83.3	9	77.6
10	82.8	10	69.8
Mean $\pm S_{\bar{x}}$ = 78.8 $\pm$ 2.1		Mean $\pm S_{\bar{x}}$ = 67.8 $\pm$ 1.7	

[1]Phosphohexose isomerase activity is expressed as the mg of glucose-6-phosphate converted to fructose-6-phosphate per hour per gram of brain protein.

TABLE XV

SUCCINIC DEHYDROGENASE ACTIVITY[1] IN CANINE AND CAPRINE BRAIN HOMOGENATES

Dog	Activity	Goat	Activity
1	6.5	1	8.2
2	5.3	2	9.0
3	7.2	3	7.5
4	7.2	4	7.0
5	9.5	5	5.2
6	6.2	6	5.3
7	5.9	7	5.6
8	4.7	8	5.7
9	6.7	9	6.1
10	5.6	10	4.6
Mean $\pm S_{\bar{x}}$ = 6.5 $\pm$ 0.4		Mean $\pm S_{\bar{x}}$ = 6.4 $\pm$ 0.5	

[1]Succinic dehydrogenase activity is expressed as the ml of oxygen uptake per hour per gram of brain protein.

TABLE XVI

^{14}C ACTIVITY IN THE CARBOHYDRATE FRACTION FROM CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	11,233	1	16,209
2	11,849	2	10,510
3	16,383	3	10,816
4	13.330	4	13,355
5	20,129	5	14,853
6	20,614	6	13,008
7	11,913	7	15,016
8	15,847	8	26,707
9	14,866	9	12,742
10	20,569	10	17,103
Mean $\pm S_{\bar{x}}$ = 15,673 $\pm$ 1,171		Mean $\pm S_{\bar{x}}$ = 15,032 $\pm$ 1,462	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

TABLE XVII

^{14}C ACTIVITY IN THE CARBON DIOXIDE FRACTION FROM CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	210	1	694
2	274	2	314
3	376	3	375
4	430	4	1127
5	473	5	275
6	591	6	514
7	345	7	615
8	504	8	718
9	213	9	536
10	419	10	741
Mean $\pm S_{\bar{x}}$ = 384 $\pm$ 39.7		Mean $\pm S_{\bar{x}}$ = 591 $\pm$ 79.5	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

TABLE XVIII

^{14}C ACTIVITY IN THE CHOLESTEROL FRACTION FROM CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	45	1	62
2	61	2	61
3	44	3	48
4	39	4	34
5	48	5	48
6	74	6	41
7	35	7	55
8	45	8	135
9	99	9	80
10	74	10	63
Mean $\pm S_{\bar{x}}$ = 56 ± 6.4		Mean $\pm S_{\bar{x}}$ = 63 ± 9.0	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

TABLE XIX

^{14}C ACTIVITY IN THE GLYCEROLPHOSPHOLIPID FRACTION FROM THE CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	24	1	36
2	19	2	59
3	39	3	16
4	13	4	13
5	27	5	22
6	26	6	18
7	19	7	52
8	20	8	34
9	20	9	18
10	43	10	61
Mean $\pm S_{\bar{x}}$ = 25 $\pm$ 3.0		Mean $\pm S_{\bar{x}}$ = 33 $\pm$ 5.8	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

TABLE XX

^{14}C ACTIVITY IN THE SPHINGOLIPID FRACTION FROM THE CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	17	1	28
2	21	2	24
3	33	3	22
4	25	4	31
5	45	5	31
6	39	6	26
7	23	7	27
8	33	8	55
9	34	9	38
10	46	10	45
Mean $\pm S_{\bar{x}}$ = 32 $\pm$ 3.2		Mean $\pm S_{\bar{x}}$ = 33 $\pm$ 3.3	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

TABLE XXI

^{14}C ACTIVITY IN THE CRUDE PROTEIN FRACTION FROM CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	81	1	279
2	166	2	132
3	31	3	39
4	73	4	121
5	47	5	29
6	13	6	12
7	33	7	25
8	32	8	39
9	148	9	125
10	58	10	36
Mean $\pm S_{\bar{x}}$ = 68 $\pm$ 16.2		Mean $\pm S_{\bar{x}}$ = 84 $\pm$ 26.1	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

TABLE XXII

^{14}C ACTIVITY IN THE CRUDE NUCLEIC ACID FRACTION FROM CANINE AND CAPRINE BRAIN HOMOGENATES AFTER INCUBATION WITH U-^{14}C-GLUCOSE FOR 18 HOURS

Dog	^{14}C Activity	Goat	^{14}C Activity
1	12	1	16
2	13	2	12
3	15	3	13
4	17	4	14
5	18	5	15
6	17	6	11
7	15	7	22
8	17	8	33
9	27	9	21
10	25	10	28
Mean $\pm S_{\bar{x}}$ = 18 $\pm$ 1.5		Mean $\pm S_{\bar{x}}$ = 19 $\pm$ 2.3	

[1]The ^{14}C activity is expressed as the counts per minute per mg of brain protein.

VITA

3

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Candidate for the Degree of

Doctor of Philosophy

Thesis: IN VITRO ENZYMATIC ACTIVITY AND ^{14}C-GLUCOSE METABOLIC STUDIES WITH CAPRINE AND CANINE BRAIN HOMOGENATES

Major Field: Physiology

Biographical:

Personal Data: Born in Louisville, Kentucky, February 2, 1942, the son of Mr. and Mrs. Charles H. Jones, Sr.

Education: Attended Brinkerhoff Elementary School and Johnny Appleseed Junior High School in Mansfield, Ohio; graduated from Upper Arlington High School, Upper Arlington, Ohio, in 1960; received the Bachelor of Science degree from the Ohio State University, Columbus, Ohio, in June, 1965, with a major in Physiology; received the Bachelor of Science degree in Education from the Ohio State University, Columbus, Ohio, in December, 1965, with a major in Chemistry; completed the requirements for the degree of Master of Science at Oklahoma State University in January, 1969, with a major in Physiology; completed the requirements for the degree of Doctor of Philosophy at the Oklahoma State University in May, 1969, with a major in Physiology.

Professional Experience: Employed as a graduate teaching assistant in the Department of Physiology and Pharmacology at the Oklahoma State University from January, 1966 to June, 1966; National Science Foundation graduate fellow in the Department of Physiology and Pharmacology at the Oklahoma State University from August, 1966 to September, 1967; National Institutes of Health graduate fellow in the Department of Physiology and Pharmacology at the Oklahoma State University, Stillwater, Oklahoma, from September, 1967 to May, 1969.