# GLUCOSE HOMEOSTASIS IN THE NEWBORN DOG

by

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# I

## General features of the fetal and neonatal metabolism:

### I The fetus:

The nutrition and metabolism of the fetus has aroused the interest of scientists for several hundred years, even before direct investigations concerning the nature of metabolites themselves became possible. In recent years however, the development of a number of new experimental techniques such as the exteriorized fetus, the implantation of indwelling catheters into the umbilical vessels (Meschia et al, 1965), the perfused placental preparations (Alexander et al, 1955), and extra-corporeal perfusion systems that maintain the isolated fetus (Alexander et al, 1955; Nixon, 1963), made it possible to investigate the metabolism of the fetus directly under more or less physiological circumstances. With these techniques and by the use of newly established chemical micro-methods for the analysis of various biologically important substances present in the body fluids of the mother, fetus and newborn, much has been learned about the fetal metabolism.

### A. Carbohydrate metabolism:

Claude Bernard (1859) discovered glycogen in embryonic tissues including liver more than a hundred years ago. More recently Villee (1954, 1955) found significant amounts of glycogen in liver, kidney, lung, brain and diaphragm of mammalian primates including man, by 9 to 10 weeks of gestation. Bohr (1900) established experimentally that

the respiratory quotient in the fetal guinea pig was about unity. Similar results were reported by Dickens and Simer (1930), in the rat embryo. Since then carbohydrate metabolism in the fetus has become of great interest to embryologists, physiologists, biochemists and paediatricians.

Sources of circulating glucose in the fetus:

Glucose is actively transported by facilitated diffusion from mother to fetus across the placenta (Widdas, 1952). This ensures a faster rate of transport than predicted by the laws of diffusion. In most species studied plasma glucose concentration in the fetus was found to be about half of that present in the mother. This concentration gradient for glucose is thought to be due, at least partially, to the utilization of glucose by the placental tissue itself. Placental perfusion studies in sheep showed that the placenta removes glucose from the perfusate at the rate of about 10 mg/Kg-min (Nixon, 1963). In addition to the actively transported glucose from the mother, the fetus has its own stores of carbohydrate in the form of glycogen. Glycogen was found to be present in the germinal disk of a thirteen day old human fetus (Villee, 1960). The fetal liver at this early stage of development has very little or no glycogen. As the pregnancy advances the amount of glycogen in the liver increases. At term the fetal liver glycogen is about 40 to 60 mg per gram of wet liver weight, about twice the concentration that exists in the mother. (Shelley, 1961, 1964).

Phosphorylation of glucose:

As in the adult, glycogen synthesis occurs mainly from glucose in the fetus. In most adult mammalian species two hepatic enzymes, glucokinase and hexokinase, are found to be present which phosphorylate glucose to glucose-6-phosphate (Walker, 1962; Sols, 1964).

The liver of the fetal rat and guinea pig have no glucokinase activity, which in the normal adult animal is responsible for about 85% of glucose phosphorylating activity. In rats glucokinase activity appears after birth, by about the time of weaning (Walker and Holland, 1965).

The activity of hepatic hexokinase was found to be highest in the late stages of pregnancy and to decline after birth in rats (Burch et al. 1963). The high levels of hexokinase before birth would indicate that even in the absence of glucokinase, the fetal liver has a high capacity for the phosphorylation of glucose.

Glycogen synthesis:

The enzymes involved in the synthesis of glycogen, namely phosphoglucomutase, uridine-diphosphate-glucose (UDPG) pyrophosphorylase, and glycogen synthetase show high levels of activity in fetal rat liver in the latter part of gestation (Dawkins, 1963; Ballard and Oliver, 1963). In spite of the existence of the enzymes necessary for glycogen synthesis, glycogen in the liver of fetal rat appeared only on the 17th day of gestation, reaching its highest level immediately before birth (Jacquot and Kretchmer, 1964; Ballard and Oliver, 1963). Both the adrenals and the pituitary seem to be involved in the control of enzymes responsible for the accumulation of glycogen in the liver late during

gestation, since combined fetal decapitation and maternal adrenalectomy were required to prevent glycogen synthesis in rabbit and rat fetuses (Jost, 1961).

Glycogenolysis:

The enzymes responsible for glycogenolysis, phosphorylase and glucose-6-phosphatase have low activities until very close to birth in embryonic rat and guinea pig livers but increase rapidly at term and in the immediate post natal period (Dawkins, 1963, Kornfeld and Brown, 1963). Glucose-6-phosphatase activity was detected in the liver of fetal rhesus monkey four weeks before term and in the human fetal liver beginning with 12th gestational week (Villee, 1953; Dawkins, 1963).

B. Fat Metabolism:

Radioisotopic studies have shown the placenta to be relatively impermeable to lipids present in maternal blood. (Goldwater and Stetten, 1947; McBride and Korn, 1964). Only a very small amount of labelled palmitic acid was found to pass across the placenta in the sheep and rat. Accordingly the concentration of free fatty acids is much higher in maternal than in fetal plasma (Van Duyne et al., 1960). Large differences in the relative maternal and fetal blood levels of FFA were also reported in humans (Van Duyne and Havel, 1959; Keele and Kay, 1966). Measurements of umbilical arterio-venous differences in acetate concentration in the sheep showed that very little acetate crossed the placenta when this substance was infused into the ewe (Pugh and Scaribrick, 1955). A relative impermeability of the placenta to infused ketone

bodies into the ewe was also reported by Alexander et al. (1969). However, after injection of C-14-labelled sodium acetate into the sheep fetus, a substantial amount of label was found to be incorporated into carbon dioxide in the umbilical vein (Alexander, Britton and Nixon, 1966, 1967) indicating that the fetus is capable of oxidizing acetate. The perfused sheep fetus was also shown to utilize long chain free fatty acids rapidly (Alexander et al., 1967).

Thus, it appears that although fats are not usually available to the fetus in amounts sufficient to become a major source of energy, they may be utilized by the fetus.

In humans there is some evidence that ketone bodies can cross the placenta and may serve as a source of energy (Sabata et al., 1968).

C. Protein metabolism:

The study of concentration of individual amino acids after their perfusion in the fetal sheep show that they could play only a minor role in providing the energy requirements for the fetus (Alexander et al., 1970). It is likely that the amino acids are used mainly for synthesis of proteins rather than for oxidation or gluconeogenesis. Hepatic gluconeogenesis is virtually absent in the rat fetus (Ballard and Hanson, 1967). The key gluconeogenic enzyme - phosphopyruvate carboxykinase (PEP-CK) was absent in the fetal liver (Yeung and Oliver, 1967). Pyruvate-carboxylase, although present in the fetal liver, showed very little activity until shortly before birth (Yeung et al., 1967).

The enzymes involved in amino acid catabolism and in the urea cycle are also of low activity in the fetal liver (Dawkins, 1966).

Thus, it appears that the mammalian fetus derives most of its energy requirements from the oxidation of carbohydrates, primarily glucose.

II The Neonate:

In most species after birth there is a period of starvation. The newborn does not get a proper supply of milk for several hours or even days after birth. During this period the energy requirements for respiration, muscular activity and maintenance of body temperature in the newborn are derived from its own resources.

A. Carbohydrate metabolism:

The glycogen content in both the liver and skeletal muscle falls precipitously within few hours after birth. In the cat liver glycogen loses 90 percent of its concentration at birth within 2 hours. This loss is effected by an increase in hepatic phosphorylase activity (Cake and Oliver, 1969). It was speculated that the activation of phosphorylase may be due to a rise in cyclic AMP which occurs due to an increased sympathetic activity or glucagon secretion consequent to neonatal hypoglycemia (Exton, 1972). Adrenalin and glucagon were shown to activate phosphorylase in slices of newborn rat liver (Dawkins, 1963).

The rapid fall in liver glycogen concentration after birth in many species suggest that its mobilization is the only source of glucose that maintains the blood glucose level at this early age. Hepatic glucose-6-phosphatase activity also shows a significant and rapid increase after birth in most species (Shelley, 1969). In spite of this increased glycogen breakdown however, in newborn humans the blood glucose level falls to low values. A similar fall in plasma glucose occurs in most mammalian species. Although glycogen stores

are substantial, they are not sufficient to meet the energy requirements of the newborn for long, and thus it shifts towards metabolizing fat increasingly. It appears that blood glucose levels are restored to adult values mainly (a) by a replacement of glucose by FFA as a fuel (b) by gluconeogenesis and finally by (c) intake of carbohydrates.

B. Fat metabolism:

In the immediate postnatal period there is a rapid rise in the concentration of plasma FFA, glycerol and ketone bodies in the sheep, (Van Duyne and Havel, 1959), and in man (Keele and Kay, 1966), indicating an increased mobilization of fat. Fat mobilization was also observed in prematurely delivered sheep fetuses (Alexander et al., 1969). In human infants the fat reserves at birth are quite substantial. Wolf et al., (1969), calculated these reserves to be sufficient to supply 5000 calories which would enable physiological activities to continue for several days in the absence of exogenous supply. That considerable lipolysis occurs after birth is also indicated by a progressive fall in the RQ, which is about unity at birth, to about 0.7. In infants this level is reached by about third day of life, the RQ then increases again gradually when the carbohydrates are being supplied by the diet (Benedict and Talbot, 1915; Cross et al., 1957). The cause of lipolysis after birth was attributed to an increase in sympathetic activity since lipolysis could be blocked by hexamethonium in the newborn lamb (Van Duyne et al., 1960, 1965). Cold exposure leading to sympathetic stimulation of adipose tissue was also proposed as a possible mechanism for increased

lipolysis after birth (Scopes, 1965). It is also likely that growth hormone level which is high in the newborn may lead to increased lipolysis. The administration of growth hormone was shown to increase plasma FFA concentrations in infants (Raben and Hollenberg, 1959) and newborn sheep (Manns and Boda, 1965).

C. Protein metabolism:

Soon after birth the circulating amino acid concentration in blood increases rapidly, (Lindblad and Baldesten, 1967). The levels of lysine and tyrosine rise more than those of other amino acids. The increase is transient, afew hours after birth the elevated levels of amino acids start declining. The concentrations of branch - chained amino acids show a greater decrease than those of the others. This early decrease of the branched - chain amino acids from the blood is not associated with their excretion in the urine or with an increased uptake by the liver (McMenamy et al., 1962). It seems there is a considerable extrahepatic uptake of amino acids. This might be due to the high level of growth hormone present in the blood during fetal and early neonatal periods, which would favour extra hepatic transfer of amino acids to various tissues in the body (Westphal, 1968). The branch-chained amino acids are metabolized via acetyl-Co-A and it is possible that they could also contribute towards supply of energy to the newborn during the first few hours of life when it is in a state of starvation.

Gluconeogenesis:

In the newborn infant the amino acids are very actively used for protein synthesis. However, in rats utilization of amino acids for

gluconeogenesis, while absent in the fetus, is initiated on the first day after birth, reaches a peak level on the fifth day, and then decreases gradually to adult rates at about the thirtieth postnatal day. (Yeung and Oliver, 1967). The amino acids derived primarily from the proteins supplied by milk are utilized in gluconeogenesis.

At birth a marked increase occurs in the activity of several gluconeogenic enzymes, aspartate-aminotransferase (Yeung, Stanley and Oliver, 1967), phosphoenol-pyruvate-carboxykinase (Yeung and Oliver, 1968), tyrosine-aminotransferase and serine dehydratase (Greengard and Dewey, 1967) and glucose-6-phosphatase (Dawkins, 1963). Except glucose-6-phosphatase, the other gluconeogenic enzymes are absent or are present only in very small amounts in the liver of the fetus. The increase in their activity begins at birth.

Phosphoenol-pyruvate carboxykinase (PEP-carboxykinase) appears to play a major role in the development of gluconeogenesis in the newborn rat. This belief is supported by the presence of a highly significant correlation between the synthesis of glucose or glycogen from pyruvate and the increase in the activity of phosphoenol-pyruvate carboxykinase during the first week of postnatal life (Ballard, 1971).

It was shown that starvation and diabetes which favour gluconeogenesis also increase the level of PEP-carboxykinase activity by a factor of two in the fetal rat (Yeung and Oliver, 1967). Glucose infusion in the newborn rat supresses the increase in PEP-carboxykinase activity (Yeung and Oliver, 1968).

PEP-carboxykinase activity could be prematurely evoked by glucagon or epinepherine administration to the fetus. (Yeung and Oliver, 1968) or simply by inducing premature delivery. The levels of glucose-6-phosphatase in the rabbit fetus (Dawkins, 1961), and tyrosine aminotransferase in the rat fetus (Holt and Oliver, 1968) could also be increased by inducing premature birth. The activity of glucose-6-phosphatase was shown to remain low if pregnancy was lengthened by three days, but immediately increased after birth. Glucose administered after birth exerted an inhibitory effect on the activity of glucose-6-phosphatase (Dawkins, 1963), together with those of tyrosine aminotransferase and serine dehydratase (Greengard and Dewey, 1967).

There is an increase after birth in the activities of many other enzymes involved in gluconeogenesis such as pyruvate carboxylase, fructose 1-6-diphosphatase, aspartate aminotransferase but since they are already present in significant amounts in the fetal liver their importance in the activation of gluconeogenesis after birth is only contributory.

Enzyme induction in the fetus and the neonate:

It is worth noting that hormones are not able to induce the formation of enzymes in the fetal liver before the fetus reaches a certain age. Cyclic AMP or its dibutyryl derivative on the other hand are effective in evoking tyrosine aminotransferase in fetuses too young to respond to glucagon or epinephrine (Yeung and Oliver, 1967). The capacity of the liver to synthesize and accumulate cyclic AMP in response to glucagon or epinephrine develops after the ability to respond to administered cyclic AMP.

Some of the enzymes present in the liver and some other tissues regress late in fetal life or in the early postnatal period. This regression might be followed by a second increase that occurs in the human approximately three weeks after birth. It is difficult to ascertain the cause of these enzymic changes. No new hormones appear during this period, but the responses by the target organs develop and thus the endocrine integration and regulation become more efficient. It is likely that the change in the diet of the newborn animal may be of some importance. By the third week of life most animals start nibbling their mothers' food also, which might influence the release of hormones and their effects on enzymes. Insulin secretion is known to be affected by starvation and possibly by the composition of the diet. It is likely that hepatic glucokinase activity might be influenced by insulin. Glucocorticoids may also be involved in the accumulation of glucokinase around the third week. On the other hand the reason for some of the enzymes remaining dormant after birth could be attributed to the high estrogen levels derived from the mother at the time of birth. Herzfeld and Greengard (1969), showed that estrogen administration prevented the normal accumulation of ornithine aminotransferase in the rat.

In summary, it appears that development and accumulation of enzymes occurs in at least three stages. (1) The majority of the hepatic enzymes appear during the late fetal period around 16th to 20th day of gestation in rats. (2) Some enzymes appear in the early postnatal period, i.e. on the first day after birth. (3) Whereas some enzymes increase their activity later during postnatal life (suckling period). The

activity of several enzymes show more than one phase of rise and decline. They may first suddenly appear in early fetal period and, after a slow rise during the late fetal period, a second abrupt rise follows immediately after birth. The stimuli responsible for the stepwise development of the enzymes are not definitely determined but certain likely explanations have been offered. The three stages of enzyme development are associated with major physiological events. (1) At late fetal stage the pituitary, thyroid, the adrenal cortex and beta cells of pancreas begin to function. (2) At early neonatal period the glucagon secreting alpha cells of pancreas develop. Further there must be complex changes in the endocrine control mechanisms to enable the newborn to make various adjustments required for its separate existence. (3) At late suckling stage the full activity the pituitary-adrenal axis is established. Other endocrine regulatory processes may also be altered by a change in the diet from a diet rich in proteins and fat to one predominantly of carbohydrate.

# II

## The pathophysiology of neonatal hypoglycaemia

Neonatal hypoglycaemia was described as early as in 1929 by VanCreveld who reported that the concentration of glucose in the blood in premature infants was generally low as compared to that in normal adults. Eight years later Hartman and Jaudon (1937) reported that hypoglycaemia during the first four or five days of life occurs quite regularly in normal newborn infants and suggested that it is due to an imperfectly developed regulatory mechanism which creates a state of relative hyperinsulinism. They also suggested that the frequent occurence in the normal newborn infants of cyanosis, irritability, listlessness, and muscular disorders, such as hypotonicity, hypertonicity, and twitching might very well be due to undetected hypoglycaemia, an essentially "normal" occurence during the first few days of life. Insulin tolerance tests performed shortly after birth on such infants suggested that hypoglycaemia developed principally because of an incompletely developed opposition to insulin activity (Hartman and Jaudon, 1937). Since then there have been numerous studies and reports of very low blood sugar levels observed in the newborn of several mammalian species including man (Norval et al., 1949; Cornblath et al., 1956; Farquhar, 1963; Shelley and Neligan, 1965), rhesus monkey and lambs (Shelley, 1960; Bassett and Alexander, 1971, Dawes et al., 1963), calves (Comline and Edwards, 1968), rat (Dawkins, 1963) and pups (Allen et al., 1966).

In apparently healthy newborn babies blood sugar levels between 20 and 50 mg/100 ml are common. In premature infants the blood glucose level tended to be even lower (Haworth and Ford, 1960). In a nursery unit for premature infants the incidence of hypoglycaemia was 6% (Wybergt et al., 1964). In low birth weight babies with neurological symptoms the incidence of hypoglycaemia was as high as 15% (Wybergt et al., 1964). In normal neonates the low blood sugar values are usually maintained for several hours or days before they gradually rise to a normal adult level. Paradoxically, the clinical signs of hypoglycaemia are relatively rare (Baird and Farquhar, 1962; Farquhar, 1962). However, whereas some neonates seem to be quite unaffected by a low blood sugar level, others may exhibit serious clinical manifestations as convulsions, pallor, profuse perspiration, apneic spells or even circulatory failure (Cornblath et al., 1966; Cornblath and Schwartz, 1966; Hartman and Jaudon, 1937; Hawarth et al., 1963). Symptomatic hypoglycaemia is now established as a pathological entity. It is more common in newborn infants whose weight is low for the period of gestation. The males are affected more than the females. In most cases the hypoglycaemia may spontaneously subside. In other cases it may persist or have a tendency to recur. (Zetterström, 1961). Infants of diabetic mothers are more prone to develop symptomatic hypoglycaemia. In some cases the low blood sugar level may be due to defects of hepatic glycogen storage, hereditary fructose intolerance, galactosemia and hepatic glycogen synthetase deficiency. These conditions may be associated with the absence of specific enzymes in the liver. In a few cases of symptomatic

hypoglycaemia pancreatic islet cell tumours have been reported (Sherman, 1947). Respiratory Distress Syndrome (RDS) and neonatal cold injury are also often associated with symptomatic hypoglycaemia (Usher, 1961; Mann and Eliott, 1957). Often the low blood sugar values were observed to coincide in time with low hepatic glycogen in infants (Shelley and Neligan, 1966).

However, a large number of cases of even symptomatic hypoglycaemia in the neonatal period may not be associated with any other abnormality. They are truly "idiopathic".

It is not known why in some infants low blood sugar level does produce clinical signs whilst in others does not. Scopes (1964) found in calves that clinical signs of hypoglycaemia were absent as long as their blood lactate levels were high. However, Cornblath et al., (1966) found no signs of relief in hypoglycaemic human infants when sodium lactate was administered in quantities sufficient to cause a large increase in blood lactate concentration. Baens et al., (1963) suggested that the length of time during which an infant is hypoglycaemic may be responsible for appearance of neurological symptoms, whereas Hawarth and McRae (1965, 1967) deny this.

<u>Blood glucose in the neonate</u>:

Before birth the fetus receives a continuous supply of glucose from its mother through the placenta and its principal metabolic fuel is carbohydrate. At birth there is a sudden stoppage in the delivery of nutrients from the mother. After birth there is a transitional period

until proper suckling is established during which the newborn has to rely on its own resources. Thereafter its principal fuel is fat. Thus, there occurs a major change in metabolism at birth. This is reflected also by a fall in RQ from approximately 1.0 in the fetus to 0.9 during the first few hours of life and then to 0.73 by third day.

At the time of birth, the blood sugar is usually within adult range. It falls rapidly within a few hours in normal full-term infants. Blood sugar values of 15 - 30 mg/100ml were observed occasionally in otherwise normal infants. In some premature infants blood glucose levels may reach levels as low as 10mg/100ml without apparent clinical signs. (Wachter, 1949, Hawarth and Ford, 1960). Normally the blood sugar reaches a value of about 70 mg/100ml by the third postnatal day. This pattern of postnatal changes in plasma glucose is common to other mammalian species also, viz. rat, rabbit, dogs, sheep and rhesus monkey (Shelley, 1960). Allen et al., (1966) reported that blood sugar values ranged between 42 and 207 mg/100ml in puppies during the initial 48 hours after birth. Thereafter the values stabilized between 70 to 120 mg/100ml. While, hypoglycaemia seems to be a common occurence in the immediate postnatal period, occasionally high levels of blood sugar are also observed. The extreme lability of the plasma glucose level in the newborn would suggest a defect in the regulatory mechanisms which normally in the adult maintain glucose homeostasis. Before discussing the possible sites of defect the factors which control the level of blood sugar in the adult will be briefly reviewed.

The regulation of blood glucose level in the adult:

In an adult the blood sugar remains at a fairly steady level in the post absorptive state. In this state the liver is the main source of glucose. The glucose released continuously into the circulation is being removed therefrom by most tissues of the body. The balance between the rates of glucose production and utilization underlies the steady level of glucose observed in the plasma. The fact that blood sugar remains at a fairly steady level indicates that the balance between the production and uptake of glucose is regulated very precisely. If the rate of appearance of glucose exceeds the rate of its disappearance, the plasma glucose concentration will rise. Conversely, if the rate of disappearance exceeds the rate of its appearance the concentration of glucose in the plasma will fall.

The main regulator of glucose in the plasma is the blood sugar itself. A negative feed-back exists between the plasma glucose concentration and the rate of glucose production and utilization (DeBodo et al., 1963, Hetenyi et al., 1973, Urquhart 1970.) Any disturbance which tends to lower the plasma glucose concentration increases the rate of glucose production by the liver and also tends to decrease its rate of utilization due to the "mass action" of glucose. Conversely hyperglycaemia suppresses hepatic production and enhances utilization of glucose (Hetenyi et al., 1961, Hetenyi and Wrenshall, 1968). These adaptive changes are responsible for the re-establishment of normoglycaemia. However, it is somewhat controversial as to how much of this adaptation is due to the intrinsic activities of liver and tissues as

modified by plasma glucose level and, how much of it is mediated through the release of hormones or the activation of nevous mechanisms.

One hormone at least has a most decisive and central role in this adaptive process. As a direct response to hyperglycaemia insulin is produced from the beta cells of the islets of pancreas and is released into the circulation. Insulin exerts an inhibitory effect on the production of glucose by the liver provided hypoglycaemia is prevented (Madison et al., 1959 ; Hetenyi, Wrenshall and Best, 1961; Steele et al., 1965). Insulin infused into the portal vein was found to be more effective in supressing the hepatic glucose production (Madison et al., 1959; Hetenyi and Ishiwata, 1968) than insulin infused into a peripheral vein. Ishiwata et al., (1969) also reported that release of extra insulin over and above the basal rate of secretion, is required for the adaptive reduction of hepatic glucose production. Insulin secretion may be stimulated by metabolites other than glucose, such as ketones and fatty acids (Greenbourgh et al., 1967, Sanbar et al., 1965). Insulin increases the metabolic clearance rate but, due to the ensuing hypoglycaemia, not necessarily the net utilization or uptake of glucose by peripheral tissues (Forbath and Hetenyi, 1966, Steele et al., 1956; Wall et al., 1957). The metabolic clearance of glucose i.e. the uptake at a given level of glucose in plasma is closely correlated with the amount of insulin released into the circulation. (Vranic et al., 1971). Thus the central role of insulin in re-establishing glucose homeostasis when the glucose concentration in blood tends to rise is well established.

Hypoglycaemia on the other hand increases the rate of hepatic glucose production very markedly. This reaction is triggered by an inadequate supply of glucose to the brain (Hetenyi, 1972). Generally hypoglycaemia is brought about by the administration of a large amount of insulin. The effect of hypoglycaemia on the liver overrides the direct hepatic effect of insulin, at least in dogs (Hetenyi, 1972).

Hypoglycaemia triggers the release of at least three hormones: epinephrine, glucagon and cortico-steroids. Epinephrine suppresses the release of insulin thereby transiently increasing hepatic glucose release and reducing the metabolic clearance rate of glucose. This latter effect is believed to be primarily responsible for epinephrine induced hyper-glycaemia, since its effect on hepatic glycogen mobilization being transient, it does not appear to contribute to sustained hyperglycaemia produced by epinephrine infusion (Altszuler et al., 1967).

Glucagon is generally regarded as the main physiological regulator by which normoglycaemia is being re-established should the blood sugar level fall for any reason. Glucagon is released during hypoglycaemia (Unger et al., 1962). It increases the breakdown of hepatic glycogen due to the activation of phosphorylase mediated by cyclic-AMP (Sutherland and Rall, 1960) in a much smaller molar con-centration than epinephrine (Sokal et al., 1964). The metabolic clear-ance of glucose is not decreased (Cherrington et al., 1972), although paradoxically, glucagon is capable of releasing insulin (Candela 1960) in doses as low as 2 - 5 μg.

The balanced secretion of glucagon and insulin is believed to be of major importance in the maintenance of normoglycaemia. The simultaneous release of these two hormones into the circulation in the molar ratio of 1 : 2.6 at higher than normal rates will increase the turnover of glucose without any significant effect on the concentration of glucose in the blood (Vranic 1972). A model of a dual feedback system to control glucose homeostasis based on graded rates of release of glucagon and insulin by changes in the concentration of glucose in plasma has been suggested (Vranic 1972). Glucocorticoids are also being released during hypoglycaemia. (Steeples and Jensen, 1949). The physiological significance of this reaction however is uncertain.

Glucoregulatory mechanisms in the neonate:

As it appears even from a brief review, the pathogenesis of neonatal hypoglycaemia appears to be complicated. Theoretically, a decreased rate of glucose production or an increased metabolic clearance of glucose or a combination of the two could account for the low blood sugar levels observed in the newborn. Several factors could lead to a decreased glucose production: viz. insufficient glycogen stores, defective glycogen breakdown, absence of gluconeogenesis from all or some specific type of sources. Similarly various conditions, such as increased metabolic rate, increased muscular activity, elevated body temperature, change in plasma pH, excessive insulin secretion, could cause an increased glucose utilization. The relative significance, if any, of these factors has not been adequately assessed.

A high level of insulin secretion could account for an elevated metabolic clearance of glucose and thus for hypoglycaemia. Further, the inadequacy of homeostatic mechanisms (the release of epinephrine, glucagon, perhaps also of cortocoids, growth hormone) could explain the persistence of hypoglycaemia in the newborn.

However, insulin levels while high at birth were not elevated in 12 - 48 hours old rats (Blazquez et al., 1970), or rabbits (Hardman et al., 1971). Also, the rate of fall in plasma glucose concentration after i.v. injected glucose was less than that in adults (Bowie et al., 1963), suggesting that little if any insulin was being released by injected glucose. Similar results were reported in newborn puppies (Allen et al., 1966), and more recently in newborn mice (Lavine et al., 1971). It is evident that hypoglycaemia in the normal newborn cannot be explained on the basis of an increased peripheral utilization or metabolic clearance rate of glucose.

Turning to the hormones of hyperglycaemic effect, Cheek et al., (1963) showed that the chromaffin tissue of normal full term and premature newborn infant was fully capable of producing catecholamines and that epinephrine was secreted in response to a sudden drop in blood sugar level. Induction of hypoglycaemia was shown to produce an exaggerated release of growth hormone as well (Cornblath et al., 1966). Thus at least some of the mechanisms that are activated in the adult by hypoglycaemia are already present in the newborn.

# III

## Aims of the investigation to be reported

The present study was undertaken to examine quantitatively in the newborn dog the homeostatic mechanisms that are responsible for the maintenance of normoglycaemia in the fully grown animal.

(1) The rates of glucose production and utilization were determined by tracer methods in newborn pups. As discussed previously, in the grown animal a very sensitively regulated inverse relationship exists between the rate of glucose production and the concentration of glucose in the plasma. This phenomenon is recognized to be of primary importance in the maintenance of normoglycaemia in the adult. If this mechanism were to be absent or defective in the newborn, the persistence of hypoglycaemia could be accounted for.

(2) In addition a search has been carried out for other mechanisms adapting rates of glucose production to changing metabolic needs. In this context the importance of plasma FFA was also examined.

(3) The sensitivity to injected and endogenously released insulin was examined in the newborn, and the overall efficiency of the homeostatic mechanisms that, in the adult, normally counteract externally induced hyperglycaemia was assessed quantitatively.

(4) Finally the effects of a glucocorticoid were studied. Methylprednisolone has been known to increase gluconeogenesis thereby increasing the rate of production of glucose in the adult. (Ninomiya et al., 1965; Issekutz et al., 1972). This increased production however does not lead

to hyperglycaemia since due to the simultaneous increase in the release of insulin the metabolic clearance of glucose increases too maintaining a normal plasma glucose level with a considerably increased turnover rate of glucose. These experiments were repeated on newborn pups in order to examine whether (a) do pups respond to adrenal steroids with an increased gluconeogenesis? (b) if they do, will insulin too be released thereby maintaining normoglycaemia? (c) if adrenal steroids were found to be ineffective will insulin be released nevertheless? A dissociation of increased insulin secretion from increased glucose formation would point towards a sequence of the events that occur in the adult.

The experimental work and the pertinent discussion will be presented in five parts in this thesis. The first part deals with the relationship between plasma glucose level and hepatic glucose production in the newborn pup; the second part with its response ("sensitivity") to injected or endogenously released insulin. The third part is concerned with the homeostatic responses evoked by externally induced hyperglycaemia. In the fourth part the mechanism by which hypoxia affects the mobilization and overall utilization of glucose and free fatty acids will be examined. Finally in the last series of experiments glucose homeostasis under a well explored situation of endocrine imbalance will be studied.

# MATERIALS AND METHODS

The experimental work was divided into five main parts. Special aspects of the methodology and experimental design pertinent to the individual parts will be dealt with, subsequent to the description of the methods that were used in all five parts.

## I. GENERAL

### Animals:

73 inbred beagle (13 litters) and 77 mongrel pups (14 litters) of both sexes were used in this study. Their ages ranged from 2½ hours to 55 days. Their weights ranged between 156 gm to 2.48 kg.

Experiments were carried out on 14 adult dogs: 11 mothers of beagle and 3 mothers of mongrel litters. These animals were all 3 - 4 weeks post-partum. The weights of the mothers ranged from 7.9 to 21.2 kg. The younger pups were removed from the mother 2½ - 3 hours and older pups, 4 - 5 hours before the experiment. The adult dogs were fasted for 16 - 18 hours prior to the experiment.

### Anesthesia:

Pups were anesthetized by intraperitoneal administration (25 - 30 mg/kg) of a freshly prepared solution of sodium pentobarbital in 0.9 percent NaCl. The mothers were anesthetized by intravenous injection of 30 mg/kg of Nembutal. The anesthesia was maintained by injecting additional doses of 4 - 5 mg/kg of Nembutal during the course of the experiment whenever required.

Most of the younger pups were allowed to breathe with their heads in a funnel through which 95% $O_2$ + 5% $CO_2$ (Carbogen) was allowed to flow resulting in the inhalation of an unspecified mixture of this "gas mixture" and room air.

The body temperature of the pups was maintained at 37.5 $\pm$ 0.5°C. by the use of an automatic feedback control system involving a rectal thermometer and an electric heating pad. In adult dogs no special precautions were taken concerning oxygenation. Their rectal temperature was monitored and a heating pad was activated manually if the temperature fell below 36.5°C.

<u>Cannulation</u>:

In the experiments upon pups a Polyethylene (Becton and Dickinson) catheter was inserted into a femoral or carotid artery. In some animals the left jugular vein was also cannulated for infusion purposes. The catheter sizes ranged from PE 50 to PE 90, according to the size of the vessel cannulated. Blood samples were drawn via the catheter inserted into the artery (or in two cases, femoral vein). Generally the amount of blood drawn in each sample varied from 0.15 to 0.3 ml, and the volume was replaced by 0.9 percent NaCl. Care was taken to keep the amount of blood withdrawn to a minimum, especially in the younger pups, where 3 ml was the largest total amount taken in any experiment. In younger animals the amount of blood withdrawn in the last sample was 0.7 ml to permit estimation of insulin values. In older pups a similar sample of blood was taken at the end and also once during the earlier part of the experiment. The animals were sacrificed at the end

of the experiment by an overdose of sodium pentobarbital. At the end of each experiment, the liver was carefully and quickly dissected out and weighed.

In adult dogs a cephalic vein was cannulated by a polyethylene catheter for giving the infusions. Blood was withdrawn by a catheter inserted into the inferior vena cava via the saphenous vein.

Treatment of blood samples:

Blood samples were withdrawn into dry sterile disposable plastic 1 ml syringes and transferred to heparinized small glass tubes. The tubes were immediately placed in the refrigerator (4C$^{o}$). Within one hour the tubes were centrifuged for 20 minutes at 2000 r.p.m. (450 g). Plasma was aspirated with a Pasteur pipette into another set of tubes.

Plasma glucose determinations:

In earlier experiments glucose was determined enzymatically from deproteinized (Somogyi, 1945) plasma by the method described by Huggett and Nixon (1957). This method is based on the specific oxidation of glucose by glucose oxidase. The resulting peroxide is determined by a peroxidase reaction in the presence of chromogenic oxygen acceptor. Usually 50 µl of plasma were deproteinized resulting in a dilution of 1:61. In younger animals 20 µl of plasma was diluted 1:121. In adult dogs the dilution was 1:10 and 0.5 ml of plasma was used. Both radioactive and cold glucose were determined from the same filtrate. All determinations of unlabelled glucose were done in duplicate.

0.5 ml of the filtrate was mixed with 3 ml of mixed enzyme reagent. The tubes were placed in the water bath at 37 $c^{o}$ for one hour. Maximum chromogenesis takes place within 60 minutes and the colour is stable for several hours. The colour developed was read in a Hitachi - 111 Spectrophotometer at wavelength of 420 m $\mu$, together with standard glucose solutions and blanks subjected to a similar procedure.

Glucose analyzer:

In the later experiments the plasma glucose was determined by using a Beckman Glucose Analyzer. 10 - 20 $\mu$l of plasma was employed for each determination. When glucose reacts under the influence of glucose oxidase, gluconic acid and hydrogen peroxide are formed. In this process oxygen is consumed and its decreasing concentration is detected by an oxygen electrode. The glucose analyzer measures the maximum rate of this oxygen consumption, which is directly proportional to the glucose concentration. The results are displayed directly and digitally in milligrams per 100 ml.

Determination of labelled glucose:

Labelled glucose was determined from deproteinized plasma, as described by Hetenyi and Mak (1970). 1 ml of deproteinized filtrate of each plasma sample was evaporated in glass counting vials in a vacuum oven at a temperature of about 80$c^{o}$. The volume was reconstituted with 1 ml of water. 10 ml of Bray's solution (Bray, 1960) was added to each vial. The vials were cooled and counted in a liquid scintillation

spectrometer (Nuclear Chicago, Mark II) for 20 minutes each. A computer programme was used to calculate the number of disintegrations per minute (d.p.m.) from the raw data for each sample.

Determination of plasma FFA:

Free fatty acids in the plasma were estimated by a colorimetric micromethod described by Laurell and Tibbling (1967). This method is based on the formation of FFA - Cu soaps. The extraction of FFA from plasma was done by a mixture of chloroform, heptane and methanol. Activated silicic acid was used to remove the phospholipids from plasma. Copper was determined colorimetrically by the use of Diphenylcarbazide. This method requires only 50 µl of plasma for determination of FFA and it was therefore found to be suitable to be used for the pups. Further, Laurell and Tibbling (1967) determined the precision of this method from 53 double determinations of plasma. They reported a standard deviation of ± 0.017 in the range of 0.04 - 0.85 mEq/L and a S.D. of ± 0.027 in the range of 0.86 - 1.55 mEq/L. It was also found that when using 50 µl of plasma, FFA concentrations below 0.034 mEq/L were indistinguishable from zero.

Determination of Serum IRI:

Insulin was determined from 0.2 ml serum by a radio-immunological method of assay described by Hales and Randle (1963). In this method an antibody specific to insulin is employed. A known amount of radioactive insulin (insulin iodinated with iodine - 125 at a minimum specific activity of 50 µCi/ng) was added to each sample. Both labelled

and unlabelled insulin in the sample compete for binding sites on this antibody resulting in the formation of insoluble insulin - antibody complex. The insulin - antibody complex was filtered out and measured for radioactivity in the scintillation counter. The amount of radioactivity present is inversely related to the amount of insulin present in each sample. This method has the advantage of accurately measuring very low concentrations of immuno-reactive insulin in small volumes of serum, blood plasma and other fluids. The Insulin Immunoassay Kit supplied by Amersham/Searle was employed in the assays.

<u>Tracer methods</u>:

Glucose labelled with tritium on the second carbon position (Glucose-2-$H^3$) was used in isotopic tracer determinations of the rates of glucose production ($R_a$) and glucose disappearance ($R_d$). These determinations involved two techniques, whose choice was dictated by the closeness to conditions of dynamic steady-state ($R_a$=$R_d$=Constant). Both techniques are based upon the Stewart-Hamilton dilution principle.

In some experiments where conditions closely approximated dynamic steady state the turnover rates for glucose in blood were determined using a tracer injection method (Wrenshall and Hetenyi, 1959), with integration of the area under a double exponential function (Shipley et al., 1967). The injection method, as described by Wrenshall and Hetenyi, has been shown to be valid within 7% in normal dogs near dynamic steady states for glucose (Cowan et al., 1969) and yields results similar to those obtained by the primed tracer infusion method under these conditions (Hetenyi et al., 1966). The double exponential functions were

fitted to data pairs of isotopic (tracer) glucose concentration in plasma and time by an iterative Fortran programme on an IBM 360-65, which also performed the integration. In this formulation

$$R_a = R_d = \frac{\bar{C} \quad M^*}{\int_0^{\infty} C^* dt}$$

where:

$\bar{C}$ is mean plasma glucose concentration during the experiment

M* is injected amount of labelled glucose (isotope)

C* is plasma concentration of isotope at anytime t and t is time.

In those experiments where the constraints of dynamic steady state were not well fulfilled, as well as in a number where they were met, the primed infusion of tracer method was employed (Steele et al., 1956; Steele 1964). The ratio of priming dose to tracer infusion was selected in such a way that tracer equilibrium was reached within 40 min. of the start of the experiment. In a number of experiments where exogenous glucose administration occurred, sufficient tracer was added to the cold glucose to prevent extensive perturbation of the tracer equilibrium (Cowan and Hetenyi, 1971).

In this formulation

$$R_a = (F - G \cdot \frac{dSA}{dt}) \cdot \frac{1}{SA}$$

or more practically

$$R_a = (F - p \cdot G. \frac{SA_2 - SA_1}{t_2 - t_1}) \cdot \frac{2}{SA_1 + SA_2}$$

where:

F is tracer infusion rate

G is glucose mass in system calculated as a product of plasma glucose concentration and the size of the apparent distribution space of glucose.

p is pool fraction of central compartment

SA is specific activity of glucose at time t

t is time

In order to avoid the taking of large amounts of blood from the pups, the apparent distribution space of glucose was estimated at 25% of body weight rather than determined by extrapolation of multiple early samples.

# MATERIAL AND METHODS II

## PART 1

In this part of the study the turnover rates of glucose in newborn dogs were determined in the absence of any externally applied perturbation.

### Animals:

30 mongrel pups from 6 litters (Table 2) and 13 inbred beagle pups from 4 litters (Table 1) were used in this series. The age of the mongrels and beagles ranged from 3 to 112 hours and 2½ to 32 hours respectively. Their weights ranged between 192 to 670 g and 186 to 345 g respectively. The details of the general animal care taken during the experiments has been described above.

### Experimental design:

Glucose - 2 - $^{3}H$ (50 µCi in 0.5 ml) was injected via the catheter and 6-9 blood samples were withdrawn between 5 and 120 minutes after tracer injection.

### Analytical methods:

The blood samples were treated as described above. The concentrations of labelled and unlabelled glucose in the plasma were determined from all samples taken. Selected samples of blood were also analyzed for Immunoreactive insulin, and free fatty acid (FFA) concentrations.

Calculations:

The rate of appearance of glucose (the rate of hepatic glucose production) and that of overall utilization in a steady state was calculated from the specific activity and/or tracer concentration curve versus time, based on the method described by Shipley et al., (1967).

## PART 2

## Responses to injected and released insulin

In this part of the study the sensitivity to exogenously administered and endogenously released insulin in the neonatal pups was examined. In one group of animals the hypoglycaemic response to injected or infused insulin was observed. In the second group the hypoglycaemic response to insulin released by an infusion of D-ribose was observed.

Group I:

Insulin was administered to 27 pups (15 beagles, 12 mongrels) between the ages of $2\frac{1}{2}$ to 112 hours. Their weights ranged from 210 to 670 g. (Table 5 and 6). Six mothers (5 beagle, 1 mongrel) of the litters were also injected with insulin.

Dose of insulin:

Bovine insulin (Toronto) 0.12U/Kg. was injected in all beagle pups. Out of 12 mongrel pups, 5 received insulin injections of 0.06U/Kg., 2 received 0.12U/Kg of insulin injection. In the remaining 5 insulin

was infused; in 2, the amount infused was 1 mU/Kg-min; in one, 2 mU/Kg-min and in the fourth and fifth pup a priming dose of 0.15U/Kg was followed by infusion of 241 mU/Kg-min and 194.8 mU/Kg-min of insulin, respectively (Table 6).

The amount of insulin injected to the mothers was either 0.12U/Kg (beagles) or 0.06U/Kg (mongrel).

Experimental design:

(a) Animals injected with insulin:
One blood sample was withdrawn during the control pre-injection period for the determination of initial plasma glucose and FFA concentrations. The tracer and insulin were injected rapidly and serial blood samples were withdrawn 5, 10, 20, 30, 45, 60, 90 and 120 minutes after the injection. Littermates of the same age and approximately similar weight acted as the controls. They were treated similarly to the test animals except that tracer alone was injected.

(b) Animals receiving a continuous infusion of insulin:
One blood sample was withdrawn in the pre-injection period and infusion of insulin was started. Tracer was injected 20 minutes after the start of infusion and 4 or more blood samples were withdrawn. The time scale of the samples was based upon a prediction of the approximate glucose turnover time, which was strongly correlated to initial plasma glucose concentration, determined during the pre-injection control period(Appendix). An error analysis of this approach using only four samples shows performance is as good as conventional approaches with many more samples (Corney and Heath, 1970).

In the last two pups a single blood sample was withdrawn during the control period and the primed infusion of insulin was started. After 40 minutes of the start of infusion, another blood sample was withdrawn and tracer was injected. Six more serial blood samples were withdrawn 10, 20, 45, 60, 75 and 90 minutes after the tracer injection. The control animals were infused with 0.9 percent NaCl. The rest of the procedures were similar to those in the test animals. The blood samples taken during the control period were analyzed for glucose and FFA concentrations. The rest of the samples were analyzed for both labelled and unlabelled glucose concentrations. Plasma FFA and serum Immunoreactive insulin concentrations were determined from selected samples.

The experimental design in the adult dogs (mothers):

Tracer was injected at the start of the experiment and serial blood samples were withdrawn 1, 2, 5, 10, 15, 30, 60 and 90 minutes after the injection. This was followed by a rapid injection of insulin and blood samples were withdrawn 10, 25, 40, 55, 70 and 100 minutes after the insulin injection. The plasma from samples withdrawn before insulin administration were analyzed for glucose and labelled glucose concentrations. The rest of the samples were analyzed for glucose concentration alone. FFA and IRI determinations were made from selected samples. Turnover rates of glucose were calculated as described above.

Group II:

D-ribose was infused to a group of 9 pups (4 beagles and 5 mongrels). The age and the weights of the mongrels ranged between 6 - 108 hours and 236 - 585 g respectively. The beagle pups used were

between the ages of 4 - 12 hours and their weights were between 186 - 303 g. The mongrel pups were from 3 litters and each beagle pup was from a different litter. The amount of D-ribose infused ranged from 3.7 to 17 mg/min-Kg. (Table 9).

Experimental design:

A blood sample was withdrawn 2 minutes prior to start of D-ribose infusion. Six more samples of blood were withdrawn after 10, 20, 40, 60, 80 and 100 minutes from the start of the infusion. Tracer was not injected. The blood sample drawn during the control period was analyzed for plasma glucose, FFA and immunoreactive insulin concentrations. The rest of the samples were analyzed for plasma glucose and D-ribose concentrations (Roe and Rice, 1948). This method is based upon the formation of furfural from pentose in 83 percent acetic acid containing thiourea at 70$^{o}$C. The furfural is made to react with p-bromoaniline acetate to form a pink coloured product which is read in a photoelectric colorimeter, using a 520 mu filter. The pentose concentration is calculated from the readings of the pentose standards used.

## PART 3

### Responses to induced hyperglycaemia

In these experiments the alterations produced in the rates of hepatic production and utilization of glucose in response to induced hyperglycaemia in newborn and young dogs were studied. Hyperglycaemia was produced by infusion of glucose. In a group of 7 animals, insulin

was infused together with glucose with a view to modifying the responses seen with infusion of glucose alone.

Animals:

Experiments were carried out upon 34 pups; 27 inbred beagles from 6 litters and 7 mongrel pups from one litter. The age of the beagles ranged from 4½ hours to 55 days and that of the mongrels from 12 hours to 16 days. The weights of the beagle and mongrel pups were between 156 g to 2.387 Kg and 372 g to 1.64 Kg respectively (Table 10).

Experiments were also carried out upon the mothers of 5 of the beagle litters.

Experimental procedures:

A pair of similar polyethylene catheters were inserted together into the left jugular vein through which "load" glucose and tracer solutions were infused. The blood samples were withdrawn through the catheterized left carotid artery.

Experimental design:

In the 27 beagle pups a primed tracer infusion was started at the beginning of the experiment. A priming dose of 5 to 10 µCi was followed by the infusion of 0.1 - 0.25 µCi/min. This infusion lasted throughout the experimental period. The first 50 minutes were treated as the control period during which tracer alone was infused. This was followed by an infusion of unlabelled "load" glucose at rates ranging between 8 - 21 mg/Kg-min, lasting for 130 - 160 minutes. Three blood samples were withdrawn during the control period, 30, 40 and 50 minutes

after the start of the tracer infusion. The glucose infusion was begun within 2 minutes after the withdrawal of the third sample. The infused load of cold glucose also contained sufficient additional tracer glucose to bring its specific activity close to that of the circulating plasma glucose at the beginning of its administration. The amount of tracer added to the glucose load infusion was based upon a rapid determination of the specific activity of plasma glucose in sample taken 20 minutes before starting that infusion. The steady state in the initial control period was sufficiently reliable that the fractional error in matching of the specific activities of infused glucose load and circulating plasma glucose at 50 minutes was 0.14 $\pm$ 0.02. As a result the tracer equilibrium was not greatly distrubed, making it possible to calculate valid $R_a$ and $R_d$ values beginning very shortly after the start of the glucose infusion. The tracer infusion permitted the calculations of $R_a$ and $R_d$ during the initial steady state control period and the infusion of "load" glucose. The metabolic clearance rates were calculated during these periods as the ratio of $R_d/c$, where c is the plasma glucose concentration (mg/ml) and these clearance rates were normalized by body weight (ml/Kg-min). The details of the tracer method and calculations have been described previously. During the glucose infusion, up to 9 blood samples at 20 minute intervals were withdrawn. In 7 of the 27 beagle pups between the ages of 38 hours and 40 days insulin was added to the glucose infused. In 5 the rate of insulin infused ranged between 550 - 710 µU/Kg-min. In the other 2 it was 2400 and 5880 µU/Kg-min respectively.

The experiments on the 7 mongrel pups were of similar design except for a very short control period preceding the infusion of glucose. Only in the experiment on the oldest (16 days) mongrel pup was this of 50 minutes duration.

All plasma samples were analyzed for glucose and labelled glucose. Selected samples were analyzed for FFA and for IRI concentrations.

Adults:

In 4 of the 5 beagle mothers the glucose infusion was started after a short control period of 5 minutes during which 2 blood samples were withdrawn. After the start of glucose infusion 8 - 10 samples were withdrawn at intervals of 10 minutes. This was followed by an injection of 60 uCi of tracer and serial blood samples were withdrawn after 1, 2, 3, 5, 8, 12, 20, 30, 45, 60, 75 and 90 minutes from the time of tracer injection.

All blood samples withdrawn were analyzed for plasma glucose. FFA and IRI concentrations were determined in selected samples. The samples withdrawn after the tracer injection were analyzed in addition for labelled glucose to enable calculation of the turnover rate of glucose. In the fifth adult dog the design of the experiment was similar to that used in the beagle pups.

## PART 4

### Glucose production and utilization during hypoxia

The objective of the experiments in this section was to examine the role of hypoxia as a possible regulator of hepatic glucose production in the newborn dogs.

Animals:

The experiments were carried out on 11 inbred beagle pups from 2 litters and 2 mongrel pups from one litter. Their ages ranged between 22 hours to 30 days and they weighed between 307 g and 1.628 kg (Table 15).

Experimental procedures:

The left jugular vein and left carotid artery were cannulated. The trachea was intubated and the animal was ventilated with a Palmer small-animal respirator. The gas mixture to be inhaled was mixed, warmed and saturated with water vapour in a plexiglass mixing chamber. Both $O_2$ and $N_2$ were directly led from their respective tanks into this chamber through calibrated flow meters.

Experimental design:

Most experiments consisted of 4 periods. During the first period, which lasted for 50 minutes, the pups were ventilated with room air. In the second period of 45 minutes duration they were ventilated with a mixture of 10% $O_2$ and 90% $N_2$. This was followed by the third 60 minute recovery period during which the pups breathed

room air again. During the fourth period, lasting for 45 minutes, they were ventilated with a mixture of 5% $O_2$ and 95% $N_2$. In three pups the order of the two hypoxic periods was reversed. In 2 beagle pups the experiment was terminated after the second period. In 2 beagle pups propranolol (Inderal, Ayerst Laboratories, Montreal) was infused at constant rates of 62 and 84 µgm/min in order to produce an adrenergic B-receptor blockade.

The rate of ventilation was kept constant at 30/min. The appropriate tidal volume was calculated based on the body surface area, and the respiration pump was correspondingly adjusted, keeping the pH of the blood between 7.35 and 7.42 during the first period.

Three arterial blood samples of approximately 0.25 ml each were withdrawn during each of the 4 periods. In the middle of each period an additional blood sample of about 100 ul was taken for the determination of the arterial $O_2$ content, pH and haemoglobin concentration. Plasma FFA concentrations were determined from the third samples taken during the 1st and 2nd periods.

Analytical methods:

Haemoglobin concentration of the blood was measured by the cyanmethaemoglobin method from a 20 ul blood sample. A spectrometer (Bausch and Lomb, Spectronic 20) was used for reading the colour intensity.

The pH of blood was determined from a 50-75 µl sample withdrawn into a heparinized capillary tube, with Acid-Base Analyzer (Radiometer microelectrode Unit, Type PHM 7 1 b). The $O_2$ content of blood was measured by Galvanic coulometric technique from a 20 µl sample using

the Lex $O_2$ CoN (Lexington Instrument Corporation, Waltham, Mass.). This method has been described recently by Valeri and coworkers, (1972). The sample of blood was withdrawn anerobically into a Hamilton syringe.

The oxygen saturation of haemeglobin was calculated from the oxygen content of the blood and haemoglobin concentration using a factor of 1.36 for the solubility of oxygen in blood at 37°C.

Tracer methods:

The rates of appearance ($R_a$) and disappearance ($R_d$) of glucose, were calculated by the method of the primed infusion of tracer (Steele et al., 1956) and modified as described earlier. At the start of the experiment a priming dose of 2.5 μCi of glucose-2-$^3$H was injected and was followed by infusion of 0.11 μCi/min. The collection of samples during the early decay of the specific activity (SA) versus time curve was omitted. The selected ratio of the priming dose/infusion rate of the tracer led to quick equilibration of tracer and tracee and the calculation of the rates were begun during an initial period in which steady state and tracer equilibrium prevailed. Later periods involved departures from this steady state. The apparent distribution space of glucose, a factor which influences the calculation of $R_a$ in proportion to the degree of departure from a steady state was assumed to be 25% of the body weight.

The rates of appearance and disappearance of glucose were expressed as mg glucose/min per kg body weight. The metabolic clearance rate of glucose was calculated as in part 3. No attempt was made to account for glycosuria, although in some experiments during the latter part of the 2nd and 4th periods, glycosuria might have contributed to the calculated value of the rate of disappearance of glucose.

## PART 5

### Effect of methylprednisolone on turnover rates of glucose

In the experiments to be reported in this section the effect of methylprednisolone (MP) on glucose homeostasis was examined in newborn pups.

Animals:

The experiments were carried out on 20 mongrel pups from 3 litters and 2 beagle pups from 1 litter. The age of the pups ranged from 4 to 47 days and they weighed between 370 g and 4524 g.

Two mongrel mothers of the litters were also experimented upon 2 and 4 weeks postpartum. The general care of the animals taken during the experiment has been described earlier. The pups were fasted for 4 - 6 hours prior to the experiments.

Experimental design:

The left carotid artery was cannulated and glucose-2-$^3$H (20-30µCi) was injected as a single injection to the pups. The dose of the tracer injected to the mothers was 60 uCi, and it was injected through a cannula inserted into the inferior vena cava via the saphenous vein.

Pups of the same age and litter were paired. One pup in each pair received a daily injection of 4 mg/kg DepoMedrol (methylprednisolone, MP) intramuscularly for three consecutive days. The other pup served as the control.

Blood samples were withdrawn at 2, 5, 10, 20, 30, 45, 60, 75, 90 and 120 minutes after the injection of the tracer.

Analytical methods:

The treatment of the blood samples has been described earlier. The concentrations of labelled and unlabelled glucose in the plasma were determined from all blood samples withdrawn. Free fatty acid concentration in the plasma was determined from the samples taken at 2, 60 and 120 minutes. Serum IRI concentration was determined from the samples taken at 2 and 120 minutes. In larger (older) pups a third sample for IRI determination was withdrawn at 60 minutes also. The specific methods for the above mentioned estimations have been previously described.

At the end of the experiment a part of the liver was clamped with Wollenberger clamps, pre-cooled in a mixture of acetone and dry ice, and stored in a deep freeze for the estimations of total proteins, Ribonucleic acid (RNA) and Desoxyribonucleic acid (DNA). The rest of the liver was removed and weighed. A small piece of liver was dried in an oven at 120°C for 24 - 48 hours to determine the water content of hepatic tissue.

Estimation of RNA and DNA:

The RNA, DNA determinations were made according to a modified method of Schmidt and Thannhauser (1945) as described by Korecky and French (1967). The RNA was assayed in the hydrolysate after precipitation of proteins and DNA by 1.4 N perchloric acid. The orcinol reaction (Dische, Z. 1955) and purified RNA as standard was used. The DNA protein pellet was hydrolyzed at 90°C with 5% TCA added. The supernatant was used for DNA determination, using the diphenylamine method (Burton, 1956) and purified DNA as standard.

Estimation of total proteins:

The total protein in the liver were estimated by using the method described by Lowry et al., (1951). In this method there are two distinct steps which lead to the development of the final colour with proteins: (a) reaction with copper in alkali and (b) reduction of the Folin Phenol-reagent by the copper treated proteins. The developed colour is read after 30 minutes at 750 mμ in a spectrometer.

Tracer method and calculations:

The turnover rate of glucose was determined by the single tracer injection method described by Shipley et al., (1967). The details of this method have been described previously.

Two adult animals (mothers) were subjected to two such experiments each, one before and one after a 3-day treatment with methylprednisolone. The design of the experiments was similar to those in the pups except that the liver was not removed in the adult animals.

# RESULTS

## PART 1

### The rates of glucose production and utilization in the newborn dog:

Tables 1 and 2 show the ages and the respective mean plasma glucose concentrations in the beagle and mongrel pups. Their plasma glucose concentrations varied widely during the first 12 hours after birth. These values ranged between 34 - 187 mg/100 ml and between 29 - 300 mg/100 ml in the beagle and mongrel pups respectively. The range of plasma glucose values narrowed as the age of the pups increased, being 51 - 128 mg/100 ml and 52 - 87 mg/100 ml in beagle pups between the ages of 13 - 24 hours and 25 to 32 hours respectively. In 13 - 24 hours old mongrels the range of plasma glucose values was from 37 to 190 mg/100 ml; in 25 - 36 hours old pups it was from 32 to 119 mg/100 ml and in pups older than 36 hours the range of these values was from 56 to 185 mg/100 ml. The mean plasma glucose concentrations in the three age groups in beagle and mongrel pups are shown in Tables 3 and 4 respectively.

The calculated rates of glucose production ($R_a$) normalized per gram of liver weight are shown in Table 1, for the beagle pups and for the mongrels in Table 2. The mean $R_a$ values were higher in the mongrels than those in the beagles. There was no significant correlation between age and $R_a$. In beagles, the correlation coefficient was 0.35 for n = 13, and in mongrels the correlation coefficient was 0.10 for n = 30.

Fig. 1 shows the calculated rates of glucose production plotted against the concentration of glucose in the plasma in 43 pups (mongrels and beagles).

The constancy of the rates of glucose production by the liver was striking. No significant correlation was observed between the glucose concentrations in the plasma and the rates of hepatic glucose production in these pups: the correlation coefficient was calculated and found to be 0.15 for n = 43.

In 4 mongrel pups from one litter (litter number 6) the rates of hepatic glucose production were exceptionally high, ranging between 0.30 to 0.34 mg/g liver per min. It is striking to note that the variations in $R_a$ in this litter were virtually absent although the plasma glucose concentrations varied from 16 to 145 mg/100 ml in the four pups.

The basal concentrations of immunoreactive insulin in the serum, and plasma free fatty acid in beagle and mongrel pups at different ages are given in Tables 3 and 4. There was no significant difference between beagles and mongrels.

## PART 2

**The sensitivity to injected and endogenously released insulin:**

The number and age of beagle and mongrel pups receiving insulin are shown in Tables 5 and 6 respectively. The changes in plasma glucose concentration after an intravenous injection of 0.12U/kg of insulin at sequential time intervals in beagle pups are shown in Table 7. Those observed in mongrels following either an intravenous injection or during an infusion of insulin are shown in Table 8. No clearcut hypoglycaemic response to insulin injection was observed in beagle pups aged less than 4 hours and in mongrel pups aged less than 8 hours. In beagle pups between the ages of 4 to 16 hours and in a 9 hour old mongrel pup the hypoglycaemic response to injected insulin was observed but the response was sluggish in onset and the recovery to normoglycaemia was either absent or incomplete. In both beagle and mongrel pups older than 16 hours the well known hypoglycaemic effect of insulin was observed though the recovery to preinjection plasma glucose concentration was still delayed. Figure 2 shows the changes in plasma glucose concentrations following an intravenous injection of 0.12U/kg of insulin in three beagle pups aged 2½, 8 and 30 hours, and also in their mother. A typical hypoglycaemic response to injected insulin is seen in the mother, in which the plasma glucose concentration falls to its minimum within 15 - 20 minutes, and begins rising soon thereafter, reaching the preinjection level at about 70 minutes after the injection of insulin.

An analysis of variance was performed on the plasma glucose concentrations at sequential time intervals following insulin injection for all pups so treated. For the 2 - 5 hour old, the probability of the plasma glucose concentration changes with time occuring by chance alone was 0.999. This would indicate that insulin had no demonstrable effect in pups at that age. In the age group between 5 - 15 hours the probability of an effect occuring by chance alone was still 0.768, still not indicating a significant effect. In the age group between 16 to 32 hours old pups the probability of an effect occuring by chance alone was less than 0.001, indicating a significant effect of insulin on the glucose concentration in the plasma in the high doses applied.

The number, age, sex and weights of mongrel and beagle pups of different ages infused with D-ribose are shown in Table 9. Infusion of D-ribose (1.9-3.0mg/kg-min) in adult dogs is known to produce hypoglycaemia by the release of endogenous insulin (Hetenyi and Ishiwata, 1968; Ishiwata et al., 1969). Ribose infused at higher rates (3.7-17 mg/kg-min) to newborn pups did not produce hypoglycaemia, although the concentration of ribose in their plasma rose to 26 - 183 mg/100 ml and the concentration of immunoreactive insulin in the serum increased in all but one pup. The peak concentrations of serum IRI ranged between 36 - 90 micro U/ml.

A typical course of plasma glucose concentration during infusion of 16 mg/kg-min of ribose in a 12 hour old beagle pup is shown in Figure 3. Concentration of ribose in the plasma increased from a pre-infusion value of near zero to 176 mg/100ml and the serum IRI from

7uU/ml to 37uU/ml during the infusion of ribose, but the plasma glucose concentrations was not altered significantly.

## PART 3

### Responses to induced hyperglycaemia:

Table 10 gives detailed information about the age, sex, litter and weights of 27 beagle pups (#1 - #27) together with the infusion rates of glucose and insulin administered, 7 mongrel pups (#28 - #34) and 5 beagle mothers (#35 - #39) infused with glucose.

The upper panel of Figures 4, 5 and 6 show typical courses of plasma glucose concentration during glucose infusion in beagles of different ages, while on Figure 7 the response obtained in the experiment on an adult beagle, mother of one of the litters is shown. In the pups the plasma glucose concentration rose almost linearly at first, with a tendency to moderation of the slope at higher concentrations. The shapes of the plasma glucose concentration curves obtained in 36 hour, 60 hour and 8 day old pups following glucose infusion did not differ significantly. The mother, on the other hand, exhibited the typical adult response (Hetenyi et al., 1973), in which the initial rise in glucose concentration is checked and reversed by regulatory mechanisms which permit a new, somewhat elevated equilibrium to be reached within 75 to 90 minutes after the start of the glucose infusion. On the average, in the 5 adult beagles, the basal plasma glucose concentrations were between 89 - 109 mg/100 ml. Glucose infusions of 8 - 21 mg/kg-min caused transient rises of 82 - 115 mg/100 ml in the plasma glucose values, after which the plasma glucose level settled between 106 - 132 mg/100 ml after 90 minutes from the start of the infusion.

In all the pups receiving infusions of glucose, the curves of plasma glucose concentrations could be viewed as being of three types: (1) increasing linearly; (2) initially increasing linearly, with some attenuation of the slope at higher concentrations (modified linear); (3) similar to modified linear, but reaching an eventual plateau (rise to plateau). The distribution of these curves with age among the 20 beagle and 7 mongrel pups infused with glucose alone is shown in Table 11. It can be seen from the table that the older pups were much more capable of moderating or halting the rise in plasma glucose than were their younger littermates.

Seven beagle pups received glucose infusions together with insulin. Of the 5 pups that received insulin at a relatively lower rate (559.7 to 711.5 uU/kg-min), 3 showed nearly adult pattern of glucose response, similar to that shown in Figure 7. The ages of these three pups were 28, 35 and 40 days respectively. The other two pups were younger ($18\frac{1}{2}$ and 21 days); one showed a modified linear curve, while the other showed a rise to a plateau.

The two pups receiving insulin at the rates of 2400 μU/kg-min and 5400 μU/kg-min were 42 days and $1\frac{1}{2}$ days old respectively. The 42 day old pup showed a completely adult response in plasma glucose, while the younger pup also showed a near adult pattern of response. The plasma glucose curve of the younger pup is shown in Figure 8, where it is compared to the response of $2\frac{1}{2}$ day old pup which received glucose infusion without insulin.

Rates of production and disappearance of glucose during an i.v. glucose infusion:

In adult dogs, an infusion of glucose which raises plasma glucose concentration by 50 mg/100 ml or more is known to cause an almost total cessation of hepatic glucose production. (Steele, 1959 ; (Steele et al., 1965; Hetenyi et al., 1973). The results observed in the five beagle mothers confirm this. In four dogs (mothers), the hepatic glucose production ($R_a$) was indistinguishable from zero (Figure 7), while in the fifth, in which the smallest transient rise in plasma glucose concentration had occured, the hepatic production of glucose was 0.82 mg/kg-min, about 20 - 25% of the average normal value (Hetenyi et al., 1973; Issekutz Jr., 1972).

In the pups, the compensatory fall in endogenous glucose production in response to hyperglycaemia was much slower and smaller (Figure 9), although the rise in plasma glucose levels was far greater than 50 mg/100 ml, ranging between 61 - 295 mg/100 ml. In the 20 beagle pups who received infusions of glucose without insulin, the rates of glucose production ($R_a$) fell slowly, finally reaching steady state values with a mean of 50.8 $\pm$ 4.2% of pre-infusion control period rates.

Figure 10 shows the courses of plasma glucose concentration curves during glucose infusion in 4 mongrel pups aged 14 hours, 36 hours, 8 days and 16 days respectively. Rates of glucose production normalized per gm of liver tissue and serum IRI values (μU/ml) at different times during the glucose infusion are also shown. The single 16 day old mongrel pup in which there was an initial control period showed a rise in $R_a$ of

only 3% (Figure 10), while his six littermates showed $R_a$ values during glucose infusion equal to or higher than beagles of the same age or weight. In fact, the $R_a$ values during glucose infusion in the six mongrels (9.9±1.1mg/kg-min) were not significantly different from those in the control periods of the 27 beagle pups (8.2±0.3mg/kg-min). In the 7 beagle pups which received infusions of glucose plus insulin the decrease in $R_a$ was of about the same degree as in the 20 pups which were infused with glucose only (Figure 9).

The rate of disappearance ($R_d$), increased during the infusion of glucose (Figure 9). However, the metabolic clearance rate of glucose remained constant, except for a small but consistent rise during the initial 30 minutes of infusion (Table 12). The addition of insulin (550-710 µU/kg-min) to the glucose infusion did not increase the metabolic clearance rate above the level observed in pups infused with glucose only. Significantly higher rates of metabolic clearance were found during the entire duration of the infusion in the two pups to which glucose was infused together with 2400 or 5880 µU/kg-min insulin (Table 12).

<u>Serum IRI and plasma FFA concentrations:</u>

Insulin concentrations in the plasma increased in all but one of the twenty beagle pups during infusion of glucose. The average basal preinfusion concentration was 11.3±1.3µU/ml and the peak concentration, reached usually about 130 minutes after the start of glucose infusion, was 47.7±6.7µU/ml. These values are quite comparable to the serum IRI values observed in the adult dogs during similar infusions. The seven pups receiving supplementary insulin had comparable basal

values (9.9$\pm$0.9μU/ml) and almost identical peak values of serum IRI (47.7$\pm$8.2μU/ml). The seven mongrel pups showed higher basal (pre-infusion) and peak insulin concentrations with much greater variations than those of the beagles during the infusion of glucose. Their basal and the peak values were 19.2$\pm$4.5μU/ml and 62$\pm$15μU/ml respectively. The results are summarized in Table 13.

Concomitant with the rise in plasma insulin concentrations, the plasma free fatty acid levels tend to fall. In the beagles, the plasma FFA concentrations before the infusion of glucose or glucose and insulin were 737$\pm$163μEq/litre, with one animal below 200μEq/litre, 20 between 200 and 800μEq/litre, and 6 above 800μEq/litre. In the 7 mongrel pups the preinfusion values were 979$\pm$434μEq/litre with 6 of the 7 values between 350 - 1100 μEq/litre, and one value of 3491 μEq/litre. The relative changes in plasma FFA levels during infusion of glucose alone or glucose plus insulin in the pups and the beagle mothers are shown in Table 14.

## PART 4

### Glucose production in hypoxia:

The number of, and basic information about the pups employed in part of the study are given on Table 15.

### Total $O_2$ content, $O_2$ saturation, pH and hemoglobin concentration of blood:

Table 16 shows the changes in total $O_2$ content, $O_2$ saturation, pH and hemoglobin concentration in the arterial blood of beagle and mongrel pups, during ventilation with room air and during the two hypoxic periods produced by ventilation with a mixture of 10% $O_2$ and 90% $N_2$ or a mixture of 5% $O_2$ and 95% $N_2$ respectively. A drift toward acidosis was observed during hypoxic periods, which was more marked in the second hypoxic period when the $O_2$ content and saturation were lowest. There was no appreciable change in the concentration of hemoglobin during the experiment. In the 60 minute recovery period between the two exposures to hypoxia, the values of total $O_2$ content, $O_2$ saturation and pH of blood did not recover completely to basal resting levels.

### Plasma glucose concentration, rates of appearance and disappearance of glucose:

Figures 11 and 12 show two individual experiments on beagle pups 22 hours and 16 days of age respectively. During the first three periods the changes in plasma glucose concentration and rate of glucose production ($R_a$) in both dogs followed essentially the same pattern, showing an increase during ventilation with 10% $O_2$ and 90% $N_2$. The changes in the rate of disappearance ($R_d$) were smaller and their comparison is made difficult by the fact that in the older pup the plasma

glucose concentration reached high levels at which glycosuria was expected to occur. The responses to the inhalation of 5% $O_2$ and 95% $N_2$ were different in the two pups. In the younger pup plasma glucose concentration and the rate of glucose production increased only transiently and soon dropped to a lower level. In the older pup the increase in both glucose concentration and production were maintained, resulting in a similar, although more marked response than the one observed in the first hypoxic period (10% $O_2$ + 90% $N_2$).

The data obtained from all the experiments of this design are summarized in Figure 13 and Table 17. The plasma glucose concentration increased from an initial average control value of 94.7±8.6 mg/100ml to 204.9±35.1mg/100ml by the end of the first hypoxic period. The mean rate of glucose production in the initial control period was 10.3±1.2 mg/kg-min and it increased to a mean peak value of 18.5±3.4mg/kg-min during the last 15 minutes of the first hypoxic period. The mean initial rate of glucose utilization was 10.5±1.67mg/kg-min, and it did not change significantly during the first 15 minutes of hypoxia. During the last 15 minutes of this period it increased to an average of 18.9±3.0mg/kg-min, showing a rise of 8.4mg/kg-min. During the recovery phase the mean plasma glucose concentration diminished somewhat, stabilizing at a mean value of 122.7±16.4mg/100ml which is higher than the initial basal concentration by 28mg/100ml. During this phase the rates of glucose production and utilization returned to 8.1±0.7mg/kg-min and 8.3±0.6mg/kg-min respectively, lower than the initial rates. In the 2 pups older than 10 days, the second

exposure to hypoxia (5% $O_2$ and 95% $N_2$) caused the plasma glucose concentrations to increase to 401mg/100ml and 431mg/100ml respectively. Concomitantly, the rates of glucose production and disappearance also increased ($R_a$ = 19.7mg/kg-min and 26.5mg/kg-min; $R_d$ = 14.8mg/kg-min and 21.5mg/kg-min respectively). In younger pups of less than 10 days of age these parameters either declined or stayed unchanged after an initial transient increase. At the end of this period the plasma glucose concentration was 103.3$\pm$18.06mg/100ml; the $R_a$ was 10.1mg/kg-min and $R_d$ was 10.3$\pm$0.82mg/kg-min in these younger pups.

Table 18 summarizes the percentage changes in glucose concentration as well as in the rates of appearance and disappearance of glucose in the pups.

The results of the experiments in which the sequence of ventilation with 10% $O_2$ and 5% $O_2$ was reversed are shown in Table 19. Because of the age differences among the three pups no averages were calculated. Apparently exposure to severe hypoxia led to very marked increases in plasma glucose concentration.

<u>The metabolic clearance rate (Table 17):</u>

The mean metabolic clearance rate during the initial control period was 12.02$\pm$0.91ml/kg-min. By the end of the first hypoxic period, it fell to 8.78$\pm$1.1ml/kg-min; decreasing further to 7$\pm$0.9ml/kg-min during the recovery period. During ventilation with 5% $O_2$ and 95% $N_2$ the metabolic clearance rate declined to low values of 3.9ml/kg-min and 5.0ml/kg-min respectively in the 2 older pups ($>$10 days). Although it increased in the younger pups to a value of 9.2$\pm$0.23ml/kg-min, it remained still lower

than the initial metabolic clearance rate of 12.02±0.9ml/kg-min.

The effect of propranolol:

The responses to the exposure to hypoxia was not abolished by propranolol. Neither pup treated with propranolol survived the exposure even to the milder hypoxia. In both pups the premortal concentration of glucose was higher than the basal level by an average of 204%. The plasma FFA level increased by an average of 38%.

## PART 5

### Effect of methylprednisolone on turnover rates of glucose:

Table 21 shows the number of animals, their respective ages, sex and weights employed in this study. Their plasma glucose concentrations, serum IRI, plasma FFA concentrations, the turnover rates of glucose and the metabolic clearance rates are shown on Table 22. Methylprednisolone treatment caused a small increase in the concentration of glucose in all the pups so treated, excluding one pair (#7 and #8) in which the control pup (#8) exhibited a very low basal plasma glucose concentration. Excluding this pair the average differences in plasma glucose concentrations was 12.6 $\pm$ 3.27 percent. The smallest differences were observed between the two oldest pairs of pups. A negative correlation was observed between the increase in plasma glucose concentration and the age of the pups but it was not statistically significant ($r = -0.56$ for $n = 10$, Figure 18). The difference in the plasma glucose concentration of the two adult animals before and after treatment with MP was 5.9 and 0.2 percent (Table 23).

The free fatty acid levels in the plasma also increased after treatment with methylprednisolone in both adult animals and in eight of the eleven matched pairs of pups. In two pairs there was no change in the plasma FFA and in one pair a decrease of 40 percent was observed. The average increase in plasma FFA amounted to 65.6 $\pm$ 34.2 percent (Table 24).

Figure 15 shows the turnover rates of glucose in methylprednisolone treated pups and their littermates of the same age (controls). The effect of MP treatment on the turnover rates of glucose of the two adults (mothers) is also shown. It is evident that in the pups younger than 32 days the effect of MP treatment was absent or very small as compared to that observed in adult animals. This difference is more clearly shown in Figure 16, where the percentage difference between the turnover rates of glucose in treated and their matched control pups is plotted against their respective ages. It is remarkable that in the very young pups of less than 6 days of age MP produced a small but consistent increase in $R_a$. Relatively greater increases were obtained in pups older than 23 days and the effect was largest in the two adult animals.

Figure 17 shows the percentage differences in metabolic clearance rates of glucose between the treated and control pups plotted against a similar difference in plasma IRI levels. The age (days) of the pups are also indicated on this graph. It seems that in the younger pups the treatment with MP does not increase the level of serum IRI as much as it does in older ones (Fig. 17, Table 22). The rank correlation coefficient ($r'$) between the ages of the pups and the increase in the level of serum IRI after methylprednisolone treatment was 0.761 ($p < 0.02$). However, the elevated serum IRI levels failed to increase the metabolic clearance rates to the same extent as in adults. Thus in very young pups the insulinotropic effect of MP is absent or small. At a later age this effect appears but the increase in metabolic clearance rate induced by the extra amount of released insulin in much less than that in adults.

The adult dogs release less insulin to achieve a greater effect on their metabolic clearance rate. In this respect the glucose homeostasis in the adults is more efficient.

Effect of MP on the weight, DNA, RNA and protein content of the liver:

The data are summarized on Table 25. The treatment with MP increased the relative weight of the liver from 3.77% of body weight to 6.43%. The water content of the liver was decreased. A 37.9% decrease in the DNA concentration indicates that the "Cellularity" (Weber, 1964) of the liver decreased too, that is, the treatment decreased the number of cells per gm weight of the organ. These cells however are larger than in control pups and contain more RNA and protein, as indicated by the last two columns of Table 25. The total amount of protein in the whole liver was also considerably increased over that of the matched controls indicating stimulation of net protein synthesis. All the percentage differences shown on Table 25 are statistically significant. Except for the overall hepatic protein content (gm/whole liver), no correlation with age could be detected in any of the values shown on Table 25.

## DISCUSSION

### 1. Glucose production and utilization in the newborn dog.

The basal plasma glucose concentration was found to be very unsteady in newborn pups especially during the first 12 hours of life. Concentrations as low as 29 mg/100ml and as high as 300 mg/100ml were observed in the experiments reported. Our observation is in agreement with that of Allan et al., (1966), who reported that in newborn dogs the blood glucose concentration was generally below that found in normal adult dogs, although occasionally high levels in excess of 200 mg/100 ml were observed. This is in sharp contrast to the almost steady concentration of plasma glucose in adults, and would suggest that the glucoregulatory mechanisms are not as precise in the newborn as they are in the adult dog (Hetenyi, Norwich and Zelin, 1973).

The calculated rates of glucose production plotted against the average plasma glucose concentration (Fig. 1) showed a complete lack of correlation between the plasma glucose concentration and the rates of glucose production in the newborn pups.

This finding is in sharp contrast to the one observed in adult dogs where the existence of a negative correlation between the rates of glucose production ($R_a$) and the plasma glucose concentration has been well established. In the adults effective feedback mechanisms regulate the rates of hepatic production and overall utilization of glucose and thus rapidly restore normoglycaemia when the concentration of glucose is raised or lowered (Hetenyi et al., 1968, 1973). Glucose production increases in hypoglycaemia and decreases or stops during hyperglycaemia (de Bodo et al., 1963, Hetenyi and Wrenshall, 1968). Thus the relation between the

concentration of glucose in plasma and its rate of production are part of a negative feedback mechanism with the normal glucose level as the set point. Our observations suggest a complete absence of these corrective negative feedback influences exerted by the plasma glucose concentration on $R_a$, in the newborn pups. Since during the period of observation the animals were in or near a steady state in which the rate of glucose production equals the rate of its utilization ($R_d$), therefore, glucose utilization, $R_d$, was also un-influenced by plasma glucose concentration.

The plasma glucose concentration in such a system is a reflection of the "metabolic clearance" of glucose - that is, the factor relating concentration and utilization (utilization/concentration). In the adult dog the metabolic clearance has been shown to be primarily determined by the plasma concentration of insulin (Vranic et al., 1971). This may not be the situation in the pup, which is less sensitive to insulin than are adult dogs. It is possible that in pups, factors other than insulin (e.g. oxygenation, temperature) determine the metabolic clearance rate. As the animal develops the impact of these factors vanishes mainly due to the development of control systems that maintain homeostasis efficiently. At this stage insulin, itself a metabolic regulator, becomes effective and the vigorous control of blood glucose concentration becomes established.

The relationship between the body temperature and plasma glucose concentration was not studied, since in all the experiments the body temperature was kept approximately constant at $37 \pm 0.5^{o}C$.

The absence of a feedback between the concentration of glucose in the plasma and hepatic glucose production could account for the <u>persistence</u>

of neonatal hypoglycaemia. Any interference, be it physical or metabolic, that would tend to decrease the concentration of glucose will be compensated by an increased glucose production in the adult, where therefore hypoglycaemia will likely not even become manifest. The lack of this feedback however will permit the blood glucose to reach low levels un-opposed by the liver. Since the trigger of this mechanism is the fall in the glucose supply to the brain (Hetenyi 1972) it seems that the brain of the neonate is less sensitive to the effects of hypoglycaemia than that of the adult. It might be that in the neonate other metabolites (e.g. ketones) penetrate the blood brain barrier more easily than in adults, or that the energy requirement of the brain is less, or again the neonatal brain may be able to utilize endogenously produced oxidizable substrates e.g. fatty acids.

## 2. Sensitivity to insulin in the newborn dog.

In the experiments described, the administration of relatively high doses of bovine insulin to pups revealed a marked insensitivity to the injected insulin. During the first 4 hours of life an injection of insulin (0.12 U/kg) failed to produce appreciable hypoglycaemia in the pups. The hypoglycaemic response to insulin appeared in pups older than 4 hours but the recovery of normoglycaemia was absent. (Fig. 2). This slow return to the pre-injection plasma glucose concentration can be accounted for by the lack of the feedback mechanism operating between the plasma concentration of glucose and its hepatic production. In the absence of these corrective - adaptive mechanisms it is the gradual decrease of the metabolic clearance, which causes the glucose concentration to rise again as insulin disappears gradually from blood.

The pups are not only insensitive to injected insulin but also to their own endogenous insulin, released by infusion of D-ribose. In pups the infusion of D-ribose was found to increase serum IRI concentration to 36-90 µU/ml, without appreciable changes in plasma glucose concentration. Therefore the poor response to injected bovine insulin is not due to an insensitivity to heterogeneous insulin but to a reduced response by the normally insulin sensitive peripheral tissues.

The basal immunoreactive insulin concentrations in the plasma of newborn pups were about the same as in adult dogs. (Table 3 and 4). Similar results were reported by Sabata et al. (1969) who found the post-absorptive plasma insulin values to be relatively stable at 12-18 uU/ml in infants after few hours or days of birth. These observations suggest that hypoglycaemia during the early neonatal period cannot be due to a hyper-secretion of or a hypersensitivity to insulin. Thus the conjecture that increased insulin secretion or an increased sensitivity of the tissues to insulin are causes of hypoglycaemia in the newborn cannot be maintained. The cause of the reduced sensitivity to insulin in the newborn is not known.

3. Homeostatic responses to glucose loading in newborn dogs.

As it has been discussed previously, in adult dogs, any change in plasma glucose concentration initiates changes in overall glucose utilization and in glucose production by the liver (Steele et al. 1965, Cowan and Hetenyi, 1971). These changes are homeostatic since their combined effect results in the re-establishment of the normal steady level of glucose in the plasma. In the case of hyperglycaemia, the hepatic production of glucose falls to or near to zero, while peripheral utilization of glucose

rises (Steele et al. 1965, Hetenyi et al. 1973). Increased secretion of insulin clearly plays an important role in both these responses (Steele et al., 1965).

In the newborn dog, however, natural excursions of blood glucose to unusually high or low values do not alter significantly the rate of hepatic glucose production, and insulin-induced hypoglycaemia did not cause hepatic output to rise. This marked difference between adult and neonatal regulation led to the assumption that hyperglycaemia produced by glucose administration might also be ineffective in suppressing the endogenous production and in increasing the rate of utilization of glucose.

The net rate of disposal of injected glucose was found to be lower in the newborn than in adults in many mammalian species (Mintz et al., 1960, Lavine et al., 1971, Adam et al., 1969) including the dog (Adam et al., 1969, Allen et al., 1966) and man (Bowie et al., 1963, Gladtke et al., 1968). Glucagon or epinephrine was shown to give rise to prolonged hyperglycaemia in babies (Allen et al., 1966). In agreement with these findings it was found that in newborn pups the concentration of glucose in plasma rises monotonically during a steady infusion of glucose similarly to the response observed in diabetic patients by Moorhouse et al., (1967). This response is different from the one observed in the majority of normal men (Moorhouse et al., 1967, or dogs (Hetenyi et al., 1973) where the concentration of glucose passes through a maximum and settles at an elevated steady level for at least two hours. In a minority of adult humans or dogs the concentration of glucose in plasma rises rapidly to a plateau which is then maintained for some time. These normal responses

appear to depend on the ability of the normal animal to release insulin thereby turning off hepatic glucose production and increasing the metabolic clearance rate of glucose, that is, increasing the rate of glucose utilization out of proportion to the increase in the concentration of glucose in plasma. Diabetic dogs are unable to reduce significantly the rate of hepatic glucose production (Cowan and Hetenyi, 1971) and the sustained increase in the metabolic clearance was demonstrated to depend on the ability to release extra insulin during hyperglycaemia (Ishiwata et al., 1969).

In the experiments described, neither did the $R_a$ decrease to zero in pups, nor did the metabolic clearance of glucose increase, except transiently, during infusion. In these respects the response of the pups paralleled the response of adult dogs in which the pancreas had been replaced by a continuous infusion of insulin at a rate sufficient to maintain a constant normal level of glucose (Ishiwata et al., 1969). However, the level of serum IRI was found to increase in pups during hyperglycaemia to a degree comparable to that observed in adult dogs. This finding is in agreement with those reported by Adam et al., (1969). Therefore either the insulin released is less active biologically, or the sensitivity to insulin is less in pups than it is in adults. Although the first possibility cannot be excluded with absolute certainty our experiments furnish strong indirect evidence for the second. Beef insulin added to the glucose infusion at rates two or three times the estimated basal rate of release (Vranic and Wrenshall, 1969) did not improve the response significantly. These amounts of insulin were insufficient to raise serum IRI concentration significantly above the level observed with glucose infusion alone: indicating an increased

turnover rate of insulin as described by Adam et al., (1969). Only when exogenous insulin was infused at rates 10 or 25 times the estimated basal secretion, did the plasma glucose curve resemble that observed in normal adults and did the metabolic clearance rate of glucose increase significantly during the entire duration of the infusion of glucose. A slow improvement in insulin sensitivity with age is evident from the data presented on Table 11 but only 3 out of 6 beagle pups of age between 20 and 55 days were able to reach a steady plateau, and in no pup was a transient maximum ("hump") observed as it was in all five adult beagles i.e. the mothers of the litter.

As shown by Table 14 most pups did respond to hyperglycaemia with a decrease in the FFA concentration in their plasma, even if their response was less consistent and generally of lesser magnitude. In some respects the newborn pup resembles the third-trimester simian fetus. Chez et al., (1972), have demonstrated that in these fetuses, a moderate amount of insulin decreases the concentration of FFA in plasma but not the concentration of glucose, although the latter will decline if a much higher amount of insulin is injected.

The experiments reported demonstrate unequivocally that young animals are not only lacking an effective regulation that would restore normoglycaemia when the plasma glucose falls to low levels, they are also less efficient in handling excess glucose. This incapacity manifests itself in an incomplete shut down of hepatic glucose production and in an inability to raise the metabolic clearance rate of glucose. Since IRI

appears to be released in pups to the same extent than it is in mothers, the reason for the incomplete regulation lies with a decreased sensitivity to insulin. This is also clearly demonstrated by our experiments in which beef insulin was infused together with glucose. The simultaneous infusion of 10 or 25 times as much beef insulin as the estimated basal secretion rate tends to at least approximate to some extent the response of pups to that seen in adult dogs.

This decreased sensitivity might be of some biological value to the newborn in which a number of environmental (e.g. cold) and metabolic (e.g. low hepatic glycogen content) factors tend to favour the occurence to hypoglycaemia. Low sensitivity to insulin may prevent excessive hypoglycaemia and thus secure an adequate supply of glucose to the brain should insulin be mobilized for any reason in an already hypoglycaemic neonate. It is noteworthy that, in the absence of hypoglycaemia, an approximately fourfold increase in serum IRI is inadequate to raise the metabolic clearance of glucose but is still to some extent capable of decreasing the plasma FFA level and presumably suppress lipolysis. Thus at an elevated plasma glucose level the neonate is capable of storing fat under the influence of insulin preferentially utilizing glucose, since the constancy of the metabolic clearance allows the increase of glucose utilization only in direct linear proportion to the increase in the concentration of glucose in plasma.

4. Glucose production and utilization during hypoxia in the newborn dog.

Our observations discussed previously indicated that in pups, as opposed to adult dogs, plasma glucose concentration is not a precise and sensitive regulator of hepatic glucose release. While the cause of the unresponsiveness to hyperglycaemia is likely to be due to a decreases sensitivity to insulin, the failure of the liver of the newborn to respond to hypoglycaemia is less well understood.

Two possibilities may account for an apparent lack of regulation: (1) the liver in the newborn lacks the enzymic machinery to increase glucose production; or (2) in the newborn the main stimulus regulating glucose production is a factor different from the concentration of glucose in the plasma. The second possibility seemed to be more likely a priori, since the liver in many newborn mammals contains the enzymes necessary to form and release glucose in amounts comparable to those in adults, and plasma glucose levels were observed to increase in newborn rabbits in sustained hypoxia (Stave 1968) and in newborn babies in respiratory distress (Kerpel-Fronius et al., 1964).

In our experiments, ventilation with a gas mixture of 10% $O_2$ + 90% $N_2$ decreased the average oxygen content of blood from 14 to 5 ml/100 ml in the newborn and young pups. This led, in all pups, to a considerable increase in the concentration of glucose production accompanied by a lesser increase in the rate of elimination of glucose from the circulation. It appears therefore that the liver of the newborn dog is capable of producing excess glucose, as indeed was expected on the basis of the activities of its glycogenolytic and to some extent gluconeogenetic enzymes (Cornblath

and Schwartz 1966). As opposed to the adult dog the stimulus for this enhanced production is not hypoglycaemia but hypoxia. The effect is very likely to be mediated by an increased activity of the sympatho-adrenal system. Circulating catecholamine levels are increased at low oxygen tensions in newborn animals (Comline and Silver 1966) and in babies in respiratory distress (Cheek et al., 1963) and a local release of nor-epinephrine in the liver has also been postulated (Edwards and Silver 1969). As a result of their action, the release of glucose into the circulation is expected to rise, as indeed shown by these experiments. The release of catecholamines in hypoxia leads also to a suppression of the secretion of insulin (Baum and Porte 1972), thus enhancing the hyperglycaemic effect even further. As shown on Figures 11, 12 and 13 metabolic clearance rates did not increase during hyperglycaemia. The interpretation of some of the results is made somewhat difficult by the excessive (over 180 mg%) hyperglycaemia. At such high levels $R_d$ does not exactly reflect the rate of glucose utilization because glucose is also eliminated from the circulation by glycosuria. However, the metabolic clearance was not increased even when the elevated glucose level remained below 180mg%. This is especially evident from Figure 11 and from the first 30 minutes during period 2 (ventilation with 10% $O_2$) shown on Figure 13. In pups less than 10 days of age the more severe hypoxia (5% $O_2$) decreased the concentration of glucose in plasma together with the metabolic clearance of glucose.

The rate of glucose production by the liver is increased when the supply of oxidizable substrates to the brain becomes inadequate (Hetenyi 1972) such as in hypoglycaemia in adult dogs. The newborn dog, however, tolerates hypoglycaemia well and does not regulate against it if its

ventilation is adequate. This difference might be accounted for by a difference in cerebral metabolism. The turnover of ATP in the brain (Samson et al., 1960) and cerebral ATP-ase activity (Potter et al., 1945, Abdul-Latif and Aboud, 1964) are lower in newborn than in mature rats. Assuming this to be the case in other mammals, it is possible that during hypoglycaemia the complete oxidation of glucose available to the brain provides a sufficient amount of energy to maintain the turnover of ATP at a rate normal in the neonate. Hence no adaptive (regulatory) increase in hepatic glucose release will be initiated. In hypoxia on the other hand, the glucose supply available at a normal plasma glucose level may be inadequate to maintain the normal rate of turnover of cerebral ATP, since the anaerobic breakdown of glucose yields only about 5% of the energy that is released by complete oxidation. Therefore a signal for increased glucose production is generated, resulting in hyperglycaemia and an increased glucose supply to the brain that will become adequate to maintain cerebral ATP turnover at a normal rate even in the absence of an adequate amount of oxygen. The remarkably beneficial effect of glucose in prolonging survival and improving the function of the central nervous system in hypoxic newborn (Himwich et al., 1943, Britton and Kline, 1945, Adamsons et al., 1963) lends considerable support to this hypothesis.

In spite of the significant correlation between the increase in the release of glucose and the degree in hypoxia it is not possible to view the latter as a physiological regulator of the former, mainly because glucose production during hypoxia appears to be rather a defence mechanism activated in an emergency threatening the life of the newborn. Apparently this

emergency reaction has its limitations. It is evident from Figure 13 and Table 19 that a second exposure to hypoxia will not elicit an adequate response in the very young pup, or in any pup that was exposed to a more severe hypoxia first. It seems that the reserves that supply glucose, be they glycogen or gluconeogenic precursors, can be exhausted. The response of the pup to the re-establishment of normal oxygen supply is sluggish. After a 45 minute exposure to moderate hypoxia the plasma glucose level settles at a level considerably above the basal and the metabolic clearance rate decreases. This might well be accounted for by a difficient homeostatic response to hyperglycaemia itself as described earlier (Varma et al., 1972).

The decrease in FFA concentration during hypoxia observed in our experiments confirms the findings of Baum (1967) on somewhat older (21-35 day old) pups. In view of an increase in circulating catecholamines and a suppression of insulin secretion (Baum and Porte 1972) this response is paradoxical. The most likely explanation is that lactate accumulation during hypoxia decreases lipolysis (Issekutz et al., 1965) and this effect prevails over those of catecholamines and the lack of insulin. In any event it appears that in hypoxia the newborn animal draws more upon glucose than on FFA for a source of energy.

Propranolol did not prevent the hyperglycaemia during the anoxic periods. Hence the stimulus that raises the blood sugar level is not mediated by adrenergic $\beta$-receptors entirely. In adult dog the increase in the rate of glucose production was not prevented by propranolol but only by "double blockade", i.e. the simultaneous administration of an $\alpha$ and a $\beta$ blocking agent (Cowell and Hetenyi 1969). A similar combined blockade

was necessary to prevent acidosis and lactacidaemia in dogs in haemorrhagic shock (Halmagyi et al., 1968). The extension of our experiments to the investigation of the effects of double blockade on anoxic pups was made impossible by the interference of propranolol with cardiovascular defence mechanisms. As a result of this the two pups treated with propranolol did not survive even a period of less severe hypoxia.

## 5. Effect of methylprednisolone on glucose homeostasis in the newborn dog.

### 5.1. The effect of methylprednisolone (MP) treatment on the liver.

The relative weight of the liver (liver weight/body weight) was significantly increased by the treatment with MP. In mature dogs a 2.8 fold increase in liver weight was found after a 17 day treatment with 4 mg/kg.day MP (Campbell and Rastogi, 1968). Our results concerning the effects of MP on hepatic DNA, RNA and protein in pups are in complete agreement with those of Weber et al., (1964, 1965) who found similar effects in growing rats after a single injection of various steroids, including MP. Our results prove that these effects of MP are present in the newborn dog which is capable of synthesizing more protein and RNA under the stimulus of adrenal steroids. Since the aim of the experiments was to examine glucose homeostasis in the newborn no attempt was made for a complete analysis of the liver. There is little, if any doubt that the liver in the treated pups contained more glycogen and fat (Campbell and Rastogi, 1968) than in controls.

## 5.2. The effect of MP treatment on glucose homeostasis.

The concentration of glucose in the plasma was not markedly elevated by MP. The average increase of 12.5% observed in the pups is only slightly higher than that found by Issekutz and Allen (1972) and by Ninomiya et al., (1965) in mature animals. The two fully grown dogs (mothers of litters 3 and 4) responded with increases of 0.2 and 5.9% respectively. As judged by the relatively steady concentration of glucose in plasma, the newborn dog is about as able to maintain glucose homeostasis as the mature animal. The underlying mechanism, however, is different and changes with the age of the animal.

The insulinotropic effect of MP (Campbell et al., 1968, Perley and Kipnis, 1966) is present from the very early days of life. It becomes more marked with the age of the pup. Its presence between the 5th and 23rd days of life at which time MP had neither an effect on the rate of glucose production nor on the metabolic clearance of glucose indicates that this effect is independent of the one on gluconeogenesis. The absence of a response to the elevated serum IRI level in terms of utilization or metabolic clearance of glucose is not surprising in view of a similar ineffectiveness of endogenously released IRI in response to hyperglycaemia in pups. In view of this very low sensitivity to insulin, the assumption that the extra insulin which was released suppressed gluconeogenesis and hence hepatic glucose production seems unlikely, all the more so because in grown dogs known to be more sensitive to insulin, glucose production increases. The interpretation of the absence of an increased glucose turnover in pups, in contrast to adult dogs, must be viewed in this light. It might be assumed

that the increase in glucose release is small or absent in pups because of an absence of stimulus. If, in the grown dog, the primary insulinotropic effect were to lead to an increased metabolic clearance and utilization of glucose the increased glucose production might be due to the well-known feedback mechanism that will maintain a normal and steady glucose level in the plasma by increasing the rate of hepatic glucose production in response to increased utilization of glucose. However, the evidence presented does not bear this out. It is known however that the rate of hepatic glucose production is very significantly elevated by MP even in pancreatectomized dogs. Moreover treatment with adrenal steroids increases the activity of some enzymes involved in gluconeogensis (Exton 1972, Exton and Harper 1972, Weber et al., 1964, 1965) and, in the adult dog, the excess glucose released from the liver in dogs treated with MP originates from gluconeogenesis (Issekutz and Allen 1972). As opposed to grown rats however, in the newborn of the same species the injection of cortisol does not increase either the activity of tryptophane-pyrrolase (Goldstein and Knox 1963) or that of glucose-6-phosphatase (Dawkins, 1963). It might be assumed that the apparent key enzyme in glyconeogenesis, phosphoenol-pyruvate-carboxykinase (PEP-CK) is not being activated in newborn rats, although adrenal steroids increase the de novo synthesis (Exton and Harper, 1972) and activity (Ray et al., 1964) of this enzyme in adult animals. In the rat fetus this enzyme is absent from the liver (Ballard and Hanson, 1967) or is of very low activity (Wicks, 1969): in fetal rat liver explants its activity is not altered by cortisol (Monder et al., 1972). PEP-CK activity appears soon after birth. In the rat (a species that is assumed to mature faster than the dog) maximal

activity is reached by about a week after birth, the activity remains high until about the 16th day; then it falls rapidly to one quarter of its maximal level and remains there throughout life (Bowie et al., 1963, Exton, 1972). In view of the postnatal development of hepatic PEP-CK activity the small but persistent effect of MP treatment on glucose turn-over are puzzling. A speculative hypothesis that would account for it would be to assume that, since the sensitivity to insulin is particularly and excessively low during the first days of life, any minimal effects on PEP-CK could manifest itself freely. Such minimal effects might be suppressed somewhat later in life by the larger amounts of insulin released (Table 23) and/or by a slow improvement of insulin sensitivity. At an even later date the effect of MP on enzyme synthesis and/or activation becomes manifest and reaches its full extent in the grown dog. In summary: the lack of the effect of extra insulin on glucose utilization during MP treatment cannot by itself account for the lack of an increase in hepatic glucose production. It seems that in pups MP does not activate gluconeogenesis as it does in adult animals.

### 5.3. The effect of MP treatment on plasma FFA.

The generally higher level of FFA in the plasma of treated animals indicates that the lipolytic effect of adrenal steroids (Mahler and Stafford, 1963) appears very early in life. The differences in plasma FFA levels between treated animals and their matched pairs did not show any correlation with age and therefore the effect is independent from the gluconeogenetic effect of the steroid.

## SUMMARY AND CONCLUSIONS

Compared to adult dogs, pups are much less able to maintain the concentration of glucose in their plasma at a steady level. This inability manifests itself in a deficiency of mechanisms that in the adult dog are set into motion against impending hypoglycaemia, as well as in the inadequate disposition of glucose loads. Low blood sugar does not increase the rate of hepatic glucose production as it does in adults, indicating the lack of a feedback mechanism between glucose level and rate of glucose production. The lack of this feedback accounts for the persistence of hypoglycaemia in the newborn. Low sensitivity to injected as well as to endogenously released insulin is the cause of the impaired regulation against hyperglycaemia. High blood glucose levels mobilize IRI in the newborn to about the same degree as in the adult,but the response to insulin, as judged by the metabolic clearance of glucose and by the incomplete shutdown of hepatic glucose production is much less than in normal adult animals. It seems that in the newborn the turnover of glucose is much less efficiently regulated by the level of glucose in blood than by other factors.

Hypoxia was shown to raise the glucose level in blood by an increased glucose production not accompanied by an increase in the metabolic clearance rate of glucose. The physiological importance of this reaction has not been adequately assessed, but it would act in providing glucose preferentially to the brain since hyperglycaemia does not result in a disproportionately increased glucose uptake by peripheral insulin-sensitive tissues.

An excess of a glucocorticoid (methylprednisolone) does not alter the concentration of glucose in the plasma either in pups or in adult dogs. The turnover of glucose is however raised only in the latter. The reason of the difference lies partly in the absence of the stimulation of gluconeogenesis by methylprednisolone in pups, partly in the relative ineffectiveness of the IRI released in pups in response to the glucocorticoid. The level of serum IRI rises as high or higher in pups than in adults indicating that the insulinotropic effect of glucocorticoids is not secondary to their effect on gluconeogenesis.

TABLE 1

Mean plasma glucose concentration and glucose production ($R_a$) in beagle pups of different ages.

| Dog # | Litter | Age (hours) | Weight (kg) | Plasma glucose mg/100 ml (mean $\pm$ sem) | $R_a$ (mg/gm liver-min) |
|---|---|---|---|---|---|
| 1 | 1 | $7\frac{1}{2}$ | .300 | 84 $\pm$ 2.3 | 0.111 |
| 2 | 1 | $8\frac{1}{4}$ | .296 | 187 $\pm$ 13.6 | 0.151 |
| 3 | 1 | 15 | .258 | 127 $\pm$ 2.5 | 0.175 |
| 4 | 1 | 15 | .259 | 128 $\pm$ 6.3 | 0.176 |
| 5 | 1 | 32 | .235 | 76 $\pm$ 7.1 | 0.184 |
| 6 | 1 | 32 | .255 | 52 $\pm$ 4.3 | 0.152 |
| 7 | 2 | 3 | .192 | 129 $\pm$ 6.4 | 0.156 |
| 8 | 2 | $2\frac{1}{2}$ | .219 | 137 $\pm$ 6.6 | 0.110 |
| 9 | 2 | 8 | .269 | 45 $\pm$ 2.6 | 0.119 |
| 10 | 2 | 18 | .197 | 66 $\pm$ 1.9 | 0.097 |
| 11 | 3 | 9 | .200 | 34 $\pm$ 2.3 | 0.049 |
| 12 | 4 | 6 3/4 | .345 | 42 $\pm$ 3.5 | 0.078 |
| 13 | 4 | 30 | .287 | 65 $\pm$ 5.9 | 0.113 |

$R_a$ mg/gm liver - min (mean $\pm$ s.e.m.) = 0.128 $\pm$ 0.01

TABLE 2

Mean plasma glucose concentrations and glucose production ($R_a$) in mongrel pups of different ages.

| Dog # | Litter | Age (hours) | Weight (kg) | Plasma glucose mg/100 ml (mean ± sem) | $R_a$ (mg/gm liver-min) |
|---|---|---|---|---|---|
| 1 | 1 | 36 | 0.395 | 119 ± 4.8 | 0.183 |
| 2 | 2 | 6 | 0.325 | 300 ± 7.1 | 0.219 |
| 3 | 2 | 22 | 0.207 | 43 ± 3.7 | 0.187 |
| 4 | 2 | 24 | 0.313 | 37 ± 2.85 | 0.269 |
| 5 | 2 | 40 | 0.360 | 185 ± 7.2 | 0.248 |
| 6 | 2 | 48 | 0.310 | 123 ± 8.4 | 0.350 |
| 7 | 2 | 70 | 0.402 | 140 ± 8.9 | 0.253 |
| 8 | 2 | 72 | 0.385 | 139 ± 5.8 | 0.310 |
| 9 | 3 | 10 | 0.356 | 69 ± 3.3 | 0.157 |
| 10 | 3 | 16 | 0.405 | 75 ± 4.4 | 0.176 |
| 11 | 3 | 34 | 0.192 | 32 ± 2.1 | 0.140 |
| 12 | 3 | 36 | 0.311 | 82 ± 3.8 | 0.192 |
| 13 | 3 | 60 | 0.340 | 118 ± 2.4 | 0.183 |
| 14 | 3 | 60 | 0.340 | 128 ± 5.6 | 0.138 |
| 15 | 3 | 82 | 0.395 | 127 ± 5.3 | 0.207 |
| 16 | 3 | 84 | 0.470 | 84 ± 2.5 | 0.160 |
| 17 | 3 | 106 | 0.470 | 56 ± 1.9 | 0.234 |
| 18 | 4 | 8 | 0.295 | 46 ± 5.6 | 0.122 |

TABLE 2 (continued)

| Dog # | Litter | Age (hours) | Weight (kg) | Plasma glucose mg/100 ml (mean ± sem) | $R_a$ (mg/gm liver-min) |
|---|---|---|---|---|---|
| 19 | 4 | 8 | 0.310 | 79 ± 2.5 | 0.203 |
| 20 | 4 | 32 | 0.312 | 65 ± 1.7 | 0.180 |
| 21 | 4 | 32 | 0.346 | 47 ± 1.6 | 0.159 |
| 22 | 4 | 56 | 0.325 | 167 ± 16.4 | 0.179 |
| 23 | 5 | 7 | 0.450 | 54 ± 1.7 | 0.142 |
| 24 | 5 | 20 | 0.414 | 110 ± 8.1 | 0.131 |
| 25 | 5 | 44 | 0.467 | 110 ± 2.3 | 0.165 |
| 26 | 6 | 4 | 0.237 | 43 ± 4.7 | 0.304 |
| 27 | 6 | 4 | 0.250 | 29 ± 1.6 | 0.340 |
| 28 | 6 | 26 | 0.315 | 16 ± 0.8 | 0.300 |
| 29 | 6 | 48 | 0.330 | 145 ± 5.3 | 0.303 |
| 30 | 7 | 8 | 0.360 | 50 ± 4.5 | 0.141 |

Mean $R_a$ mg/gm liver-min (mean ± s.e.m.) = 0.209 ± .012

TABLE 3

Basal concentrations of plasma glucose, serum IRI and plasma FFA in beagle pups of different ages.

| Age in hours | 2 - 12 | 13 - 24 | 25 - 32 |
|---|---|---|---|
| Plasma glucose<br>mg/100 ml<br>Mean ± sem | 102 ± 13.1<br>(15) | 84 ± 14.1<br>(6) | 70 ± 7.5<br>(4) |
| Serum IRI<br>uU/ml<br>Mean ± sem | 16.6 ± 1.8<br>(15) | 12.2 ± 2.12<br>(6) | 19.3 ± 4.1<br>(3) |
| Plasma FFA<br>uEq/L<br>Mean ± sem | 574 ± 124.1<br>(14) | 361 ± 109.3<br>(6) | 314 ± 44.8<br>(6) |

The number of pups is shown in brackets.

TABLE 4

Basal concentrations of plasma glucose, serum IRI and plasma FFA in mongrel pups of different ages.

| Age in hours | 3 - 12 | 13 - 24 | 25 - 36 | 36 |
|---|---|---|---|---|
| Plasma glucose mg/100 ml Mean ± sem | 77 ± 21.36 (12) | 87.8 ± 17.06 (8) | 70.4 ± 10.64 (7) | 121 ± 7.47 (17) |
| Serum IRI uU/ml Mean ± sem | 11.3 ± 1.7 (13) | 17.4 ± 4.7 (7) | 10.2 ± 3.0 (5) | 12.1 ± 1.24 (16) |
| Plasma FFA uEq/L Mean ± sem | 570 ± 96.6 (10) | 403 ± 46.4 (12) | 505 ± 100.2 (10) | 419 ± 63.42 (19) |

The number of pups is shown in brackets.

TABLE 5

Number, age, sex and weight of beagle pups injected with 0.12U/kg of insulin i.v.

| Dog # | Litter | Age (hours) | Sex | Weight (kg) |
|---|---|---|---|---|
| 1 | 1 | $8\frac{1}{4}$ | M | 0.296 |
| 2 | 1 | 15 | M | 0.259 |
| 3 | 1 | 32 | F | 0.255 |
| 4 | 2 | $2\frac{1}{2}$ | M | 0.219 |
| 5 | 2 | 8 | M | 0.285 |
| 6 | 2 | 16 | M | 0.211 |
| 7 | 3 | $4\frac{1}{4}$ | F | 0.249 |
| 8 | 3 | $3\frac{1}{2}$ | F | 0.260 |
| 9 | 3 | $9\frac{1}{2}$ | F | 0.210 |
| 10 | 4 | 8 | M | 0.340 |
| 11 | 4 | 30 | M | 0.325 |
| 12 | 5 | 4 | F | 0.247 |
| 13 | 5 | 7 | M | 0.253 |
| 14 | 5 | 7 | M | 0.286 |
| 15 | 5 | 23 | M | 0.298 |

## TABLE 6

Number, age, sex and weight of mongrel pups to which insulin was injected or infused i.v.

| Dog # | Litter | Age (hours) | Sex | Weight (kg) | Treatment |
|---|---|---|---|---|---|
| 1 | 1 | 8 | M | 0.310 | Insulin infusion (1mU/kg-min.) |
| 2 | 1 | 32 | F | 0.346 | - do - |
| 3 | 1 | 56 | M | 0.365 | Insulin infusion (2mU/kg-min.) |
| 4 | 2 | 7 | F | 0.435 | Insulin injection (0.06U/kg) |
| 5 | 2 | 20 | M | 0.438 | - do - |
| 6 | 2 | 44 | F | 0.408 | - do - |
| 7 | 2 | 108 | F | 0.574 | - do - |
| 8 | 2 | 112 | F | 0.670 | - do - |
| 9 | 3 | 4 | F | 0.250 | Insulin injection (0.12U/kg) |
| 10 | 3 | 26 | M | 0.315 | Primed insulin infusion of 241mU/kg-min. |
| 11 | 4 | 9 | F | 0.380 | Insulin injection (0.12U/kg) |
| 12 | 4 | 32 | F | 0.390 | Primed insulin infusion of 194.8mU/kg-min. |

## TABLE 7

Plasma glucose concentrations (mg/100 ml) in beagle pups at different times (min) following insulin injection.

| Dog # | Age in hrs. | Before injection | 5 | 10 | 20 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2½ | 136 | 141 | 133 | 132 | 126 | 129 | 137 | 162 | 188 |
| 2 | 3½ | 144 | 135 | 120 | 114 | 105 | 92 | 82 | 50 | 34 |
| 3 | 4 | 47 | 43 | 34 | 24 | 19 | 8 | 12 | 22 | 32 |
| 4 | 4¼ | 130 | 173 | 164 | 275 | 259 | 161 | 193 | 201 | 220 |
| 5 | 7 | 89 | 80 | 70 | 52 | 45 | 31 | 23 | 42 | 48 |
| 6 | 7 | 56 | 46 | 37 | 28 | 19 | 12 | 41 | 49 | 59 |
| 7 | 8 | 128 | 130 | 130 | 118 | 110 | 83 | 70 | 50 | 51 |
| 8 | 8 | 213 | 233 | 217 | 238 | 218 | - | - | | |
| 9 | 8¼ | 234 | 226 | 215 | 206 | 200 | 184 | 166 | 135 | 115 |
| 10 | 9½ | 57 | 55 | 45 | 35 | 24 | 19 | 55 | 36 | 22 |
| 11 | 15 | 168 | 142 | 141 | 120 | 114 | 102 | 95 | 113 | 139 |
| 12 | 16 | 64 | 64 | 56 | 44 | 41 | 27 | 30 | 44 | 58 |
| 13 | 23 | 106 | 106 | 93 | 79 | 63 | 46 | 33 | 42 | 70 |
| 14 | 30 | 87 | 77 | 64 | 44 | 35 | 22 | 25 | 37 | 39 |
| 15 | 32 | 62 | 60 | 58 | 40 | 32 | 36 | 47 | 55 | 70 |

TABLE 8

Plasma glucose concentration (mg/100 ml) in mongrel pups at different times (min) following the injection of insulin.

| Dog # | Age in hrs. | Before injection | 10 | 20 | 40 | 60 | 90 | 120 | 150 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 32.0 | 30.0 | - | 28.0 | 26.0 | 24.0 | 35.0 | - |
| 2 | 7 | 41.0 | 37.0 | 30.0 | 31.0 | 63.0 | 58.0 | 58.0 | - |
| 3 | 8 | 77.5 | 85.6 | 77.0 | 73.6 | 78.0 | - | - | |
| 4 | 9 | 33.0 | 31.0 | 19.0 | 25.0 | 35.0 | 36.0 | 52.0 | |
| 5 | 20 | 101.0 | 91.0 | 78.0 | 87.0 | 92.0 | 120.0 | - | |
| 6* | 26 | 62.9 | - | - | 18.6 | 16.8 | 13.7 | 14.1 | |
| 7* | 32 | 85.1 | - | - | 43.0 | 26.2 | 34.0 | 19.7 | |
| 8 | 32 | 38.5 | 52.5 | 46.0 | 44.5 | - | 45.0 | - | |
| 9 | 44 | 81.0 | 73.0 | 54.0 | 54.0 | 74.0 | 90.0 | 94.0 | |
| 10 | 56 | 103.5 | 106.9 | 59.5 | - | 33.8 | - | - | |
| 11 | 108 | 117.0 | 115.0 | 111.0 | 133.0 | 138.0 | 378.0** | 375.0** | |
| 12 | 112 | 120.0 | 104.0 | 111.0 | 129.0 | 175.0 | 172.0 | 154.0 | 148.0 |

* Very high doses of insulin were infused

** Pup became anoxic

## TABLE 9

Age, sex and weight of pups to which D-ribose was infused.

| Mongrel Pups | Litter | Age (hours) | Sex | Weight (kg) | Treatment |
|---|---|---|---|---|---|
| 1 | 1 | 12 | F | 0.379 | Ribose infused (4.5mg/kg-min) |
| 2 | 1 | 108 | F | 0.585 | Ribose infused (3.7mg/kg-min) |
| 3 | 2 | 6 | M | 0.236 | Ribose infused (11mg/kg-min) |
| 4 | 2 | 24 | M | 0.304 | Ribose infused (8.48mg/kg-min) |
| 5 | 3 | 12 | F | 0.405 | Ribose infused (9.5mg/kg-min) |
| Beagle Pups | | | | | |
| 1 | 1 | 12 | M | 0.186 | Ribose infused (16mg/kg-min) |
| 2 | 2 | 6 | M | 0.213 | Ribose infused (10mg/kg-min) |
| 3 | 3 | 9 | M | 0.303 | Ribose infused (17mg/kg-min) |
| 4 | 4 | 4 | F | 0.270 | Ribose infused (15.9mg/kg-min) |

TABLE 10

Summary of the experiments on 27 newborn beagle pups (1 - 27), 7 mongrel pups (28 - 34) and mothers of 5 beagle litters (35 - 39) infused with glucose.

| Dog # | Litter # | Age (days) | Sex | Weight (kg) | Glucose inf. rate (mg/kg-min) | Insulin inf. rate (uU/kg-min) |
|---|---|---|---|---|---|---|
| 1 | 1 | 0.5 | F | 0.258 | 15.0 | - |
| 2 | 1 | 1.5 | M | 0.258 | 15.7 | - |
| 3 | 1 | 8.5 | F | 0.385 | 14.7 | - |
| 4 | 1 | 18.5 | F | 0.860 | 11.7 | - |
| 5 | 2 | 0.2 | F | 0.193 | 20.8 | - |
| 6 | 2 | 1.1 | M | 0.202 | 20.2 | - |
| 7 | 2 | 5 | F | 0.156 | 15.7 | - |
| 8 | 3 | 0.5 | F | 0.298 | 13.5 | - |
| 9 | 3 | 2.5 | M | 0.299 | 13.6 | - |
| 10 | 3 | 10.5 | F | 0.563 | 12.6 | - |
| 11 | 4 | 0.7 | M | 0.311 | 12.6 | - |
| 12 | 4 | 8.5 | M | 0.722 | 11.4 | - |
| 13 | 4 | 15.5 | F | 0.922 | 9.8 | - |
| 14 | 4 | 18.5 | F | 0.929 | 10.0 | 559.7 |
| 15 | 4 | 25.5 | F | 1.458 | 8.3 | - |
| 16 | 4 | 40.5 | M | 1.708 | 9.8 | 711.5 |
| 17 | 4 | 47.5 | M | 2.387 | 9.2 | - |
| 18 | 5 | 21 | F | 0.965 | 9.8 | 672.8 |
| 19 | 5 | 28 | M | 1.05 | 9.5 | 652 |
| 20 | 5 | 35 | M | 1.153 | 11.0 | 600 |
| 21 | 5 | 42 | F | 1.504 | 10.8 | 2400 |
| 22 | 5 | 47 | M | 1.188 | 8.9 | - |
| 23 | 5 | 55 | F | 1.323 | 12.6 | - |
| 24 | 6 | 1.6 | F | 0.286 | 14.0 | - |

TABLE 10 (continued)

| Dog # | Litter # | Age (days) | Sex | Weight (kg) | Glucose inf. rate (mg/kg-min) | Insulin inf. rate (uU/kg-min) |
|---|---|---|---|---|---|---|
| 25 | 6 | 1.7 | M | 0.291 | 14.0 | 5883 |
| 26 | 6 | 36 | F | 2.122 | 10.0 | - |
| 27 | 6 | 43 | F | 2.480 | 8.7 | - |
| 28 | 7 | 0.5 | M | 0.408 | 12.6 | - |
| 29 | 7 | 0.6 | M | 0.396 | 13.6 | - |
| 30 | 7 | 1.5 | M | 0.372 | 14.5 | - |
| 31 | 7 | 1.6 | M | 0.469 | 11.0 | - |
| 32 | 7 | 2.6 | M | 0.414 | 12.5 | - |
| 33 | 7 | 8.5 | F | 0.825 | 12.5 | - |
| 34 | 7 | 16 | M | 1.640 | 8.8 | - |
| 35 | 1 | | F | 10.5 | 10.5 | - |
| 36 | 3 | | F | 9.0 | 10.8 | - |
| 37 | 4 | | F | 14.3 | 10.3 | - |
| 38 | 5 | | F | 9.8 | 11.2 | - |
| 39 | 6 | | F | 12.1 | 7.9 | - |

TABLE 11

Pattern of the course of plasma glucose concentration in beagle pups during an intravenous infusion of glucose (8-21 mg/kg-min). Numbers in brackets include the results of experiments on seven mongrel pups as well.

| Age: | 0-4 days | 4-20 days | 20-55 days |
|---|---|---|---|
| linear rise: | 6 (9) | 3 | 0 |
| modified linear: | 2 (4) | 3 (4) | 3 |
| rise to plateau: | 0 | 0 (1) | 3 |

TABLE 12

Metabolic clearance (ml/kg-min) of glucose during an intravenous infusion of glucose (8-21 mg/kg-min) or of glucose plus insulin (550-5880 uU/kg-min) in beagle pups.

| Infusion | Number of pups | Pre-infusion mean | Metabolic Clearance During Infusion | | | | |
|---|---|---|---|---|---|---|---|
| | | time = | 10-30 | 30-50 | 50-70 | 70-90 | 90-180(min.) |
| Glucose only | 20 | | 11.57 $\pm$0.81 | 9.26 $\pm$0.41 | 7.68 $\pm$0.37 | 7.25 $\pm$0.35 | 6.15 $\pm$1.09 |
| Glucose + Insulin 550-710* | 5 | 8.47 $\pm$0.31 | 11.61 $\pm$1.08 | 9.57 1.00 | 8.64 $\pm$0.99 | 8.53 $\pm$1.17 | 7.96 $\pm$1.12 |
| Glucose + Insulin 2400 or 5880* | 2 | | 17.8 12.1 | 14.6 11.7 | 13.9 10.0 | 13.9 11.7 | 13.8 10.0 |

* uU/kg-min.

Only between 90 t 180 minutes was the metabolic clearance significantly higher ($P < 0.05$) in the second group compared with the first.

TABLE 13

Concentration of immuno-reactive insulin (uU/ml) before and during the infusion of glucose (8-21 mg/kg) or glucose plus insulin (550-5880 μU/kg.min) in beagles and mongrels.

| Animals/Infusions | Before infusion | After the start of the infusion (min). | | | |
|---|---|---|---|---|---|
| | | 10-30 | 40-70 | 100-150 | 170 |
| Beagle pups: glucose only | 11.3 $\pm$ 1.3 (17) | 24.9 $\pm$ 8.1 (8) | 33.7 $\pm$ 6.4 (10) | 43.0 $\pm$ 5.5 (17) | 40.5 $\pm$ 14.6 (6) |
| Beagle pups glucose + insulin | 9.9 $\pm$ 0.9 (6) | 27.7 $\pm$ 4.5 (6) | 36.1 $\pm$ 3.7 (6) | 51.9 $\pm$ 12.8 (3) | 42.1 $\pm$ 9.1 (3) |
| Adult beagles (mothers) glucose only | 9.6 $\pm$ 0.4 (5) | 24.8 $\pm$ 3.0 (5) | 43.8 $\pm$ 9.2 (5) | 41.7 $\pm$ 8.7 (5) | 31.6 $\pm$ 3.6 (5) |
| Mongrel pups glucose only | 17.4 $\pm$ 4.9 (6) | 18.4 $\pm$ 3.5 (7) | 54.0 $\pm$ 21.2 (6) | 56.8 $\pm$ 15.4 (7) | |

The numbers in brackets refer to the number of animals contributing to the respective mean and s.e.m.

TABLE 14

Changes in the concentration of FFA in the plasma of pups during an infusion of glucose (8-21 mg/kg-min) or glucose plus insulin (550-5880 uU/kg-min).

| Animals/Infusions | Rose by more than 20% | +20 to -20% | Fell by 20-50% | Fell by more than 50% |
|---|---|---|---|---|
| Beagles: glucose only | 2 | 2 | 6 | 10 |
| Beagles: glucose + insulin | 0 | 0 | 3 | 4 |
| Adult Beagles (mothers) glucose only | 0 | 0 | 0 | 5 |
| Mongrels: glucose only | 0 | 1 | 2 | 4 |

TABLE 15

Age, sex, weight and litter of pups made hypoxic.

| Dog # | Litter | Age (days) | Sex | Weight (kg) | Percent $O_2$ inhaled in the second period |
|---|---|---|---|---|---|
| 1 | 1 | 2 | M | 0.392 | 10% |
| 2 | 1 | 9 | M | 0.701 | 10% |
| 3 | 2 | 1 (22 hrs.) | M | 0.352 | 10% |
| 4 | 2 | 16 | M | 0.952 | 10% |
| 5 | 3 | 1 | M | 0.307 | 10% |
| 6 | 3 | 2 | M | 0.348 | 5% |
| 7 | 3 | 8 | M | 0.512 | 10% |
| 8 | 3 | 9 | F | 0.557 | 10% |
| 9 | 3 | 10 | M | 0.538 | 5% |
| 10 | 3 | 22 | M | 1.255 | 10% |
| 11 | 3 | 23 | F | 1.032 | 5% |
| 12* | 3 | 29 | M | 1.532 | 10% |
| 13* | 3 | 30 | F | 1.628 | 10% |

* Infused with propranolol

M = male

F = female

## TABLE 16

Total $O_2$-content, $O_2$-saturation, pH and hemoglobin concentration in arterial blood of pups ventilated with room air, 10% $O_2$ + 90% $N_2$ or 5% $O_2$ + 95% $N_2$.

| Inhaled gas | Room air | 10% $O_2$ | Room air | 5% $O_2$ |
|---|---|---|---|---|
| Total $O_2$ ml/100 ml blood | 14.0 ± 1.1 | 5.0 ± 0.6 | 13.0 ± 1.1 | 2.1 ± 0.4 |
| $O_2$ Saturation % | 83.2 ± 1.7 | 28.5 ± 2.4 | 75.21± 1.6 | 11.6 ± 1.9 |
| pH | 7.42 ± 0.01 | 7.34 ± 0.03 | 7.33 ± 0.03 | 7.21 ± 0.05 |
| Hb gm/100 ml | 12.2 ± 1.0 | —— | —— | 12.4 ± 0.9 |
| Number of determinations* | 10 | 10 | 8** | 8** |

* On one pup these determinations were not carried out.

** On two pups the experiment was terminated after the second.

TABLE 17

Changes in concentration of plasma glucose, rates of glucose production ($R_a$), and utilization ($R_d$) in pups ventilated with room air, 10% $O_2$ + 90% $N_2$ or 5% $O_2$ + 95% $N_2$.

| Ventilation | Room Air | 10% $O_2$ + 90% $N_2$ | | | Room Air | | | 5% $O_2$ + 95% $N_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time in minutes | -20-0 | 0-15 | 15-30 | 30-45 | 45-65 | 65-85 | 85-105 | 105-120 | 120-135 | 135-155 | Age of pups days. |
| Plasma Glucose Concentration (mg/100 ml) Mean ± sem | 94.7 ±8.65 | 134.5 ±23.6 | 164 ±30.0 | 204.9 ±35.1 | 121.1 ±20.0 | 114.4 ±16.7 | 122.7 ±16.4 | 292.1 | 381.15 | 416.15 | > 10 |
| | | | | | | | | 120.4 ±9.83 | 113.3 ±6.81 | 103.33 ±18.06 | < 10 |
| $R_a$ mg/kg-min Mean ± sem | 10.31 ±1.206 | 13.6 ±2.1 | 17.7 ±3.2 | 18.5 ±3.2 | 10.3 ±0.4 | 9.4 ±0.65 | 8.1 ±0.71 | 22.09 | 22.16 | 21.05 | > 10 |
| | | | | | | | | 11.1 ±1.08 | 12.5 ±2.38 | 10.1 ±1.83 | < 10 |
| $R_d$ mg/kg-min Mean ± sem | 10.53 ±1.669 | 10.7 ±1.8 | 12.7 ±2.6 | 18.9 ±3.0 | 11.8 ±0.61 | 9.9 ±0.63 | 8.3 ±0.67 | 11.35 | 13.95 | 18.15 | > 10 |
| | | | | | | | | 10.3 ±0.67 | 11.5 ±1.48 | 10.3 ±0.82 | < 10 |
| Metabolic Clearance ml/kg-min Mean ± sem | 12.02 ±0.914 | 10.6 ±1.48 | 10.9 ±1.30 | 8.78 ±1.10 | 10.1 ±1.28 | 9.7 ±1.8 | 7.0 ±0.93 | 5.15 | 4.08 | 4.5 | > 10 |
| | | | | | | | | 9.8 ±1.13 | 9.9 ±1.5 | 9.2 ±0.23 | < 10 |
| Number of pups | 8 | 8 | | | 6 | | | 2 | | | > 10 |
| | | | | | | | | 4 | | | < 10 |

TABLE 18

Percentage changes in the concentration of plasma glucose and in the rates of glucose production ($R_a$) and utilization ($R_d$) in pups ventilated with room air, 10% $O_2$ + 90% $N_2$ or 5% $O_2$ + 95% $N_2$.

100% = mean of 3 measurements during the first control period (room-air).

| Ventilation | Room Air | 10% $O_2$ + 90% $N_2$ | | | Room Air | | | 5% $O_2$ + 95% $N_2$ | | | Age of pups in days |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time in minutes | -20-0 | 0-15 | 15-30 | 30-45 | 45-65 | 65-85 | 85-105 | 105-120 | 120-135 | 135-155 | |
| Plasma Glucose Concentration | 100 ±2 | 143 ±9 | 176 ±16 | 212 ±22 | 159 ±17 | 154 ±21 | 158 ±24 | 209 | 281 | 307 | > 10 |
| | | | | | | | | 237 ±48 | 215 ±25 | 182 ±16 | < 10 |
| $R_a$ | 100 ±2 | 136 ±9 | 174 ±14 | 182 ±22 | 138 ±12 | 127 ±16 | 110 ±17 | 231 | 230 | 226 | > 10 |
| | | | | | | | | 168 ±32 | 182 ±29 | 144 ±21 | < 10 |
| $R_d$ | 100 ±2 | 102 ±6 | 147 ±12 | 156 ±20 | 152 ±15 | 126 ±13 | 109 ±16 | 123 | 147 | 193 | > 10 |
| | | | | | | | | 148 ±17 | 162 ±27 | 148 ±9 | < 10 |
| Number of pups | 8 | 8 | | | 6 | | | 2 | | | > 10 |
| | | | | | | | | 4 | | | < 10 |

TABLE 19

Percentage changes in the concentration of plasma glucose and in the rates of glucose production ($R_a$) and ($R_d$) in pups ventilated first with 5% $O_2$ + 95% $N_2$ and then with 10% $O_2$ + 90% $N_2$. These two periods were separated by 60 minutes during which they were ventilated with room air. 100% = mean of 3 measurements during the first control period (room air).

| Ventilation Time | | | Room Air | 5% $O_2$ + 95% $N_2$ | | | Room Air | | | 10% $O_2$ + 90% $N_2$ | | | Basal Values (=100%) in absolute units |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Expt. No. | Age days | Determi-nations | -20–0 | 0–15 | 15–30 | 30–45 | 45–65 | 65–85 | 85–105 | 105–120 | 120–135 | 135–165 | |
| 6 | 2 | C | 100±1.8 | 176.2 | 255.5 | 384.3 | 295.1 | 279.7 | 278.7 | 275.8 | 276.8 | 257.7 | 94.5 mg/100 ml |
| | | Ra | 100 | 180.1 | 248.6 | 193.4 | 223.2 | 187.8 | 152.5 | 170.1 | 153.6 | 150.3 | 9.05 mg/kg-min |
| | | Rd | 100 | 109.9 | 179.1 | 172.5 | 210.9 | 196.7 | 151.6 | 172.5 | 151.6 | 165.9 | 9.10 mg/kg-min |
| 9 | 10 | C | 100±1.3 | 180.2 | 273.1 | 295.7 | 268.3 | 256.9 | 251.2 | 257.5 | 253.7 | 225.8 | 149.9 mg/100 ml |
| | | Ra | 100 | 216.2 | 280.8 | 172.7 | 205.8 | 165.1 | 146.4 | 161.7 | 125.9 | 112.3 | 11.76 mg/kg-min |
| | | Rd | 100 | 137.6 | 188.0 | 150.4 | 227.3 | 174.3 | 151.3 | 156.4 | 130.7 | 141.0 | 11.70 mg/kg-min |
| 11 | 23 | C | 100±0.2 | 268.1 | 369.9 | 323.5 | 256.9 | 246.6 | 237.9 | 249.6 | 225.7 | 209.6 | 133.5 mg/100 ml |
| | | Ra | 100 | 298.2 | 328.6 | 153.6 | 169.6 | 136.6 | 148.2 | 155.3 | 101.8 | 97.3 | 11.20 mg/kg-min |
| | | Rd | 100 | 130.6 | 226.6 | 199.1 | 220.4 | 146.6 | 151.0 | 143.1 | 124.4 | 113.6 | 11.25 mg/kg-min |

TABLE 20

Changes in the concentration of plasma FFA (uEq/l) during hypoxia.

| Expt. No. | During ventilation with | | Difference | % of air inhaled during the second period |
|---|---|---|---|---|
| | Room Air | Mixture of $O_2$ and $N_2$ | | |
| 1 | 2218 | 1038 | -1180 | 10% |
| 2 | 1027 | 767 | - 260 | |
| 3 | 1431 | 796 | - 635 | |
| 4 | 933 | 846 | - 87 | |
| 5 | 2589 | 2085 | - 504 | |
| 8 | 2738 | 1513 | -1225 | |
| 10 | 386 | 561 | + 175 | |
| mean = $\pm$ s.e.m. | | | $-531\pm200$* | |
| 6 | 4000 | 2287 | -1713 | 5% |
| 9 | 964 | 649 | - 315 | |
| 11 | 615 | 471 | - 144 | |
| mean | 1859.6 | 1135.6 | - 724 | |

* $t = 2.66$; $n = 7$; $P < 0.05$

## TABLE 21

Number of pups and the adults (mothers) treated with Methyl-prednisolone (MP).

| Dog Number | Litter | Age (days) | Sex | Weight gm. | Treatment |
|---|---|---|---|---|---|
| 1 | 2 | 4 | F | 420 | MP |
| 2 | 2 | 4 | M | 396 | None |
| 3 | 4 | 4 | F | 394 | MP |
| 4 | 4 | 4 | F | 370 | None |
| 5 | 3 | 5 | F | 428 | MP |
| 6 | 3 | 5 | M | 460 | None |
| 7 | 4 | 7 | M | 409 | MP |
| 8 | 4 | 7 | M | 467 | None |
| 9 | 3 | 8 | M | 666 | MP |
| 10 | 3 | 8 | M | 593 | None |
| 11 | 2 | 12 | F | 635 | MP |
| 12 | 2 | 12 | F | 669 | None |
| 13 | 2 | 18 | F | 980 | MP |
| 14 | 2 | 18 | F | 963 | None |
| 15 | 4 | 23 | F | 1142 | MP |
| 16 | 4 | 23 | F | 1297 | None |
| 17 | 2 | 33 | M | 2060 | MP |
| 18 | 2 | 33 | M | 2344 | None |
| 19 | 1 | 33 | F | 1532 | MP |
| 20 | 1 | 36 | F | 2122 | None |
| 21 | 2 | 47 | F | 3348 | MP |
| 22 | 2 | 47 | M | 4524 | None |
| Mother 1 | 3 | Adult | F | 8900 | None |
| " | 3 | " | F | 8200 | MP |
| Mother 2 | 4 | " | F | 18800 | None |
| " | 4 | " | F | 18800 | MP |

TABLE 22

Plasma glucose concentrations, turnover rates of glucose, metabolic clearance rates of glucose, serum IRI and plasma FFA concentrations in control and treated * (methylprednisolone 4 mg/kg for 3 days) animals.

| Dog No. | Age (days) | Mean plasma glucose (mg/100ml) | $R_a = R_d$ (mg/kg-min) | Metabolic Clearance (ml/kg-min) | Mean IRI (uU/ml) | Mean FFA (uEq/L) |
|---|---|---|---|---|---|---|
| 1 * | 4 | 104 | 8.0 | 7.7 | 17.7 | 1842 |
| 2 | 4 | 86 | 5.9 | 6.8 | 13.9 | 1641 |
| 3 * | 4 | 108 | 8.3 | 7.7 | 9.8 | 896 |
| 4 | 4 | 83 | 6.6 | 7.9 | 6.7 | 878 |
| 5 * | 5 | 121 | 7.6 | 6.3 | 13.7 | 1104 |
| 6 | 5 | 109 | 6.7 | 6.2 | 12.2 | 698 |
| 7 * | 7 | 106 | 5.6 | 5.3 | 10.9 | 614 |
| 8 | 7 | 39 | 5.6 | 14.2 | 6.9 | 459 |
| 9 * | 8 | 139 | 7.2 | 5.1 | 55.5 | 1224 |
| 10 | 8 | 127 | 7.3 | 5.8 | 12.8 | 987 |
| 11 * | 12 | 133 | 6.5 | 4.9 | 22.7 | 2070 |
| 12 | 12 | 110 | 5.8 | 5.3 | 11.1 | 864 |
| 13 * | 18 | 141 | 7.9 | 5.6 | 61.1 | 2020 |
| 14 | 18 | 144 | 8.1 | 5.6 | 18.0 | 423 |
| 15 * | 23 | 124 | 4.6 | 3.7 | 29.7 | 624 |
| 16 | 23 | 112 | 3.6 | 3.2 | 9.5 | 391 |
| 17 * | 33 | 130 | 7.8 | 6.1 | 39.1 | 474 |
| 18 | 33 | 108 | 5.0 | 4.6 | 10.9 | 797 |
| 19 * | 33 | 129 | 16.3 | 12.6 | 80.0 | 324 |
| 20 | 36 | 127 | 7.5 | 5.9 | 16.5 | 203 |
| 21 * | 47 | 109 | 7.2 | 6.5 | 41.1 | 627 |
| 22 | 47 | 108 | 3.8 | 3.5 | 13.3 | 659 |
| Mother 1 | adult | 113 | 2.5 | 2.2 | 11.9 | 736 |
| Mother 1 * | adult | 113 | 6.1 | 5.4 | 21.9 | 865 |
| Mother 2 | adult | 95 | 2.2 | 2.3 | 9.2 | 315 |
| Mother 2 * | adult | 100 | 6.9 | 6.9 | 12.2 | 523 |

## TABLE 23

Percent changes in plasma glucose concentration (C), $R_a$ and liver weight of pups after treatment with methylprednisolone.

| Dog # | Age in days | Litter # | $\Delta C$ % | $\Delta R_a$ % | Liver Wt % |
|---|---|---|---|---|---|
| 1, 2 | 4 | 2 | 21.1 | 35.6 | 64.2 |
| 3, 4 | 4 | 4 | 29.7 | 25.4 | 89.3 |
| 5, 6 | 5 | 3 | 12.2 | 13.4 | 39.6 |
| 7, 8 | 7 | 4 | 169.3 * | 0.7 | 68.7 |
| 9, 10 | 8 | 3 | 10.1 | - 1.3 | 92.2 |
| 11, 12 | 12 | 2 | 20.7 | 12.0 | 76.3 |
| 13, 14 | 18 | 2 | - 2.0 | - 2.4 | 57.1 |
| 15, 16 | 23 | 4 | 11.2 | 28.4 | 46.4 |
| 17, 18 | 33 | 2 | 20.4 | 59.4 | 49.9 |
| 19, 20 | 33, 36 | 1 | 1.9 | 118.0 | 49.3 |
| 21, 22 | 47 | 2 | 0.9 | 88.1 | 17.4 |
| Mean ± s.e.m. | | | 12.6 ± 3.27 | | |

TABLE 23 (continued)

| Dog # | Age in days | Litter # | $\Delta$C% | $\Delta R_a$ % | Liver Wt % |
|---|---|---|---|---|---|
| Mother | adult | 3 | 0.2 | 144.9 | — |
| Mother | adult | 4 | 5.9 | 216.1 | — |
| Mean | | | 3.05 | 180.5 | |

* The control pup of the pair (#7 and #8) was exceptionally hypoglycaemic.

TABLE 24

Changes in plasma FFA concentration in pups after treatment with methylprednisolone.

| Dog # | Age (days) | FFA u Eq/liter | % FFA |
|---|---|---|---|
| 1<br>2 | 4 | 201 | 12.2 |
| 3<br>4 | 4 | 18 | 2.1 |
| 5<br>6 | 5 | 406 | 58.2 |
| 7<br>8 | 7 | 155 | 33.8 |
| 9<br>10 | 8 | 237 | 24.0 |
| 11<br>12 | 12 | 1206 | 139.5 |
| 13<br>14 | 18 | 1597 | 377.5 |
| 15<br>16 | 23 | 233 | 59.6 |
| 17<br>18 | 33 | - 320 | - 40.2 |
| 19<br>20 | 34.5 | 121 | 59.6 |
| 21<br>22 | 47 | - 32 | - 4.9 |
| Mean ± s.e.m. | | 347<br>±169 | 66<br>±34 |

TABLE 25

The effect of methylprednisolone on the weight and composition of the liver in pups 4 - 47 days of age.

| | $\frac{\text{Liver wt.}}{\text{Body wt.}}$ .100 | % $H_2O$ in liver | DNA mg/gm | RNA mg/gm | PROTEIN mg/gm | PROTEIN gm/liver | $\frac{\text{RNA}}{\text{DNA}}$ | $\frac{\text{PROTEIN}}{\text{DNA}}$ |
|---|---|---|---|---|---|---|---|---|
| MP Treated | | | | | | | | |
| mean: | 6.43 | 71.7 | 1.56 | 8.50 | 120.4 | 8.59 | 5.86 | 82.62 |
| s.e.m. ± | 0.22 | 0.47 | 0.14 | 0.52 | 8.1 | 2.17 | 0.56 | 8.11 |
| Control (litter mate) | | | | | | | | |
| mean: | 3.77 | 75.9 | 2.64 | 10.16 | 147.6 | 7.29 | 4.11 | 61.91 |
| s.e.m. ± | 0.11 | 0.34 | 0.27 | 0.56 | 5.9 | 2.19 | 0.35 | 7.07 |
| 100.$\frac{\text{Treat-Contr.}}{\text{Contr.}}$ | | | | | | | | |
| mean: | 71.4 | - 5.51 | - 37.9 | - 16.1 | - 18.14 | 30.4 | 45.7 | 32.3 |
| s.e.m. ± | 5.5 | 0.55 | 4.9 | 3.2 | 4.7 | 11.3 | 13.2 | 9.0 |

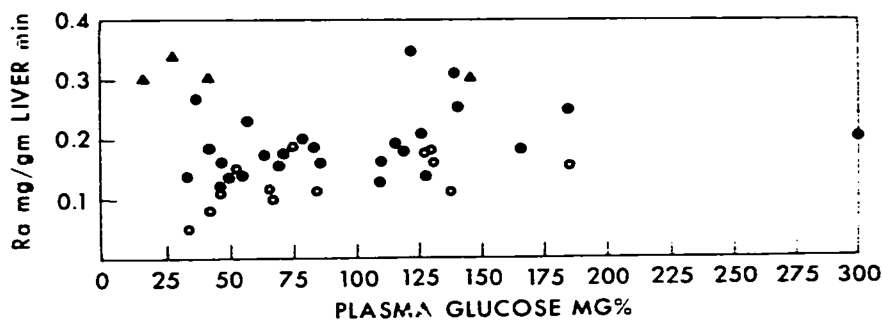


Figure 1 - Rate of hepatic glucose production (mg/g liver per min.) in 43 newborn dogs plotted against plasma glucose concentration (mg/100ml). Beagles 0, mongrels ●, and one mongrel litter ▲ with exceptionally high rates of glucose production.

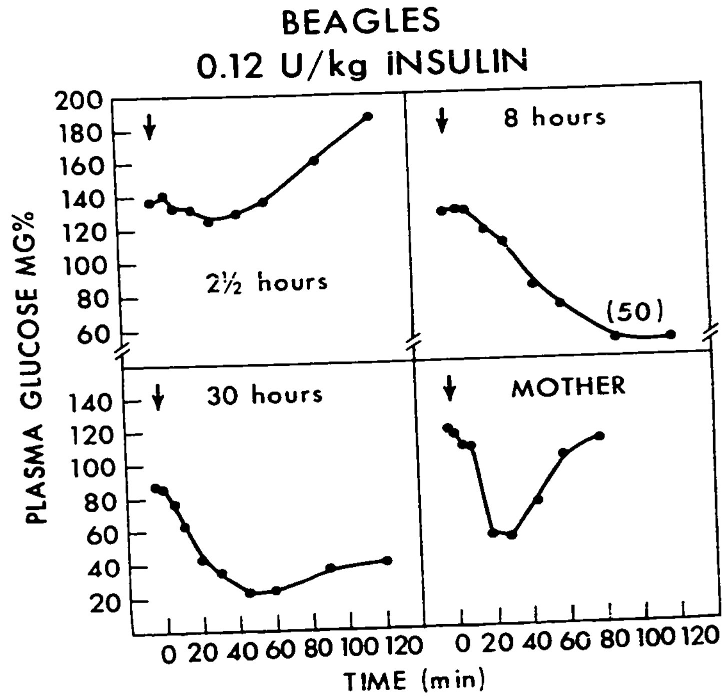


Figure 2 - Changes in plasma glucose concentration (mg/100ml) in newborn beagles $2\frac{1}{2}$, 8 and 30 hours old and in their mother during a period of two hours after intravenous injection of insulin (0.12 U/kg).

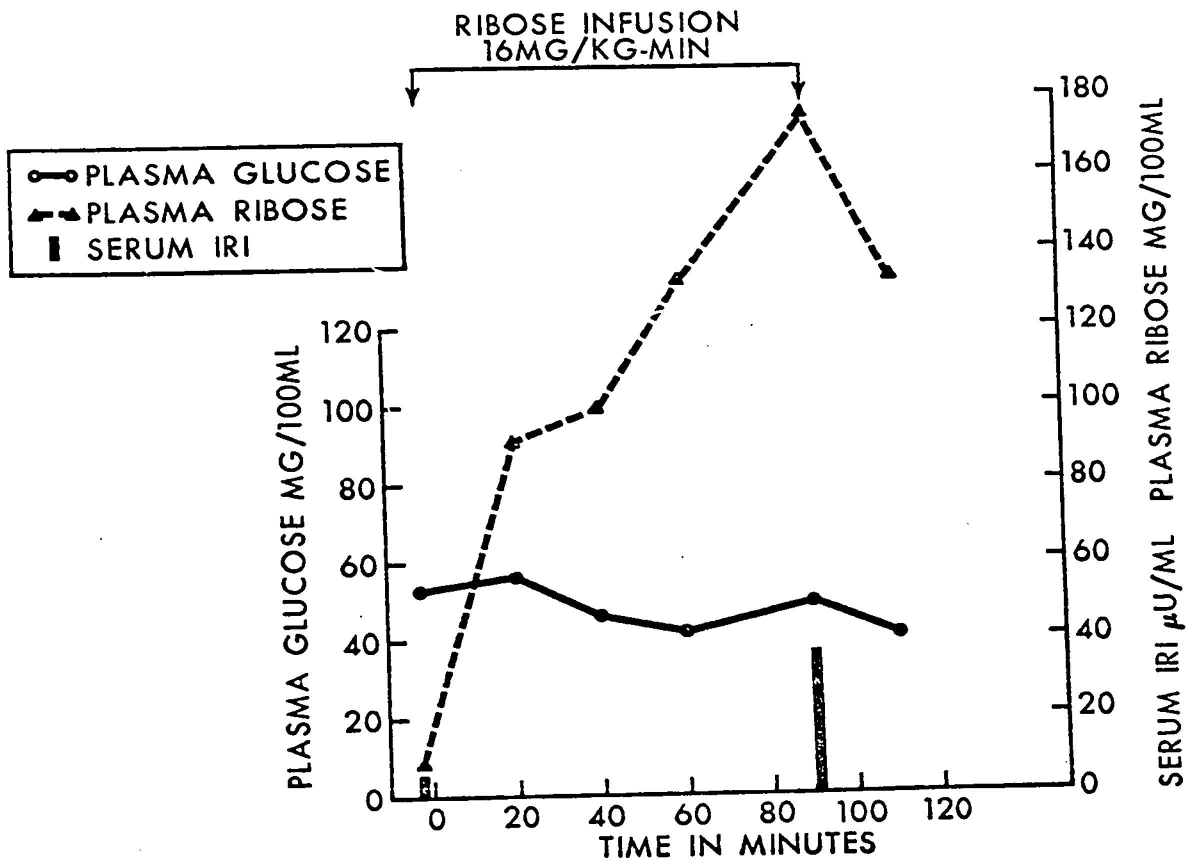


Figure 3 - Changes in concentrations of plasma glucose (mg/100ml), ribose (mg/100ml) and serum IRI (μU/ml) in a 12 hours old beagle during an intravenous infusion of D-ribose (16 mg/kg-min).

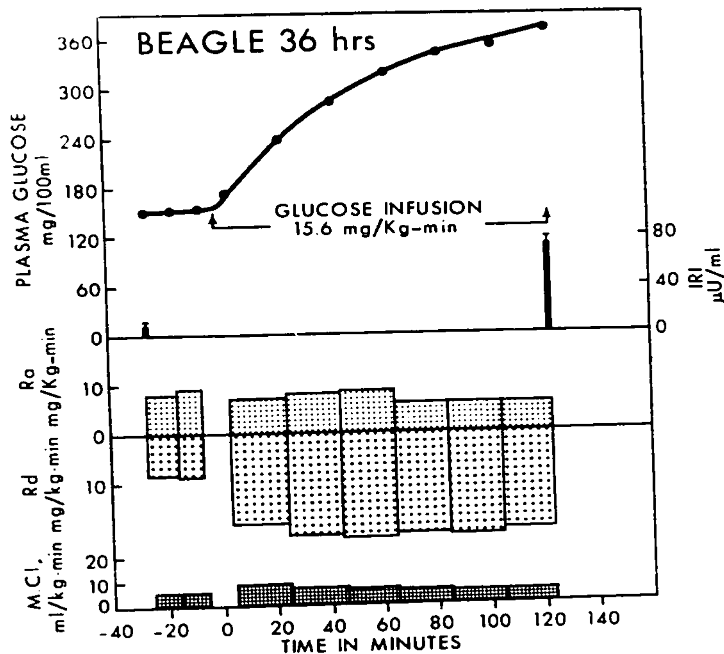


Figure 4 - The concentrations of glucose and insulin in the plasma, and the rates of glucose production and utilization, and the metabolic clearance of glucose during an intravenous glucose infusion (15.6 mg/kg-min) in a 36 hour old beagle pup. Abscissa: time in minutes. On the ordinate on the left side in descending order: plasma glucose concentration (mg/100 ml); rates of glucose production ($R_a$) and utilization ($R_d$) as mg/kg-min, and the metabolic clearance of glucose (ml/kg-min). Concentration of plasma insulin (IRI, µU/ml) is shown on the right side ordinate. On the upper panel: glucose concentration is shown together with plasma IRI (black columns). Standard errors of parallel determinations are indicated. The duration of the infusion is indicated by the horizontal line between arrows. On the lower panel $R_a$ is shown as columns above zero, $R_d$ as columns pointing downwards below zero. Columns on the bottom depict the metabolic clearance of glucose.

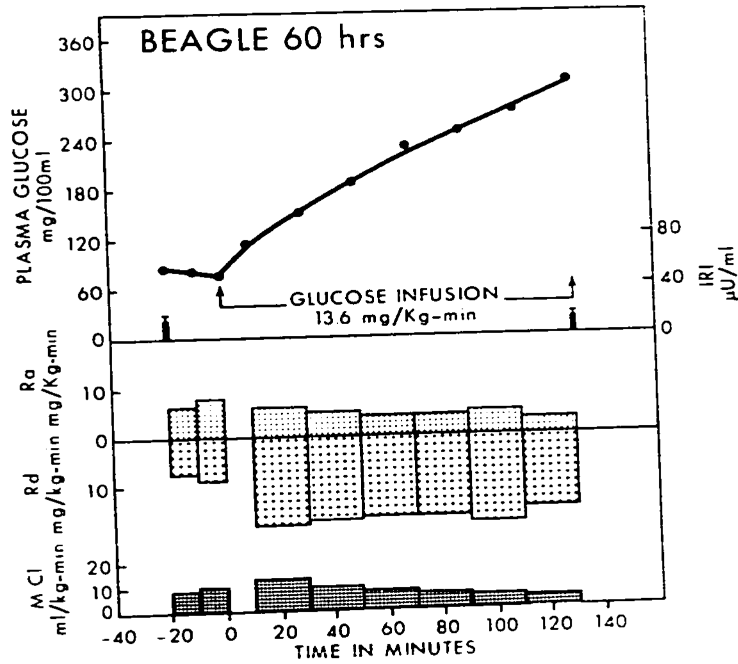


Figure 5 - The concentrations of glucose and insulin in the plasma, and the rates of glucose production ($R_a$) and utilization ($R_d$), together with the metabolic clearance of glucose during an intravenous infusion of glucose (13.6 mg/kg-min) in a 60 hour old beagle pup. For details see legend to Figure 4.

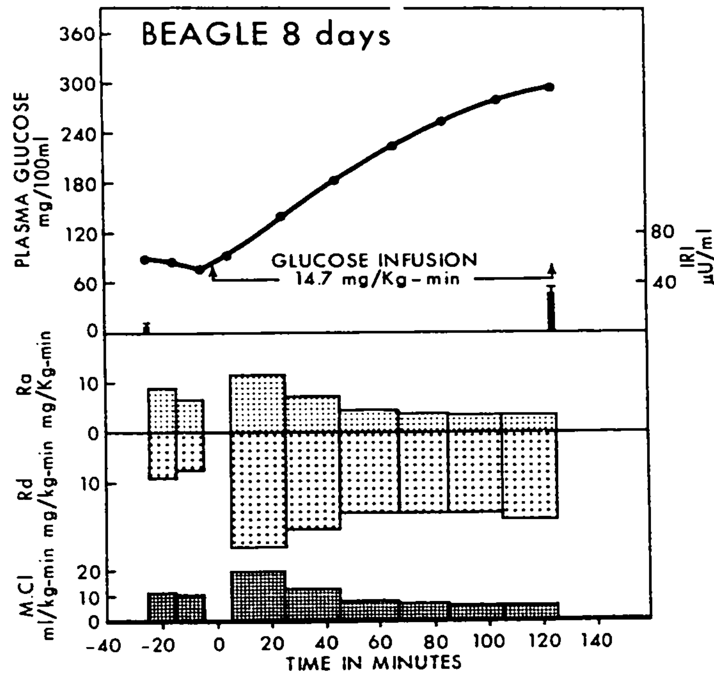


Figure 6 - The concentrations of glucose and insulin in the plasma and the rates of glucose production ($R_a$) and utilization ($R_d$), together with the metabolic clearance of glucose during and intravenous infusion of glucose (14.7 mg/kg-min) in an 8 day old beagle pup. For details see legend to Figure 4.

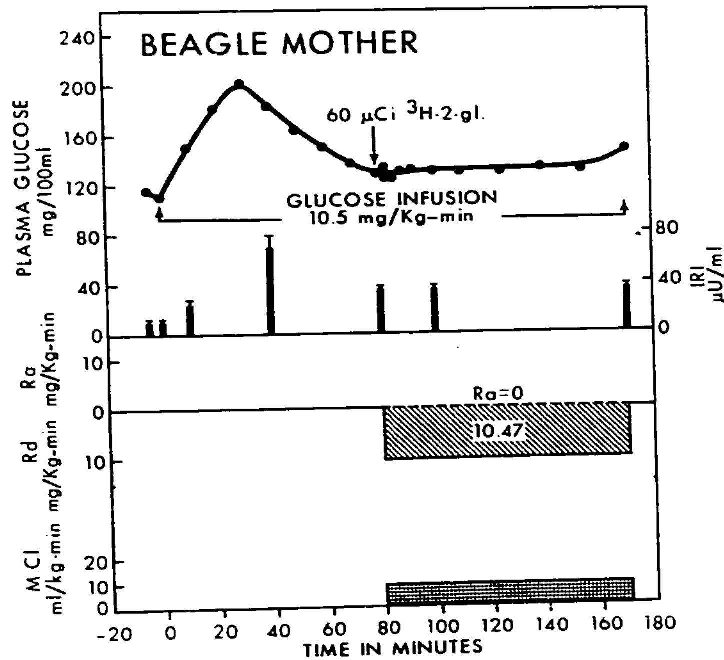


Figure 7 - The concentrations of glucose and insulin in the plasma during an intravenous infusion of glucose in an adult beagle (mother). Time (min.) is shown on the abscissa. Rates of (endogenous) glucose production ($R_a$) and utilization ($R_d$) together with the metabolic clearance of glucose were determined during the steady state period between t = 80 and t = 170 min. by a tracer injection method. Glucose and insulin concentrations are shown on the upper panel, rates on the lower one.

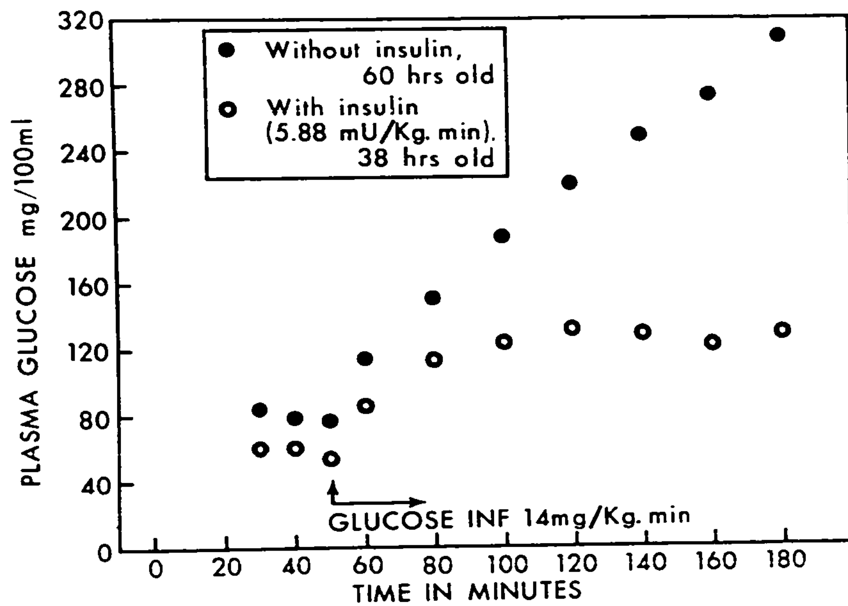


Figure 8 - Plasma glucose concentrations before and during the infusion of 14 mg/kg-min. glucose with or without extra insulin.

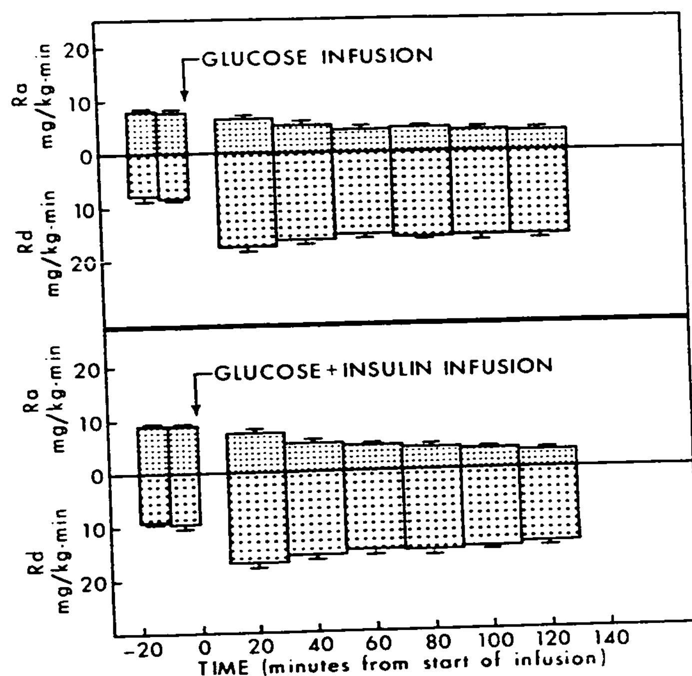


Figure 9 - The effect of an infusion of glucose (8.3-20.8 mg/kg-min) or of glucose plus insulin (9.5-14.0 mg/kg-min. plus 560-5883 μU/kg-min) on the rates of glucose production and utilization in beagle pups (0.5-55 days of age). The columns represent the mean of 20, and 7 experiments respectively. Abscissa: time in minutes. Ordinates: endogenous glucose production ($R_a$) and utilization ($R_d$). Rates are shown as columns above ($R_a$), or below ($R_d$) the zero line. Standard errors of the mean rates are indicated by lines on the top or bottom of the columns.

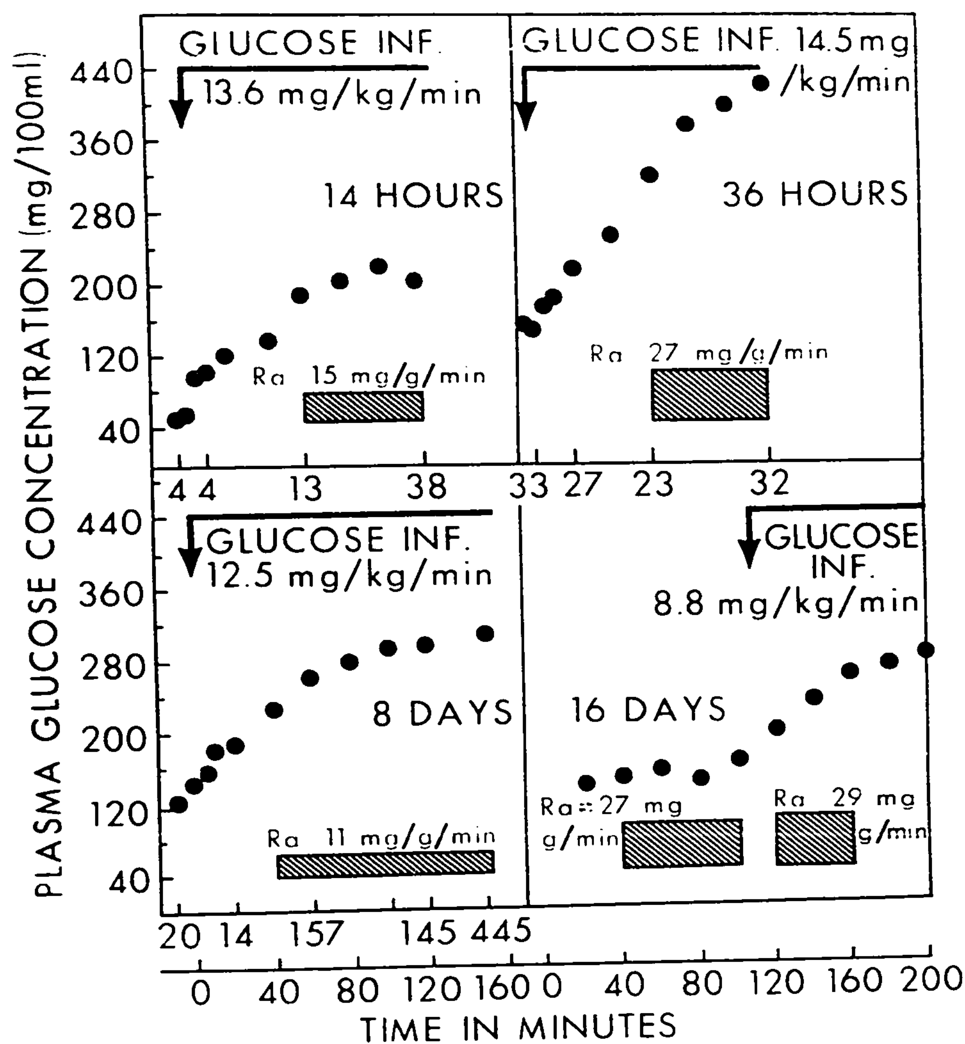


Figure 10 – The concentrations of glucose and insulin in the plasma, and the rates of glucose production during an intravenous infusion of glucose in 4 mongrel pups aged 14 hours, 36 hours, 8 days and 16 days. Abscissa: time in minutes. The numbers on the inner side of the abscissa indicate values of insulin (µU/ml). On the ordinate: plasma glucose concentration (mg/100ml). The shaded blocks show the rates of glucose production (mg/gm liver-min).

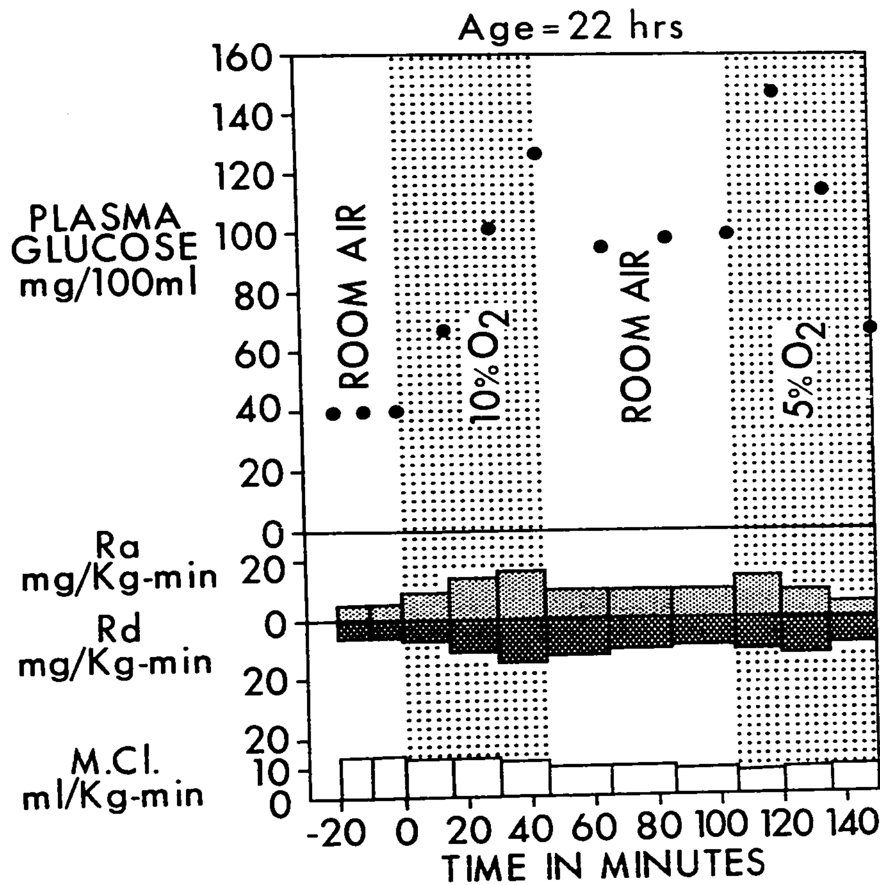


Figure 11 - The effect of hypoxia on the concentration of glucose in the plasma and the rates of glucose production ($R_a$) and disappearance ($R_d$) in a 22 hour old beagle pup. Upper ordinate: plasma glucose concentration, mg/100 ml. Middle ordinates: glucose production ($R_a$) and disappearance ($R_d$) as mg/kg-min. $R_a$ pointing upward, $R_d$ downward from a common line representing zero. Lower ordinate: the metabolic clearance of glucose as ml/kg-min. Abscissa: time in minutes. Shaded vertical bands indicate periods of exposure to a gas mixture containing 10% and 5% $O_2$. Black dots (•): glucose concentration. Histogram bars in the middle panel $R_a$ and $R_d$. Open histogram bars in lower panel: metabolic clearance.

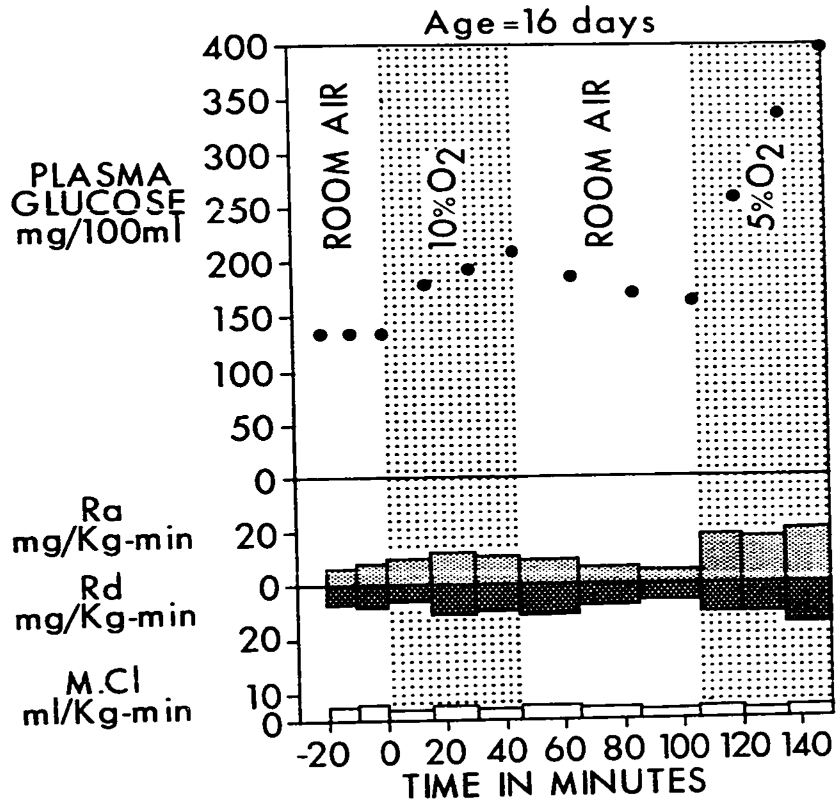


Figure 12 - The effect of hypoxia on the concentration of glucose in the plasma and the rates of glucose production ($R_a$) and disappearance ($R_d$) in a 16 days old beagle pup. Abscissa, ordinates and symbols as in Figure 11.

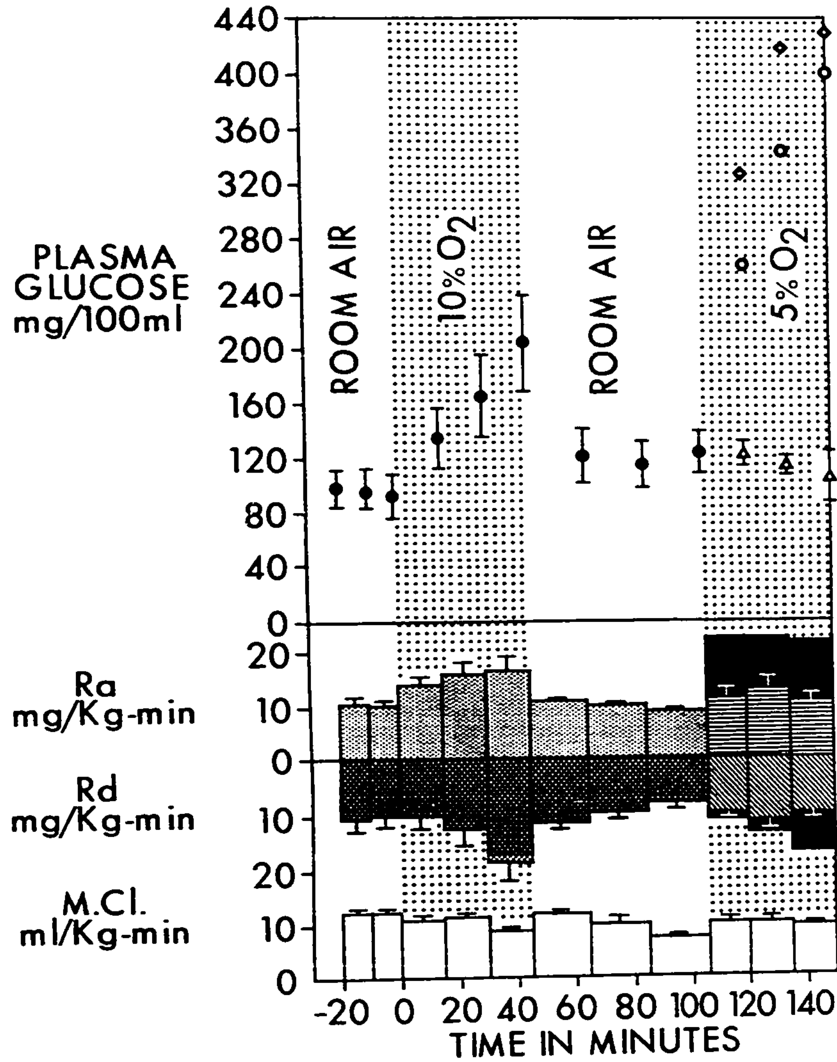


Figure 13 - Mean values and their standard errors of plasma glucose concentration, $R_a$, $R_d$ and Metabolic clearance in the six beagle pups exposed to 10% $0_2$ in the second and to 5% $0_2$ in the fourth period. During the last hypoxic period the triangles (▲) represent mean values for the four pups under 10 days of age while the open circles (o) and diamonds (◇) represent the two older pups respectively. In the histograms representing $R_a$ and $R_d$ in the last period smaller, lighter bars with their associated s.e.m. are for the four younger pups while the larger black bars represent the resp. means for the two older pups.

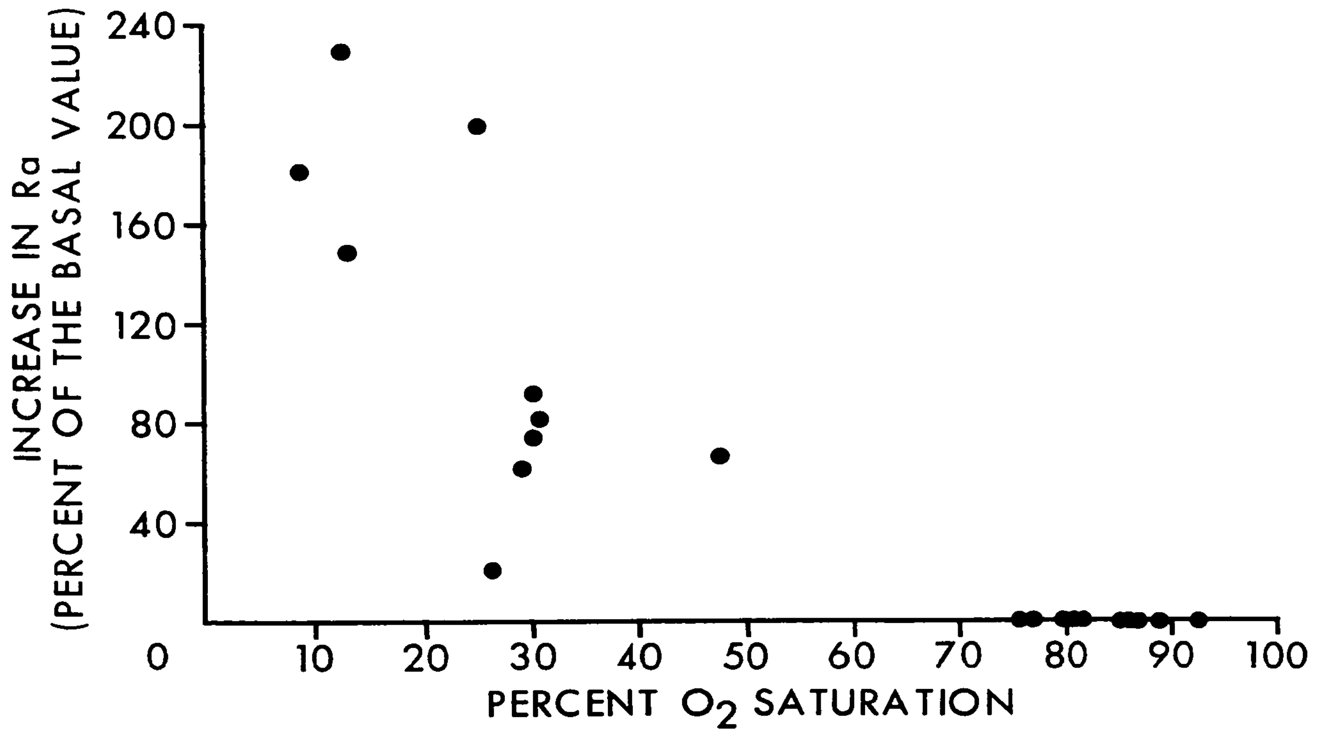


Figure 14 - Percent increase in the rate of glucose production ($R_a$) of glucose above values determined in the control period plotted against $O_2$ saturation (%) in arterial blood. Dots on the right side of the abscissa indicate $O_2$ saturation during the control period.

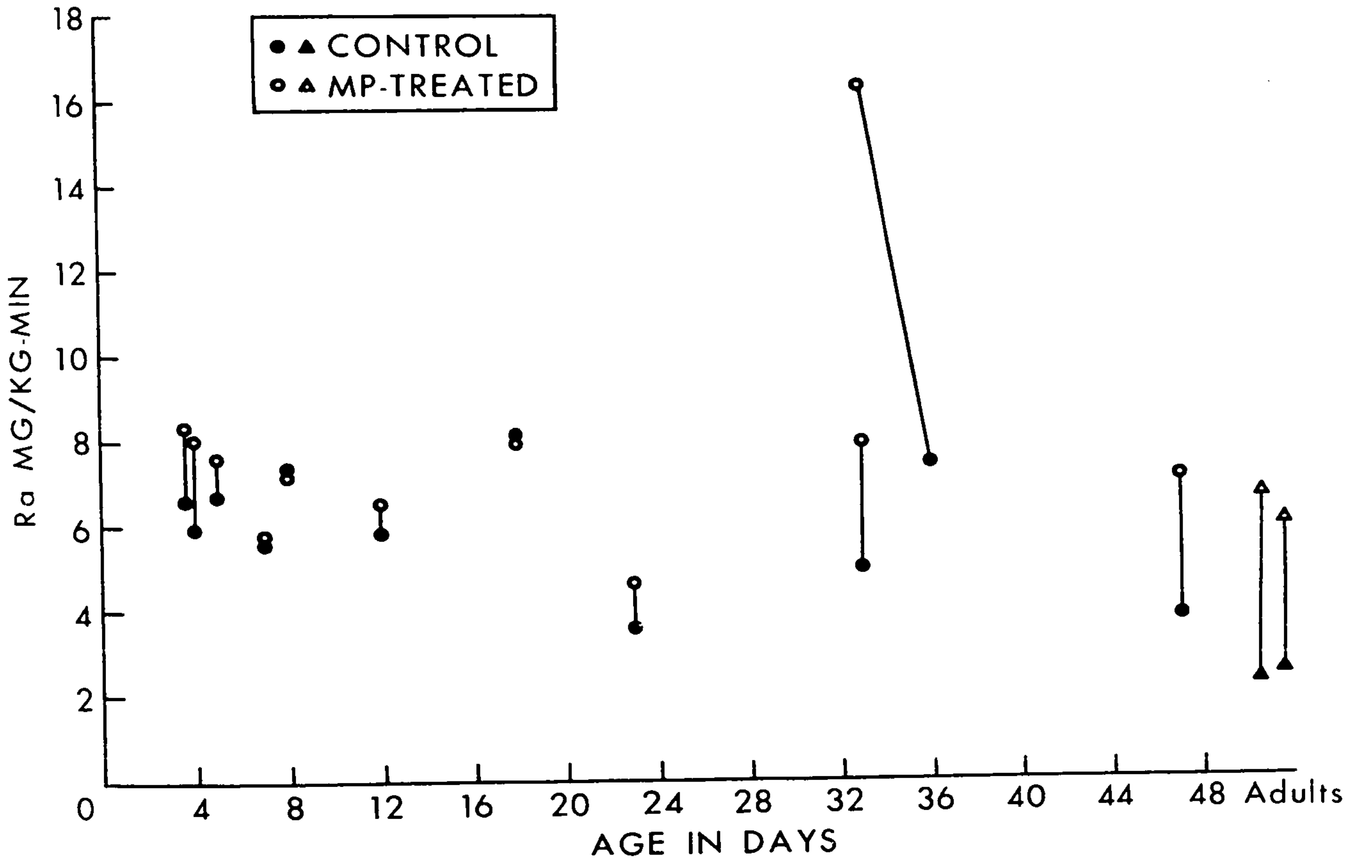


Figure 15 - Effect of a three-day treatment with MP (4 mg/kg-min) on the turnover of glucose in pups of different ages and two adult dogs.
Abscissa: Age in days. Ordinate: Rate of glucose production (= glucose turnover) as mg/kg-min. White circles: pups treated with MP; black circles: their respective closely matched litter mate. Black and white triangles: rate of glucose production in the adult dogs before and after MP treatment respectively.

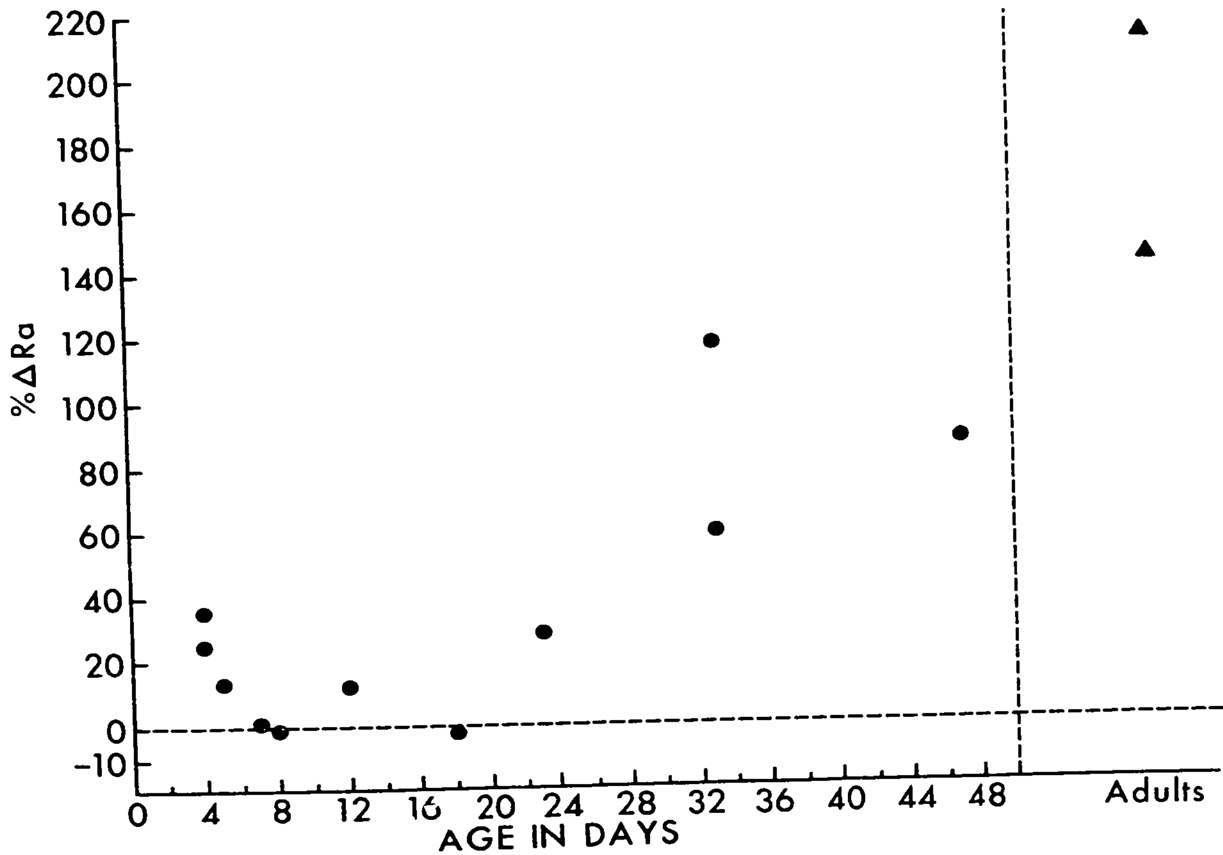


Figure 16 - The percent difference in glucose production ($R_a$) between MP treated pups and their closely matched litter mates. Abscissa: age of pups in days. Ordinate: percent increase in $R_a$ in the MP treated compared to its closely matched litter mate. Triangles refer to adult dogs and show percent increase in $R_a$ after treatment with MP.

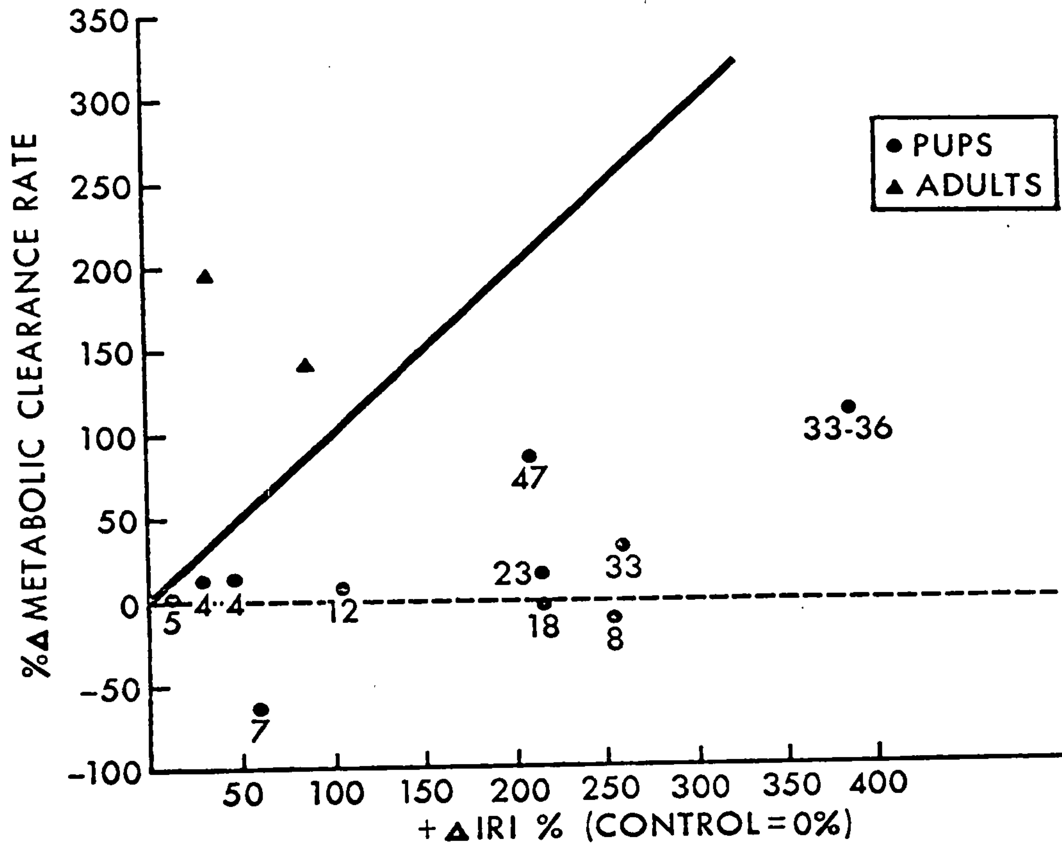


Figure 17 - The percent increase in plasma IRI plotted against percent increase in the metabolic clearance of glucose in MP treated pups compared to their closely matched litter mates. Triangles refer to adult dogs and show percent increases in the parameters after the treatment with MP. The numbers associated with points refer to the age (days) of pups.

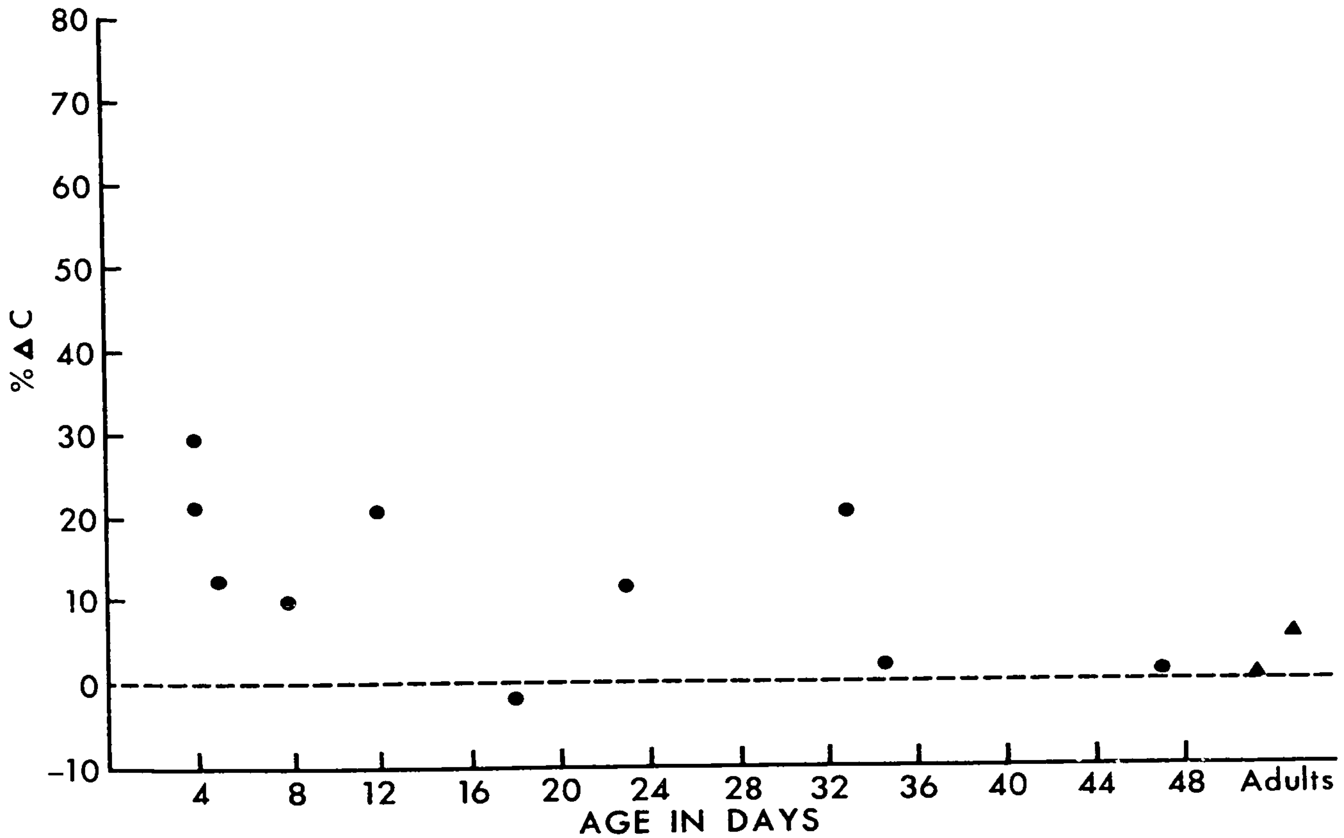


Figure 18 - The percentage change in plasma glucose concentration in pups at different ages (●) and in two adults (mothers) (▲) after a 3 day treatment with methylprednisolone (4 mg/kg-day).

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## APPENDIX

Sample times for 4 samples resulting in minimum error in calculation of area beneath a double exponential curve - Specific Activity (S.A.) versus time, as predicted from Corney and Heath technique. Sample times given for typical mean blood sugars ($\bar{c}$) based on previously determined correlation between $\bar{c}$ and S.A. curves.

| $\bar{c}$ mg/100 ml. | TIME OF SAMPLE IN MINUTES | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 20 | 5.4 | 19.0 | 38.8 | 84.0 |
| 30 | 5.6 | 19.8 | 41.3 | 87.6 |
| 40 | 5.9 | 20.6 | 43.1 | 91.4 |
| 50 | 6.1 | 21.5 | 45.0 | 95.3 |
| 60 | 6.4 | 22.5 | 46.9 | 99.4 |
| 70 | 6.6 | 23.4 | 48.9 | 103.8 |
| 80 | 6.9 | 24.5 | 51.1 | 108.2 |
| 90 | 7.2 | 25.5 | 53.3 | 113.0 |
| 100 | 7.6 | 26.6 | 55.6 | 117.8 |
| 120 | 8.2 | 29.0 | 60.4 | 128.2 |
| 140 | 8.9 | 31.5 | 65.8 | 139.6 |
| 160 | 9.8 | 34.3 | 71.6 | 151.9 |
| 180 | 10.6 | 37.4 | | |
| 200 | 11.6 | 40.7 | | |
| 250 | 14.3 | 50.3 | | |
| 300 | 17.7 | 62.2 | | |

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