REGULATION OF GLUCONEOGENESIS IN NEWBORN AND ADULT DOGS

by

Susan E.H. Hall, B.Sc.

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Ph.D. in Physiology

UNIVERSITY OF OTTAWA

OTTAWA, CANADA, 1975

ACKNOWLEDGEMENTS

This work was carried out under the supervision of Professor G. Hetenyi Jr. who provided an enjoyable and stimulating research environment, which was greatly appreciated.

Dr. Jean Himms-Hagen acted as a co-supervisor and the author is indebted to her for time spent in interesting and informative discussion. Dr. M. Vranic from the University of Toronto is also thanked for information and his involvement with parts of this work.

Some of the data analysis was carried out in the Department of Theoretical Biology at the National Institute of Health, Bethesda, U.S.A., under the supervision of Dr. Mones Berman. Thanks are extended to Dr. Berman and his colleagues, both for their hospitality, and the introduction to compartmental modelling.

The technical advice of Mr. Ross Layberry and his expert assistance, together with that of Mrs. Ileana Popescue and Mrs. Isabel Barnes is most gratefully acknowledged. Mrs. Doreen Dodge did an expert job in typing the thesis.

Particular thanks are due to Alan J. Hall, P. Eng. for his professional advice and encouragement.

The generous financial support of this work by the Medical Research Council is gratefully acknowledged.

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GLUCOSE HOMEOSTASTIS IN THE NEWBORN

Hypoglycaemia is extremely common in the newborn of most mammalian species, including man (155). This is a reflection of their inability to regulate the blood glucose concentration, a factor which is finely and precisely controlled in the adult. Any alteration of the concentration of glucose in the blood in the adult animal is compensated for by a prompt and appropriate change in hepatic glucose production and peripheral utilization (76).

The apparent absence of a glucose regulatory mechanism in the newborn may be due either to a lack of sensitivity to hypoglycaemia as a stimulus or to the inability to respond to that stimulus by increasing hepatic glucose production.

Varma et al (181) demonstrated that in response to hypoxia, the pup is capable of increased glucose production, at least in the short term, with a resultant elevation of the plasma glucose concentration. Thus it appears that all the necessary "enzymic machinery" required for glucose production is fully operative from birth.

Towards the end of gestation, the foetus' of all mammalian species, so far investigated rapidly synthesize and store glycogen in the liver, heart and skeletal muscle (154). However muscle glycogen is only available locally providing glucose for the working tissue. The hepatic glycogen concentration at term is approximately twice that of the post-absorptive adult and this glycogen is rapidly depleted after birth (154).

This lack of glycogen combined with an extremely low dietary

glucose intake threaten glucose homeostasis in the newborn..

If the newborn mammal is to be capable of maintaining any constant and suitable level of glucose in the blood, it has to rely on gluconeogenesis.

PATHWAYS OF GLUCONEOGENESIS AND THEIR CONTROL

Gluconeogenesis is defined by Krebs as the new formation of carbohydrate from non carbohydrate sources (98). In recent years there has been a resurgence of biochemical interest in the process of gluconeogenesis in the mammalian liver and kidney; in the quantitative significance of glucose synthesis in the intact animal and in the control of glucose synthesis. The development of the process during foetal life has been extensively studied and it has been shown both in vitro and in vivo that the capacity for gluconeogenesis in the rat liver is acquired only after birth, the foetal liver being relatively inactive in this synthetic system (8, 9, 142, 200). Recently the synthetic pathways have been elucidated.

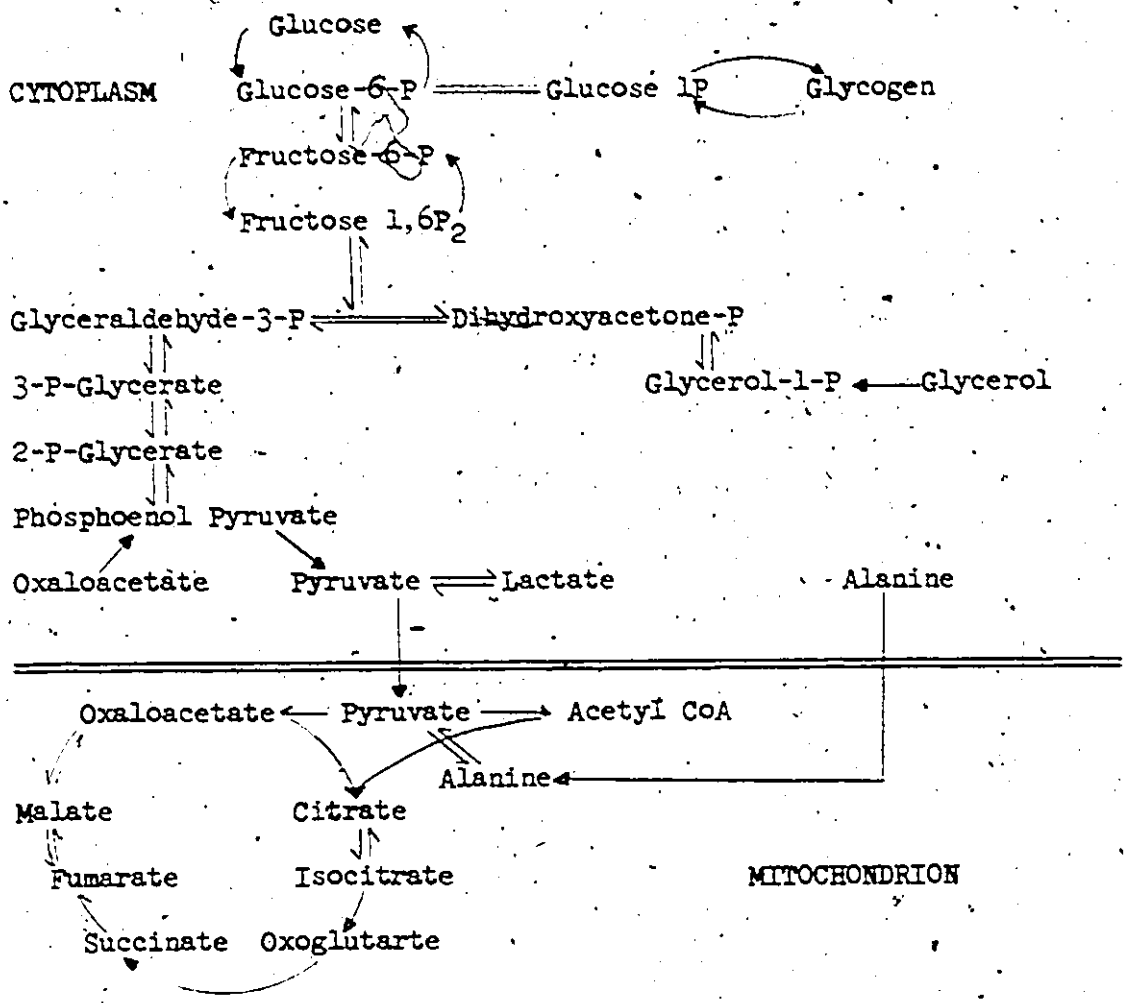
Fig. 1 shows the sequence by which lactate pyruvate, glycerol and the major amino acids are converted to glucose in the liver and kidney cortex. Nearly all investigation in this area has been into the enzymes and the metabolic pathways they form in the adult animal. Until more detailed knowledge is forthcoming it is assumed that the metabolic sequences and the enzymes which regulate them are the same in the newborn animal.

Many of the glucogenic reactions are identical with those of glycolysis; such reactions are always close to equilibrium so that the rate and direction of flow of residues can be changed by variations in the concentration of substrates and/or products of these reactions.

On the other hand, certain key reactions of glycolysis are non equilibrium reactions, enabling net conversion of glucose to pyruvate or

Fig. 1

The pathways of Glycolysis and
Gluconeogenesis in Liver and Kidney Cortex



From Regulation in Metabolism Newsholme E.A. and Start C.

Publisher John Wiley and Sons 1973.

net formation of glucose from gluconeogenic precursors to occur at any time. These reactions are predominantly unidirectional, it being thermodynamically unfavourable for their reversal (97, 81).

The important glycolytic reactions which have to be bypassed in the gluconeogenetic pathway are those reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.

The reactions catalyzed by hexokinase and phosphofructokinase are both bypassed by hydrolytic cleavage of the phosphate ester bond. Glucose-6-Phosphate is hydrolyzed to glucose and inorganic phosphate, a reaction catalyzed by glucose-6-phosphatase. The activity of this enzyme increases markedly at birth (33, 10) and its activity is increased by glucocorticoids, diabetes and fasting. However its importance as a major control site in gluconeogenesis is doubtful. Fructose 1:6 diphosphate is hydrolyzed to fructose-6-phosphate and inorganic phosphate, this reaction being catalyzed by fructose 1:6 diphosphatase. Adenosine monophosphate (AMP) in low concentration and the substrate fructose 1:6 diphosphate are negative allosteric modifiers of the enzyme (171, 131). This inhibition can be removed by accumulation of the precursors of gluconeogenesis such as lactate, malate or the α-oxoacids arising from amino acids which all accelerate synthesis of ATP and hence remove AMP (98). Control of glucoenogenesis through this reaction is also exercised by inhibition of the regulatory antagonist glycolytic enzyme phosphofructokinase. The allosteric modification of this enzyme has been discussed extensively by Mansour (120).

The pyruvate kinase bypass consists of two separate reactions. Pyruvate is first carboxylated to oxaloacetate in a reaction involving ATP hydrolysis and catalyzed by pyruvate carboxylase. Acetyl CoA is an allosteric activator of this enzyme and Utter et al have argued that regulation of gluconeogenesis can effectively be exerted at this stage with acetyl CoA as a controlling factor (176,177). Support for this hypothesis has come from the work of Krebs et al (99). Hepatic acetyl CoA level is increased in many situations associated with enhanced gluconeogenesis, such as starvation, insulin deficiency and corticosteroid treatment (103). Similarly it has been suggested that the observed stimulation of gluconeogenesis by long chain fatty acids is also brought about by increased amounts of acetyl CoA derivatives affecting pyruvate carboxylase (100). However many other effectors in addition to acetyl CoA have been reported to influence pyruvate carboxylase in vitro. These include ADP, ATP, Ca^{2+}, Mg^{2+}, pyruvate, aspartate and various CoA esters. It is difficult however to resolve the extent to which, if any, these are influential in vivo (153).

The second step in the bypass of the pyruvate kinase reaction is the conversion of oxaloacetate to phosphoenolpyruvate which involves a decarboxylation and phosphate transfer from either G.T.P. or I.T.P. and which is catalyzed by phosphoenolpyruvate carboxykinase (P.E.P.C.K.). Exton et al suggested regulation of this enzyme either directly or indirectly by cyclic 3'5'-Adenosinemonophosphate (cAMP) (41) but there have been no reports that this enzyme exists in interconvertible forms, capable of allosteric modification. The activity of this enzyme is

increased by glucocorticoids, glucagon, lactate and fasting (159). The effects of glucagon are abolished by agents which block protein synthesis (132). It is thus generally assumed that control of this enzyme is wrought by its de novo synthesis.

Alternatively pyruvate can enter the gluconeogenetic pathway via its conversion to acetyl CoA. This reaction is catalyzed by the regulatory allosteric enzyme pyruvate dehydrogenase (144). Regulation of gluconeogenesis by this enzyme is achieved by competition for pyruvate with pyruvate carboxylase.

All these regulatory enzymes are sensitive to a wide range of intracellular metabolites; thus providing a comprehensive integrated mode of control of the glycolytic and gluconeogenetic pathways.

GLUCONEOGENESIS FROM GLYCEROL

Early in this century Cremer and Lüthje reported that exogenous glycerol increases the glycosuria of diabetic dogs (31, 116). Subsequently it was shown that administration of glycerol causes hyperglycaemia (187, 55), increases the liver glycogen content (24) and counteracts insulin-induced hypoglycaemia, as rapidly and as effectively as glucose (186).

These early studies indicated that the adult mammal is able to convert glycerol into glucose and glycogen. More recently using radioactively labelled glycerol this has been confirmed *in vivo* in the adults of a variety of mammalian species including man (197, 133, 18). It is generally regarded that glycerol-C is a minor contributor to de novo glucose synthesis (44). However carbon, capable of being converted to glucose can be stored in the form of glycerol-C in triglyceride, thus providing an important homeostatic C reserve.

Steele *et al* found that in the grown dog the fraction of glucose-C derived from glycerol is related to the concentration of glycerol in the plasma, within the physiological range (196). This relationship also exists in man (18). During fasting in the adult man or dog lipolysis increases and glycerol becomes quantitatively of more importance as a glucogenic precursor (166, 18, 138).

The enzyme glycerokinase which catalyzes the phosphorylation of glycerol to glycerol-1-phosphate is apparently induced after birth

by the appearance of a sufficient concentration of its substrate glycerol (192).

During gestation triglyceride accumulates in adipose tissue, and at term approximately 5% of the body weight is accounted for by fat (157). After birth the neonate rapidly mobilizes this stored triglyceride, releasing glycerol and free fatty acids into the plasma (140, 179, 134).

This high rate of lipolysis combined with a high dietary fat intake provide a considerable amount of glycerol available for gluconeogenesis.

GLUCONEOGENESIS FROM LACTATE AND OTHER TRICARBON UNITS

In 1931 Cori demonstrated that lactate derived from glucose catabolism in extrahepatic tissues contributes to hepatic glucose synthesis (27); thus forming a cycle which now bears his name. It is now realized that recycling of carbon units originating as glucose-C return to glucose not only via lactate but also through other tricarbon intermediaries such as pyruvate and alanine.

Dunn et al using $^{3}H^{6}$-glucose simultaneously with U-^{14}C-glucose have reported that 20 - 25% of total glucose production in rats occurs via such recycled tricarbon units (38). In sheep, however, only 5 - 11% of glucose production arises from recycled tricarbon compounds and no difference is found between fed and fasted animals (13). Issekutz et al found in dogs that 30% of the glucose production could be accounted for by carbon recycled through these intermediates, this figure rising to 67% after treatment with methyl prednisolone (83). In man it appears that recycling of ^{14}C is greatly increased during starvation (204).

The extent of recycling of carbon via the Cori cycle in the newborn has not been evaluated quantitatively. However injected lactate ^{14}C results in the rapid appearance of labelled carbon as circulating glucose and in hepatic glycogen in the newborn rat (184) and lamb (193). Helmrath et al determined in vitro gluconeogenic rates from lactate in liver tissue from pigs 1, 5 or 24 days old. They concluded that although

liver slices from 1 day old piglets possessed the capability to convert lactate to glucose, glucose was generated at only one third of the rate found in slices from older piglets (73). Experiments conducted by Martin et al confirm these findings but conclude that maximum development of gluconeogenic capability is prevented by metyrapone induced cortisol deficiency (123).

Formation of glucose carbon from lactate carbon alone via the Cori cycle constitutes a re-formation rather than a new formation of glucose. Thus unless tricarbon units participating in such a cycle are themselves derived from non carbohydrate sources, their conversion to glucose can not represent a net gain of glucose-C for the animal and can not therefore be considered as gluconeogenesis in the light of the accepted definition.

GLUCONEOGENESIS FROM AMINO ACIDS AND THE ALANINE CYCLE

Almost all amino acids are potentially glucogenic although only alanine, serine, threonine and glycine give rise to significant amounts of glucose in the perfused liver (148). In fact it is probable that gluconeogenesis is the major route of metabolism for these amino acids.

There is much evidence that alanine is the major amino acid involved in gluconeogenesis. In intact man administration of ^{14}C alanine is rapidly followed by its recovery in blood glucose (51). It is taken up by the splanchnic bed in vivo (48) and by the perfused liver in vitro (56, 118) to a greater extent than any other amino acid. Its output from muscle exceeds that of all other amino acids, even though alanine comprises less than 10% of the amino acid residues in muscle protein (96, 49). An important role has been assigned to muscle in the degradation of branched chain amino acids (valine, isoleucine), which form a considerable proportion of the amino acid content of protein. These amino acids are degraded to glutamate which is either transaminated to form α ketoglutarate and alanine or aminated to form glutamine (132). Thus muscle tissues release considerable amounts of both alanine and glutamine. Alanine is transported to the liver and in this way becomes available for de novo glucose synthesis (49). In marked contrast to alanine, extraction by the gut accounts for a substantial portion of splanchnic glutamine uptake (53), thereby reducing the availability of glutamine for gluconeogenesis.

How actively such a catabolic process occurs in the newborn is unknown. Although, at birth there is a definite redistribution in the ratio of the combined plasma concentration of non-essential (glycine + serine + glutamate + taurine) to that of essential amino acids (leucine + isoleucine + valine + methionine) (125). Apparently this redistribution results in an increase in the relative concentration of the glucogenic amino acids, paralleling the postnatal changes occurring in carbohydrate and fat metabolism (126).

Cahill has postulated the existence of a glucose-alanine cycle, analogous to the Cori cycle for lactate (49). In such a cycle alanine would serve to convey amino groups and carbon substrate from muscle to liver for conversion to urea and glucose respectively and would be resynthesized in muscle by transamination of pyruvate, derived from glucose. Operation of such a cycle however would not result in a net gain of new glucose by the organism. Therefore unless alanine is derived from other amino acids its conversion to glucose does not represent gluconeogenesis. In the normal human infant there appears to be no shortage of circulating plasma alanine carbon available for conversion to glucose (109). Although the function or even the existence of the glucose alanine cycle has not been established.

ALANINE AND GLUCONEOGENESIS IN PROLONGED STARVATION

The classical studies of Benedict in 1915 revealed that prolonged starvation is characterized by a progressive decline in the rate of protein catabolism (12). More recently Owen et al found that urinary nitrogen fell from 12 - 15 g/day during the first week of fasting to less than 5 g/day after five to six weeks starvation (138).

In prolonged fasting the plasma concentrations of most amino acids ultimately decline but the rate and magnitude of the decrease in alanine concentration exceeds that of the other amino acids, falling to 70% of its original value after a five to six week fast (48,2). Felig et al reported that in man, after a fast of this length, the output of alanine from muscle fell considerably, while still remaining the major amino acid released. They therefore attributed this lack of circulating alanine to diminished output from muscle (63). Fractional extraction by the splanchnic bed, however, continues at rates comparable to the postabsorptive state (48).

Conservation of protein limits substrate (alanine) availability and hence would be expected to reduce gluconeogenesis. Concomitant with this sparing action of protein in prolonged fasted man the glucose turnover is markedly curtailed (48, 54). Similarly in the dog, Cowan et al reported a reduction in the rate of glucose turnover to 65% of the post-absorptive value after a prolonged fast (29).

Apparently despite reduction in plasma insulin and an elevation

in plasma glucagon, gluconeogenesis from alanine is limited during starvation by lack of available substrate, resulting in a diminution in the rate of glucose synthesis.

Studies by Lindblad in human babies, both in those small for gestational age and those in lower socio-economic groups of a refugee area in Karachi, found that at four hours of age there was a significant increase of the alanine levels compared with normals of the same age (110). Similarly, studies on starvation either by experimental restriction of protein or in the clinical syndrome Kwashiorkor also show significantly higher levels of plasma alanine than normal (80, 1, 6, 161, 169). However Kwashiorkor is usually associated with a relatively high dietary carbohydrate intake and such simulated conditions in the adult also result in high circulating levels of alanine. Evidence concerning alanine and gluconeogenesis in starvation in the newborn is lacking and the area is still largely unresearched.

Until more quantitative approaches are applied to this problem, it is extremely difficult to differentiate between possible causes of a reduction in plasma alanine concentration; whether it is primarily due to lack of glucose for operation of the glucose alanine cycle or whether in fact the reduction can be wholly accounted for by diminished protein turnover.

As death occurs in severe malnutrition after loss of one-third to one half of total body protein (63), it is certainly advantageous that man's adaptive mechanisms should be predominantly concerned with minimizing dissolution of total body protein stores.

HORMONES AND GLUCONEOGENESIS

Many diverse hormones, including glucocorticoids, catecholamines and growth hormone exert a powerful action on metabolism in general and gluconeogenesis in particular. Two of the most important metabolically active hormones are the pancreatic hormones insulin and glucagon, secreted by the β and α cells respectively. The action and control of insulin and glucagon are complexly and inextricably linked and there follows here a brief review of their known actions specifically on gluconeogenesis in both the adult and newborn animal.

Insulin and Gluconeogenesis

The overall metabolic effect of insulin is that of an anabolic hormone, since it does under appropriate conditions stimulate glycogen synthesis, lipogenesis and protein synthesis from glucose and amino acids (61). Moreover, in the absence of hypoglycaemia, insulin suppresses glucogenesis from all gluconeogenic precursors and also inhibits glycogenolysis.

Insulin appears to limit gluconeogenesis from glycerol, largely by decreasing substrate availability. Intravenous infusion of insulin into anaesthetized rats causes a decline in plasma glycerol concentration (78). This reduction in circulating glycerol is a reflection of reduced lipolysis and increased formation of triglyceride in adipose tissue (107), probably mediated by a direct effect of insulin on the adipocyte (61).

Long before the demonstration of a stimulating effect of insulin on protein synthesis by Forker et al in 1951 (58), it was well

recognized that insulin lowered the amino-N concentration in blood (115). If hypoglycaemia is allowed to develop, the excretion of urea (67) or the concentration of urea in the blood of nephrectomized animals increases (147) indicating an increased rate of gluconeogenesis.

Stimulation of endogenous insulin secretion by either oral or intravenous administration of glucose results in hypoaminoacidemia, its effect being most conspicuous on the concentration of essential amino acids (113, 35). Alanine is unique, being the only amino acid for which the plasma concentration does not show a consistent decline and which, in fact, may even increase under the influence of insulin (53). This anomalous behaviour of alanine is attributed to stimulation of its peripheral formation. The augmented synthesis of alanine is a consequence of the effect of insulin on glucose derived pyruvate for transamination (160, 23). The tendency for insulin to increase the uptake of circulating alanine by muscle is thus counterbalanced by augmented intracellular production and release of this amino acid.

No attempt has yet been made to validate this concept by measurement of alanine turnover during insulin-induced hypoglycaemia.

The hypoaminoacidemia of insulin, coupled with the difficulty in demonstrating a direct action of insulin lead to the notion that insulin regulates gluconeogenesis by influencing the availability of precursor substrates.

Since insulin does not decrease net availability of circulating alanine, the manner whereby insulin reduces hepatic gluconeogenesis must depend on a hepatic rather than a peripheral effect. More recent work with perfused liver as well as studies in intact man suggest such a direct hepatic effect (127). Felig and Wahren (52) calculated in man that endogenously released insulin caused a 30 - 60% reduction in the splanchnic uptake of alanine and other amino acids.

Further evidence to support the direct inhibitory effect of insulin on hepatic alanine uptake has been provided by studies in perfused liver by Rudorff et al (151).

The mechanism whereby insulin reduces gluconeogenesis at the cellular level is poorly understood. However the first step does appear to involve interaction of insulin with the cell membrane (22). Cuatrecasas has characterized specific insulin receptors on both the adipocyte and the hepatocyte (32).

It is argued that the overall metabolic effects of insulin are mediated by a reduction in cAMP levels in target tissues. The treatment of rats with insulin antiserum results in an increased hepatic content of cyclic AMP which is counteracted by the addition of insulin to the perfusate (132). Insulin can also reverse the rise in hepatic cAMP produced by glucagon (44). Several groups have also reported reduction of cAMP in the adipocyte (21, 89, 119), and the hepatocyte (45, 84). Whether this reduction is brought about by inhibition of adenylate cyclase (139) or by increasing the activity of the antagonist cyclic nucleotide phosphodiesterase (4) remains unresolved.

GLUCAGON AND GLUCONEOGENESIS

Glucagon levels have been shown to be increased during fasting, exercise (175), starvation (3) and hypoglycaemia (173), conditions associated with maximal stimulation of gluconeogenesis.

It is widely accepted that glucagon stimulates gluconeogenesis from amino acids in the perfused liver, a finding confirmed many times but most frequently in rat liver (41, 42, 47, 162).

Glucagon stimulation of gluconeogenesis however could not be demonstrated in perfused mouse (7) or guinea pig liver (163). It is possible that species differences exist with respect to the direct hepatic effect of glucagon. Evidence concerning the role of glucagon in stimulating gluconeogenesis in the dog is conflicting.

Studies by Lindsay and Madison in the intact dog suggest that glucagon does not stimulate gluconeogenesis from amino acids (111). They point out that virtually all of the evidence for the gluconeogenic action of glucagon has been obtained in *in vitro* systems and that definitive evidence that glucagon is a gluconeogenic hormone *in vivo* has yet to be presented. Chiasson *et al*, however, reported that infusion of glucagon in anaesthetized dogs, which raised the glucagon level to the upper limit of the physiological range did stimulate gluconeogenesis from alanine (26).

Examination of the effects of glucagon on individual amino acids in intact man apparently substantiates the important role of

alanine as a glucogenic precursor. Not only is it claimed that glucose production from alanine is stimulated by glucagon (62) but its uptake by liver is increased to a greater extent than that of all other amino acids (119). Similarly in postabsorptive (52) and prolonged fasted man (121) the magnitude of the decline in circulating alanine exceeds that of all other amino acids. It is presumed that the hypoalaninaemic effect of glucagon reflects stimulation of hepatic utilization of this amino acid for gluconeogenesis, since such a decline is not observed in patients with acute viral hepatitis and impaired glucose homeostasis (52). A reciprocal effect of alanine on glucagon levels also apparently exists as alanine is a powerful stimulant of glucagon secretion (130).

Glucagon has been shown to be a lipolytic agent (106) and so would be expected to increase gluconeogenesis from glycerol by increasing substrate supply.

Glucagon injection in the newborn rat hastens the normal postnatal systhesis of phosphopyruvate carboxylase (201) and Yeung and Oliver have shown that it is the postnatal synthesis of this key regulatory enzyme which completes the hepatic gluconeogenic system (199). Moreover administration of glucagon causes the precocious appearance of this enzyme in foetal life (202, 201). Similarly intraperitoneal injection of glucagon leads to the premature appearance of glucose-6-phosphatase (68). In view of this evidence Greengard has hypothesized that glucagon is the agent which causes development of these enzymes postnatally.

It appears that the mode of action of glucagon at the cellular

level, which results in the observed physiological phenomena are mediated via cyclic AMP.

All the actions of glucagon including those involving de novo enzyme synthesis can be mimicked by cyclic AMP (88). Glucagon increases the cAMP content of the perfused liver and on removal of glucagon from the perfusate the cAMP content returns rapidly to the basal level (43). Thus it is generally accepted that the metabolic actions of glucagon are mediated by an increase in the cAMP content of the target organs.

In a convincing series of experiments Rodbell has demonstrated the existence of distinct receptors, specific for glucagon on the rat hepatocyte membrane (145, 146). He suggests that the primary event which eventually results in the typical metabolic manifestations of glucagon is binding of this hormone to its receptors, followed by stimulation of the membrane bound adenylate cyclase with the resultant elevation of the intracellular cAMP content (145).

Source of Circulating Glucagon

Until recently glucagon has been regarded solely as the product of the pancreatic α cells and it was generally considered therefore that total pancreatectomy deprives mammals of glucagon.

However, it was found by Vranic et al that totally depancreatized dogs had normal, or in the absence of exogenously administered insulin, elevated levels of immunoreactive glucagon (IRG) in plasma (188). The antiserum used in the assay system was specific for pancreatic glucagon, and this finding was confirmed using three other specific antisera.

It is suggested therefore that there is an extra pancreatic source of IRG and furthermore than increased glucagon production from this source can compensate for pancreatic α cell failure.

Such a system would explain the difficulty in demonstrating glucagon deficiency in laboratory animals and the rarity of clinical disorder attributable to glucagon lack.

In 1948 Sutherland and de Duve (168) found that extracts of the upper two-thirds of the stomach of the dog have glycogenolytic activity but less was found in the duodenum and ileum. Valverde et al separated the glucagonlike immunoreactivity of the extracts from the dog gastrointestinal tract into two fractions (peak I and peak II) and they implied that peak II was the hyperglycaemic factor of gut (178).

Several researchers have indicated that cells similar or identical to pancreatic α cells can be found in the mucosa of the gastrointestinal tract in man and other mammalian species and have suggested that these cells might secrete either glucagon or glucagon-like material (59, 92, 183).

Morita et al in an attempt to locate the extra pancreatic source of glucagon assayed tissue extracts from normal and depancreatized, insulin deprived dogs, using an antiserum which was highly specific for pancreatic glucagon. IRG was undetectable in heart, kidney, liver and brain extracts and only small amounts were found in extracts from the pyloric region of the stomach, duodenum, jejunum, ileum, colon, sigma and rectum in both groups of dogs. Significant amounts of IRG were however found in stomach fundus extracts, similar amounts being found in

the extracts from the depancreatized, insulin deprived dogs (189).

It thus appears likely that a possible candidate for the extra pancreatic source of circulating IRG is the gut and specifically the fundic region of the stomach. Further investigation in this area, however is required before it can be stated unequivocally that any organ produces a hormone, functionally and structurally indistinguishable, from pancreatic α cell glucagon.

CONTROL OF GLUCAGON SECRETION

In the past few years much has been learned about the precise regulation of the secretion of pancreatic α cells in the grown animal. Electron microscopic studies indicate that pancreatic α and β cells communicate at the membrane level through junctional complexes (137).

The precise mechanism of intracellular synthesis and release of glucagon has not been elaborated but it has been suggested that a microtubular microfilamentous system is involved in the process of glucagon secretion (105, 40) analagous to that in the β cell.

In vivo blood glucagon levels are raised in response to hypoglycaemia (173), starvation, a protein meal, and infusions of amino acids such as alanine (130) and arginine (90, 94) and by the intraduodenal administration of fat (37).

Although arginine infusion has a direct stimulatory effect on the pancreatic α cells (90) triglyceride chylomicra do not act directly on the pancreas (19). It is therefore postulated by Unger that glucagon secretion in response to a fat meal is the result of an enteric signal to the islet cells, released during fat absorption (37). The identity of this hormone is as yet unknown although both pancreozymin and gastric-inhibitory peptide (174) are possible candidates.

The secretion of glucagon is decreased by insulin and or glucose. Insulin apparently promotes the entry of glucose into α cells blocking glucagon release. Such could be the mechanism underlying the

well known suppression of glucagon release during hyperglycaemia (136). Protein stimulated glucagon secretion is suppressed by intravenous (129) or oral (191) administration of glucose. Similarly intravenously administered glucose abolishes the rise in glucagon secretion in response to a fat meal.

The physiological significance of changes in blood glucagon concentration will depend on the prevailing insulin concentration or what has been called the insulin:glucagon molar ratio (203). In conditions known to be associated with reduced insulin production such as fasting and starvation, the catabolic effects of glucagon can predominate without a major rise in glucagon production. Conversely when the concentration of insulin is high after a meal the anabolic effects of insulin will previal. For this reason it has been proposed that the α and β cells be viewed as a single bicellular, bifunctional unit.

The concentration of glucagon reaches a peak on the 20th day of gestation (66). As maternal glucagon does not cross the placenta the foetus must be capable of glucagon production. However control of glucagon production in the rat foetus differs from that in the adult animal, as it is neither suppressed by hyperglycaemia, nor increased by maternal insulin-induced hypoglycaemia (66).

During the first few hours of life in the human infant glucagon concentration is not depressed by an intravenously administered glucose load (117). However infusion of insulin and glucose together does suppress glucagon secretion in babies less than 24 hours old (124).

Not only circulating catecholamines but also direct electrical stimulation of adrenergic nerves markedly increase glucagon release in the adult dog (122). Stimulation of the peripheral ends of both splanchric nerves causes an immediate increase in plasma glucagon concentration in the adrenalectomized calf three to five weeks after birth. During the first 24 hours of life however this response is absent (17).

In view of this evidence it appears that during the immediate neonatal period although glucagon is present in the plasma, the control of glucagon release is different from that in the adult.

AIMS OF THE INVESTIGATIONS

The purpose of the investigations to be reported was to:-

1. Quantitatively examine in newborn and adult dogs, both in the postabsorptive state and during fasting, the extent of gluconeogenesis from glycerol. In this investigation a compartmental model was formulated to calculate the irreversible disposal rate of glycerol, the turnover rate of glucose, compartment sizes and the rates and amounts of carbon transferred between compartments.
2. Quantitatively examine the transport of carbon atoms between alanine and glucose and calculate the turnover rate of alanine in newborn and adult dogs.
3. Quantify, in pups, the reincorporation of carbon into newly formed glucose, after cycling through tricarbon intermediaries, by determining the ratio of the glucose turnover rates, calculated using ^{3}H-2-glucose and ^{14}C-U-glucose as glucose tracers.
4. Because of the relative insensitivity of the newborn to insulin as reflected by the glycaemic response in pups (86) and in view of the importance of alanine as a glucogenic precursor in the adult animal, the effect of insulin-induced hypoglycaemia on the concentration of alanine in plasma and amino-nitrogen in blood of newborn dogs was examined.
5. Experiments were carried out to examine the effects of age, fasting, spontaneous and insulin-induced hypoglycaemia on the plasma glucagon concentration in newborn and adult dogs.

MATERIALS AND METHODS

Five different types of experiments were carried out, as previously outlined. Very few methods were common to all experiments. Therefore the methods and materials employed for each type will be described separately.

Tracer methods and data analysis, however, will be described in a separate section.

MATERIALS AND METHODS I

These experiments were carried out in order to determine the extent of gluconeogenesis from glycerol in newborn and adult dogs in the postabsorptive state and during fasting.

Animals

The experiments were performed on 30 inbred pups from 12 litters of inbred beagles. The age of the pups ranged from 7 hours to 30 days and their weights from 0.19 to 1.415 kg. Control experiments were carried out on 10 adult dogs (5.5 - 10.5 kg.), including three mothers of litters used at least three weeks after the last pup had been removed. One adult dog was subjected to two experiments, once under anaesthesia and once unanaesthetised. The experiments were performed on animals either in the postabsorptive state (first group) or during fasting (second group).

In experiments of the first group, food was withdrawn 16 - 18 hours prior to the experiment from adult dogs. Pups were taken from their mothers 2.5 - 5 hours prior to the experiment, depending on their age.

In the second group adults were fasted for 7 days and allowed access to water. Pups 2 days of age or less were fasted for 24 hours and older pups for 48 hours. During fasting the pups were kept warm in a heated box and water was provided frequently from a small feeding bottle.

All animals were anaesthetised with Nembutal (30 mg/kg.). This

injection was given intraperitoneally in pups and intravenously to adults via the cephalic vein.

A polyethylene cannula (PE50, PE60 or PE190, Becton Dickinson & Co.) was inserted into the left carotid artery of pups or into the inferior vena cava (via the left saphenous vein) of adults. This cannula was used both for the injection of tracer and withdrawal of blood samples. Blood volume was maintained by replacing blood removed with an equal volume of isotonic saline. Throughout the experiments rectal temperature was monitored and maintained at 37 ± 0.3°C. To prevent anoxia, the pups breathed a mixture of 95% O_2 + 5% CO_2 and room air (182).

Experimental Design

Trace amounts of glycerol, uniformly labelled with ^{14}C and ^{3}H-2-glucose (Amersham Searle) were administered simultaneously in a single injection (20 Uci kg^{-1}).

Blood samples were withdrawn from pups 0-4 days old at 2, 4, 7, 12, 20, 30, 40, 60 minutes: 5-11 days at 2, 4, 6, 10, 15, 20, 30, 40, 60 minutes: 12-30 days at 2, 4, 6, 10, 15, 20, 25, 30, 40, 60 minutes and in adults at 2, 4, 6, 8, 10, 12, 15, 20, 30, 40, 60, 90 minutes. Four pups and one adult dog received a single injection of ^{3}H-2-glucose and ^{14}C-U-glucose (Amersham Searle) simultaneously. Blood samples were withdrawn at 2, 4, 8, 12, 20, 30, 40, 60, 80 minutes from pups and 2, 4, 6, 10, 15, 20, 30, 40, 50, 60, 90, 110 minutes from the adult. Blood was withdrawn into dry sterile disposal plastic syringes (1 ml pups, 5 ml adults) and transferred into small glass tubes containing heparin. These tubes were cooled and within 10 minutes centrifuged.

All chemical determinations were made from a maximum of 0.05 ml. plasma. In no pup did the total volume of blood removed exceed 2.8 ml.

Analytical Methods

Plasma Glucose Assay

Glucose was determined enzymically from 0.02 ml. plasma on all samples where sufficient plasma was available, using a Beckman Glucose Analyzer.

Glucose Isolation

Glucose was isolated as glucose pentaacetate (G.P.A.)(85) prepared, in duplicate, from a deproteinized filtrate equivalent to 0.05 ml. plasma.

The radioactivity in a known weight of G.P.A. dissolved in Brays solution was determined by a double label counting technique in a liquid scintillation spectrometer (Nuclear Chicago Mark II).

Samples were each counted for 20 minutes. Counting efficiency was determined by using internal standards (0.1 ml. ^{14}C toluene 49.4 X 10^3 dpm and 0.1 ml. ^{3}H toluene 277.5 X 10^3 dpm (Amersham Searle)). Spillover of ^{14}C into the ^{3}H channel was determined by preparing two samples of G.P.A. from 100 mg. carrier glucose and 0.01 ml. ^{14}C-U-glucose containing approximately 222 X 10^3 dpm.

A computer programme was used to calculate the specific activity (S.A.) dpm mg^{-1} of glucose, the tracer concentration (C^*) dpm ml^{-1} and C^* as a fraction of the original dose (M^*) dpm. This program was executed by a Wang 600 computer.

Plasma Glycerol Assay

Glycerol concentration was determined enzymically from the first and every alternate sample up to 40 minutes and from last sample from 0.05 ml. plasma in pups and 0.1 ml. in adults. All glycerol samples were assayed, using a kit (Boerhinger Chem. Co.) within 20 minutes of withdrawal.

Isolation of Glycerol

Glycerol was isolated from 0.05 ml. plasma, from samples taken between 0 and 40 minutes, using an adaptation of the descending paper chromatographic technique of DeFreitas (36). Instead of eluting ^{14}C glycerol from the chromatogram, the strip containing glycerol was cut out, made into a pellet and oxidized to CO_2 in a Packard Sample Oxidizer. The $^{14}CO_2$ liberated was trapped in ethanolamine and dissolved in a methanol-toluene based scintillation fluid and counted in a liquid scintillation spectrometer. Three blank pieces of Whatman No. 1 chromatography paper, equal in dimension to the sample (4" X 2") were burned in the Oxidizer and used to determine background radioactivity. Counting efficiency was ascertained using 0.1 ml. ^{14}C toluene 49.4 X 10^3 dpm) as an internal standard. 88.9 $\pm$ 1.0% of glycerol ^{14}C was recovered from the chromatogram. A factor correcting for this loss was introduced into the computer programme, used to calculate the S.A. of glycerol.

No ^{14}C was lost during the burning process. When the S.A. of plasma glycerol was determined after an injection of ^{14}C-U-glucose a correction factor was introduced into the calculations to allow for the 0.41 $\pm$ 0.03% of glucose contaminating the glycerol strip.

MATERIALS AND METHODS II

Experiments to be described in this section were designed to investigate the kinetics of the glucose: alanine system in newborn and adult dogs in the postabsorptive state.

Animals

The experiments were performed on 14 pups from 5 litters of inbred beagles. Their ages ranged from 1 to 60 days, and their weights from 0.266 to 2.85 kg. Control experiments were performed on 5 adult beagles which were mothers of litters used at least 4 weeks after the last pup had been removed.

Food was withdrawn from the adult dogs 16 - 18 hours prior to the experiment. Pups were taken from their mothers 3 - 5 hours beforehand.

Anaesthesia, temperature control, prevention of anoxia and cannulation procedures were as described in section I.

Experimental Design

Trace amounts of alanine uniformly labelled with ^{14}C (New England Nuclear) and ^{3}H-2-glucose (Amersham Searle) were administered in a single injection simultaneously.

Blood samples were withdrawn from pups 0-4 days old at 3, 6, 12, 20, 40, 60, 80, 90, 120 minutes: 5-11 days at 3, 6, 12, 20, 40, 60, 90, 110, 120 minutes: 12-30 days at 3, 6, 9, 12, 20, 30, 40, 60, 80, 100, 120, 140 minutes: and from adults at 3, 6, 9, 12, 20, 25, 30, 40, 60, 80, 100, 120, 140, 180, 210 minutes.

Withdrawal and separation of plasma was as previously described.

All chemical determinations were made from a maximum of 0.075 ml. plasma.

Analytical Methods

Plasma Glucose Assay

Glucose concentration was assayed enzymically from 0.02 ml. plasma on all samples where sufficient plasma was available using a Beckman Glucose Analyzer.

Glucose Isolation

Glucose was isolated by preparation of G.P.A. as previously described.

Plasma Alanine Assay

Alanine was assayed enzymically (114) in the first and alternate samples up to 60 minutes from 0.05 ml. plasma in pups and 0.1 ml. in older pups and adults.

Isolation of Alanine

Alanine, together with other amino acids, was isolated by ion exchange chromatography by an adaptation of the method of Stein and Moore (128). It was further separated from other amino acids by conversion to pyruvate, catalyzed by glutamate, pyruvate transaminase (G.P.T.). The phenyl hydrazine derivative of pyruvate was prepared, (86) dissolved in Brays solution and the radioactivity determined by a double label counting technique in a liquid scintillation spectrometer.

No alanine was lost from the column. Approximately 50% of alanine ^{14}C was recovered from the phenyl hydrazone and the independent recovery for each sample was determined using a second tracer (^{3}H-2-3 alanine).

The reagents and procedures employed are described in "Validation of Methods".

MATERIALS AND METHODS III

In this section experiments will be described that were designed to examine the effects of insulin induced hypoglycaemia on the concentration of alanine in plasma and α-amino nitrogen in blood of newborn and adult dogs.

Animals

These experiments were carried out on 18 pups from 7 litters of inbred beagles, ranging in age from 0.3 to 25 days. Experiments were performed on adult dogs, 4 of which were mothers of litters. One adult dog was subjected to two experiments. Pups were fasted 3 - 6 hours and adults 16 - 18 hours prior to the experiment.

Infusions were given via a polyethylene cannula (PE50, 60, 190 Becton Dickinson Co.) tied into the cephalic vein of adults and the left jugular vein of pups. In pups, samples were taken via a cannula inserted into the carotid artery. In adults, the sampling cannula was forwarded into the inferior vena cava via the saphenous vein.

Details of the procedures of maintaining the animals throughout the experiment were as previously described.

Experimental Design

After two, or in smaller pups, one blood sample of 0.45 ml. was withdrawn, an infusion of 2.5 mU $kg^{-1}.min^{-1}$ insulin (Crystalline Insulin Toronto) in saline or, in control experiments saline alone was infused for 120 minutes. Flow rates were 0.0285 ml. min^{-1} in pups and 0.153 ml. min^{-1} in adults.

In adult control dogs no saline infusion was given. In pups

blood samples of 0.15 - 0.5 ml. were withdrawn at 30, 60, 90, 120 minutes. In pups older than 7 days an additional sample at 105 minutes was also taken. In adults, samples were taken at 30, 60, 90, 105 and 120 minutes during the infusion.

α amino-nitrogen Content of Blood

Blood was withdrawn and cooled immediately. 0.2 ml. was deproteinized as described by Frame et al (60) and the concentration of α amino-N was determined according to the method of Rubinstein and Price (149). The rest of the blood was centrifuged immediately.

Plasma Glucose and Alanine Determination

Glucose was assayed from 0.02 ml. plasma as previously described. Alanine was assayed from 0.05 ml. plasma in younger pups and 0.1 ml. plasma in older pups and adults.

MATERIALS AND METHODS IV

In this section, experiments were designed to examine the glucagon concentration in pups in the postabsorptive state and the effects of fasting insulin-induced hypoglycaemia and spontaneous hypoglycaemia on glucagon concentration.

Animals

These experiments were carried out on a total of 28 pups from 10 litters of inbred beagles and two adult female beagles. The pups in the postabsorptive state were taken from their mothers $2\frac{1}{2}$ - 5 hours prior to the experiment. Of these pups, four received insulin injections (0.12U kg^{-1}) and two received a 2 hour insulin infusion (2.5 mU $min^{-1}kg^{-1}$). Seven pups of four days old or less were fasted for 1 day and five older pups for 2 days prior to the experiment. The two adult dogs were fasted for 7 - 8 days before investigation.

Experimental protocol was as described in previous sections.

Experimental Design

In all dogs in both the postabsorptive state and after fasting one blood sample of 1.0 ml. was withdrawn for analysis. In those animals receiving either insulin injection or infusion, one sample was taken before insulin and a second sample (2 ml.), thirty minutes after injection or after two hours of infusion.

Analytical Methods

Plasma Glucose Concentration

Plasma glucose concentration was determined from 0.02 ml. plasma using the Beckman Glucose Analyzer.

Glucagon Determination

All glucagon assays were performed in the laboratory of Dr. M. Vranic in the Dept. of Physiology at the University of Toronto.

Blood for glucagon determination was collected in tubes containing trasylol (50 Ul/ml blood) + EDTA (50 Ul/ml blood) and immediately centrifuged. The haemotocrit was determined and recorded. The serum was stored at -20°C.

Serum levels of immunoreactive glucagon were determined in duplicate by a radioimmunoassay using the highly specific antiglucagon serum 30K (supplied by Novo Research Inst., Copenhagen, Denmark).

A correction factor:

$$\text{Uncorrected value} \times \frac{110 - \text{Haematocrit (\%)}}{100 - \text{Haematocrit (\%)}}$$

was applied to allow for the Trasylol-EDTA dilution.

MATERIALS AND METHODS V

In this section experiments were designed to assess the reincorporation of carbon into newly formed glucose by determining the glucose (RT) as calculated using ^{3}H-2-glucose and ^{14}C-U-glucose as tracers.

Animals

These experiments have been carried out on 13 pups from 5 litters of inbred beagles. The pups were taken from their mothers $2\frac{1}{2}$ - 5 hours prior to the experiment. Experimental protocol was as previously described.

Experimental Design

In 7 pups 25-30 µci each, of the two tracers was injected simultaneously into a cannula in the left carotid artery from which samples were withdrawn over the next 120 min. In the remaining 6 pups following the simultaneous injection of the tracers (2.5 µci ^{3}H-2-glucose and 2 µci ^{14}C-U-glucose) the tracers were infused into the jugular vein (0.11 µci min^{-1} and 0.1 µci min^{-1} respectively). During the next hour blood samples were withdrawn from the carotid artery.

Analytical Methods

Plasma Glucose Concentration

Plasma glucose concentration was determined enzymically as previously described.

Glucose Isolation

Glucose was isolated from a deproteinized plasma filtrate

equivalent to 0.05 ml. by preparation of G.P.A. The radioactivity in a known weight of G.P.A. was determined by dissolving in Brays solution.

DATA ANALYSIS

1. THE GLYCEROL:GLUCOSE SYSTEM

Development of a Model of the Glycerol:Glucose System

After the simultaneous single injection of ^{14}C-U-glycerol and ^{3}H-2-glucose the concentration (dpm.ml^{-1} plasma) of ^{14}C labelled glycerol and glucose were calculated at each sampling time as a fraction of the initial dose of tracer (M*) injected at t = o which had been normalized with respect to body weight (dpm ml^{-1}/dpm.Kg-1 = Kg ml^{-1}).

The tracer data were analyzed with the aid of the SAAM-26 computer modelling program (14). Glucose:glycerol kinetics were conceptualized as a multicompartmental system described by a set of simultaneous linear differential equations. By an iterative process least square estimates of the rate constants L_{ij} (min^{-1}) and plasma equivalent volumes V_i(ml.kg^{-1}) are calculated. (The plasma equivalent volume is defined as the hypothetical volume in which a mass would be distributed were it evenly distributed throughout the system at a concentration equal to that in the plasma.) Using these values together with steady state plasma glucose and glycerol concentrations the rates of release of glycerol-C (U_1) and of glucose-C ($U_3 + R_{3,1} = R_{0,3}$) into the system and rates of transport of C-atoms between compartments ($R_{i,j}$) were calculated mg C.min^{-1}.kg^{-1}. Steady state solutions for compartmental masses, M_i(mg C.Kg^{-1}) were also obtained.

Modelling was carried out on averaged data for each group and

a set of parameters of the model for each age group was established. Dogs in the postabsorptive state were allotted to four groups, according to age: i) 0-4 days, ii) 7-11 days, iii) 12-30 days, and iv) adult.

The tracer concentration in plasma versus time data for ^{14}C-U-glycerol and ^{3}H-2-glucose could each be fitted by sums of two exponentials Fig. 2 . Thus a two compartment model was proposed for each subsystem. To determine the conversion from glycerol to glucose the model shown in Fig. 3 was tested against the data. All data, including the ^{14}C glucose versus time curve were simultaneously fitted to the model to ensure that the same rate constants apply to the ^{3}H and ^{14}C labels. Since ^{3}H attached to the second carbon atom of glucose is not re-incorporated into circulating glucose, whereas ^{14}C atoms are; in the calculation $L_{0,3}$ was multiplied by a factor to correct for this difference. The factor 1.3 was chosen for pups (p113) as well as adult dogs (83).

Initially the return of C-atoms from glucose to glycerol was considered. The data however could not resolve such a pathway, therefore a separate series of experiments were carried out on seven dogs including some from each age group by injecting ^{14}C-U-glucose and following the appearance of ^{14}C in plasma glycerol. No appreciably detectable amount (p 82) of ^{14}C was found to appear in plasma glycerol.

Alternative pathways between the glycerol and glucose subsystems were also explored. All further changes in the model led to slower than the experimentally observed appearance of ^{14}C in the glucose subsystem.

Fig. 2

RESPONSE TO A SIMULTANEOUS SINGLE INJECTION OF 14C-U-GLYCEROL + ^{3}H-2-GLUCOSE IN ADULT DOGS

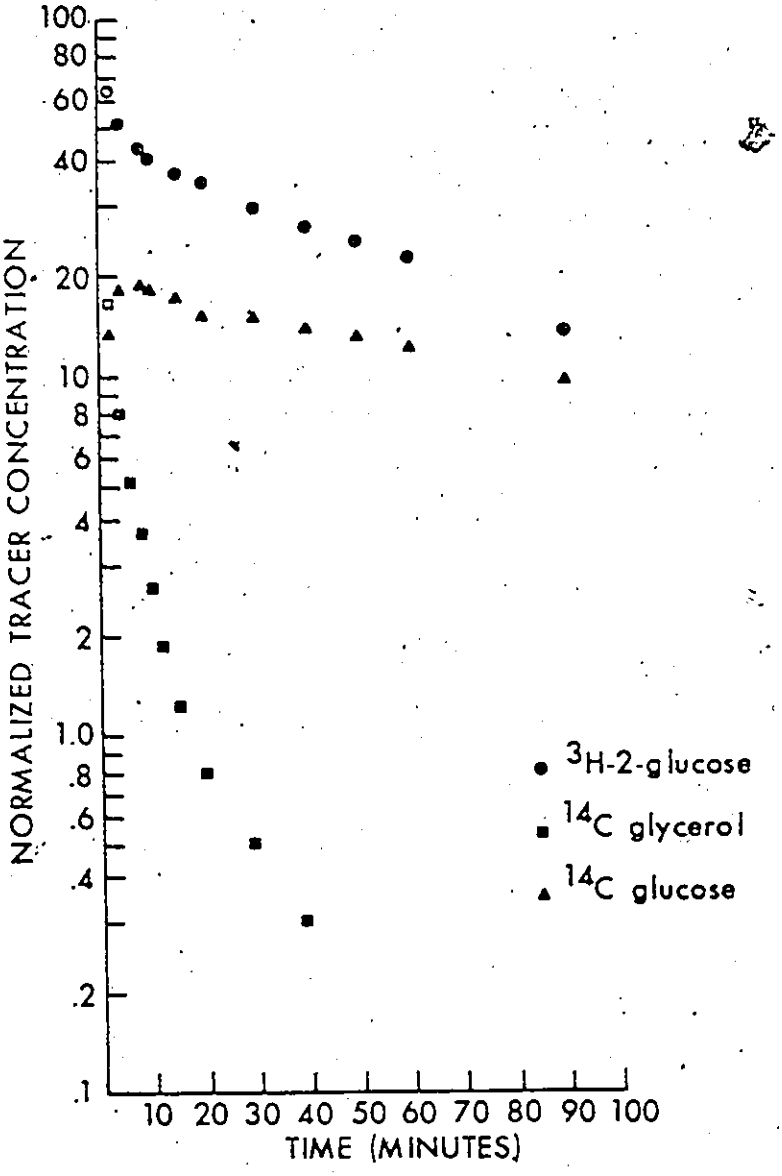
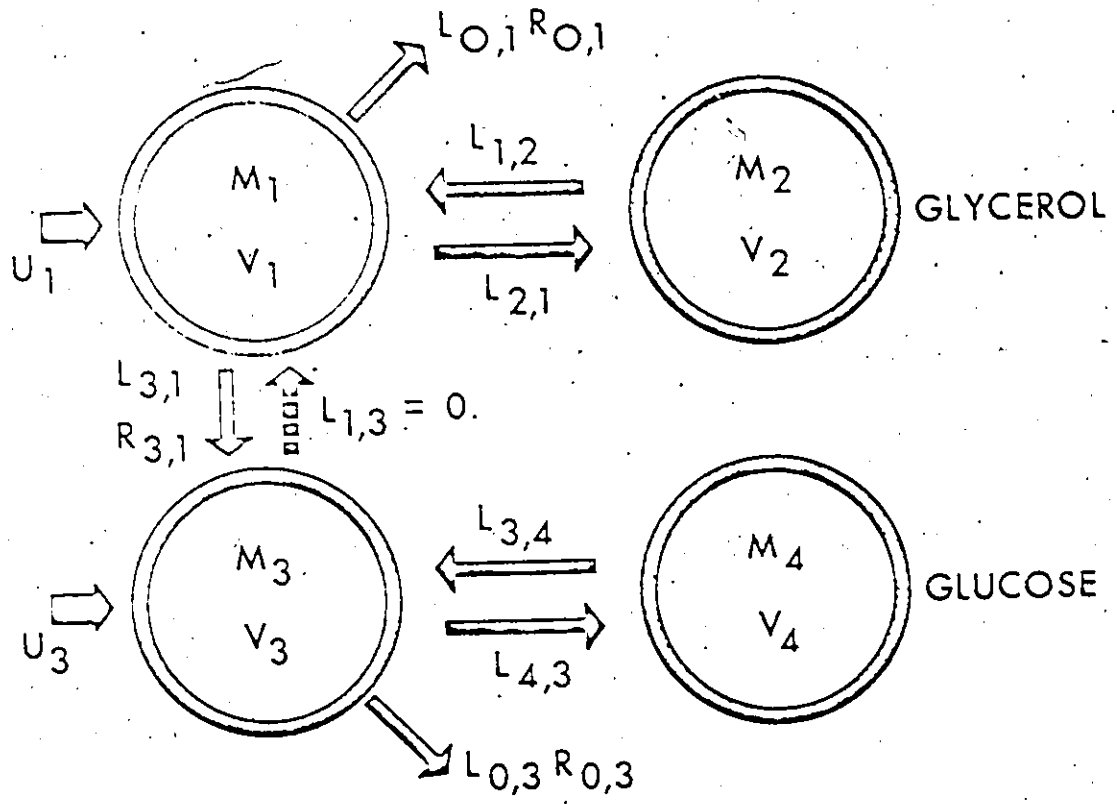


Fig. 3

Compartmental Model of the Glycerol-Glucose system



Thus pathway $R_{3,1}$ was both necessary and sufficient to fit the data.

Once the model had been established and its parameters (rate constants, compartmental sizes, rates and plasma equivalent volumes) calculated from the averaged data of each group, data from individual animals were run separately on the established model.

Among the rates calculated from the model U_1 represents the rate of inflow of unlabelled glycerol-C into the glycerol subsystem: it represents, mainly glycerol released by lipolysis. In a steady state U_1 is equal to the rate at which glycerol-C leaves the subsystem ($R_{0,1} + R_{3,1}$). Since the ^{14}C label may recycle to glycerol-C via various metabolic intermediates U_1 is equal to the irreversible disposal rate of glycerol. $R_{0,3}$ is the rate of disappearance of glucose and thus in a steady state equal to the glucose turnover rate. Because of the choice of ^{3}H-2-glucose as the tracer U_3 does not include the carbon atoms recycled via the Cori cycle into glucose.

2. THE ALANINE:GLUCOSE SYSTEM

i Calculation of Turnover Rates (RT) of Alanine and Glucose

The turnover rates of alanine and glucose in plasma were determined employing the method of tracer injection (198). This method has been shown to be valid in normal dogs in a steady state with respect to the plasma glucose concentration (28). The calculations employ the Stewart-Hamilton equation (167) which when applied to the specific activity-time curve of a labelled pool gives the production rate (Ra) for the tracee regardless of the source of the externally derived input (158). In a steady state Ra = Rd (the rate of disappearance) = RT. The form of the equation used was:-

$$RT = \frac{M^*}{\int_0^\infty SA.dt}$$

where

M^* = the injected dose of tracer (dpm)
SA = specific activity (dpm mg^{-1}).

Double exponential functions were fitted to the SA-time curves for ^{14}C-U-alanine and ^{3}H-2-glucose using an iterative program which also performed the integration and was executed by a Wang 600 computer.

ii Calculation of the Fraction of Alanine Carbon Which is Transferred To Glucose

In a linear system the tracer concentration-time curve or the unit impulse response function W(t) will predict the output C*(t), corresponding to any desired input R*(t).

The relation is

$$C^*(t) = \int_0^\infty W(t)\ R^*(t - \tau)d\tau$$

the convolution integral.

If, as in the alanine:glucose system the unit impulse response function i.e. the tracer concentration-time curve for ^{3}H-2-glucose, and the output C*(t) i.e. the tracer concentration-time curve for the appearance of ^{14}C in glucose, are known then R*(t) the input of tracer to glucose from alanine can be calculated by the process of deconvolution.

The unit impulse response function is in the general form

$$Wt = Ae^{-b_1 t} + Be^{-b_2 t}$$

and the output is in the general form

$$C^*(t) = D\left[e^{-b_3 t} - e^{-b_4 t}\right].$$

The convoluted input R*(t) in this case is given by

$$R^*(t) = \left[E_1 e^{-b_3 t} + E_2 e^{-b_4 t} + E_3 e^{-b_5 t}\right] 100$$

where $$E_1 = \frac{K(b_1 - b_3)(b_2 - b_3)}{(b_4 - b_3)(b_5 - b_3)}$$

$$E_2 = \frac{K(b_1 - b_4)(b_2 - b_4)}{(b_3 - b_4)(b_5 - b_4)}$$

$$E_3 = \frac{K(b_1 - b_5)(b_2 - b_5)}{(b_3 - b_5)(b_4 - b_5)}$$

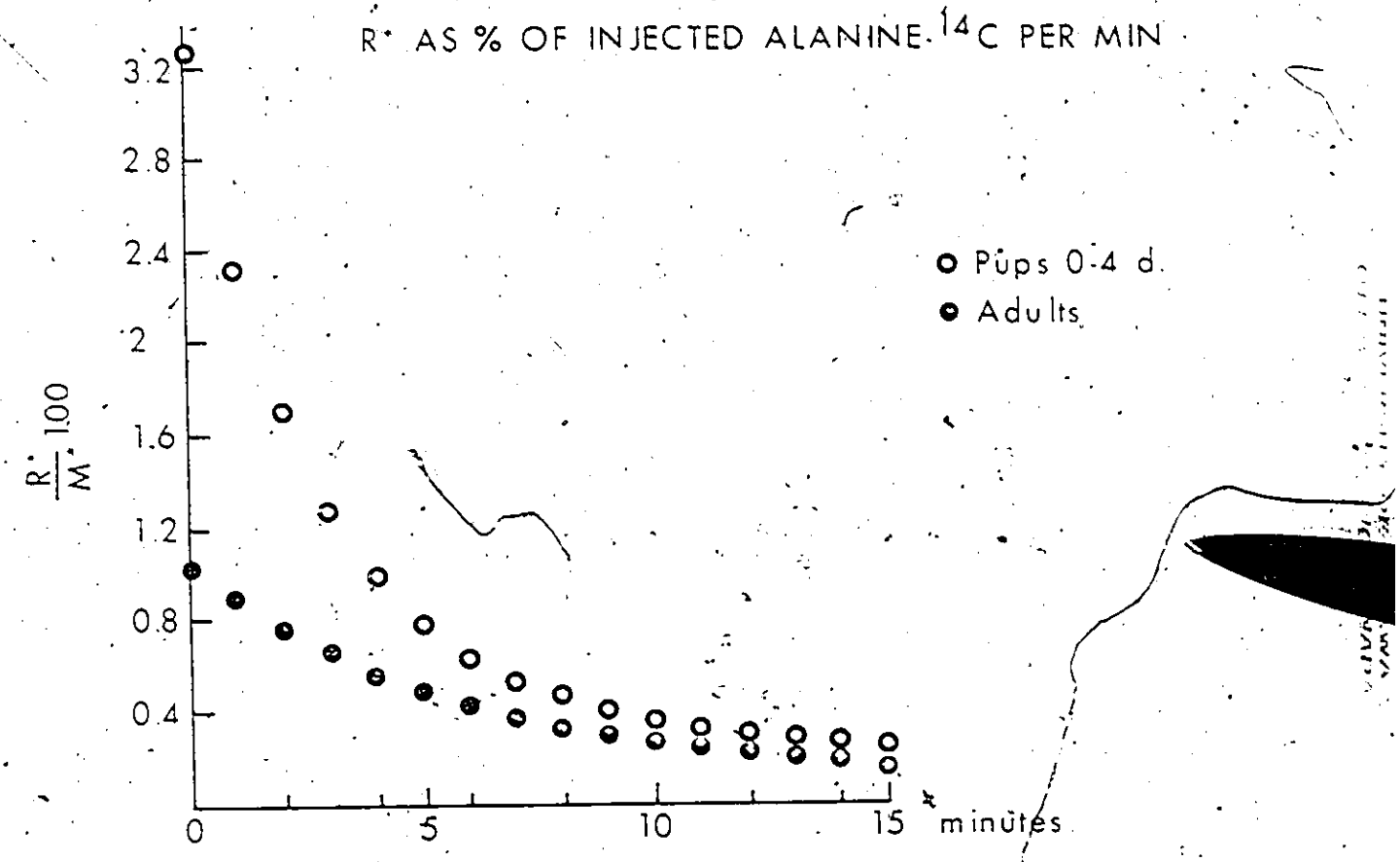
and $$K = \frac{D(b_4 - b_3)}{A + B}$$

$$b_5 = \frac{Ab_2 + Bb_1}{A + B}$$

The data are normalized so that R*(t) is the percentage of the injected dose transferred per min. Thus integrating the R*(t)-time curve gives the total percentage of the injected tracer arriving in glucose. Fig. 4.

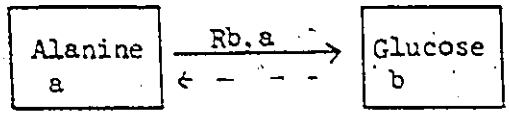
The derivation and function fitting were performed by Dr. B. Pagurek, Dept. of Systems Engineering, Carleton University.

Fig. 4


R* AS % OF INJECTED ALANINE-14C PER MIN
Pups 0-4 d.
Adults
R*/M* 100
3.2
2.8
2.4
2
1.6
1.2
0.8
0.4
0
5
10
15
minutes

iii Calculation of the Fraction of Newly Formed Glucose Carbon Arising From Alanine

In the following system:-



where Rb,a = rate of transfer from a to b

Ro,b = rate of transfer out of b

$$Rb,a \int_o^\infty SA_a dt = Ro,b \int_o^\infty SA_b dt.$$

In a steady state the rate of loss of atoms from b equals the net rate of total input which includes atoms from a and those not from a. So substituting for Ro,b in the preceeding equation and rearranging, the equation becomes:

$$\frac{\int_o^\infty SA_b dt}{\int_o^\infty SA_a dt} = \frac{Rb,a}{\text{Total net rate to b}} = \text{fraction of b from a.}$$

Applying this ratio of integrals (158) to the alanine:glucose system gives the fraction of newly formed glucose carbon which arises from plasma alanine.

iv Development of The Model of the Alanine:Glucose System

After a simultaneous injection of ^{3}H-2-glucose and ^{14}C-U-alanine, the tracer concentration (dpm.ml^{-1} plasma) of ^{14}C-alanine, ^{14}C-glucose and ^{3}H-2-glucose were calculated, normalized and averaged as described in the foregoing description of the glycerol:glucose system.

FIG. 5

RESPONSE TO A SIMULTANEOUS INJECTION OF ^{14}C-U-ALANINE AND ^{3}H-2-GLUCOSE IN PUPS 0-4 DAYS OF AGE

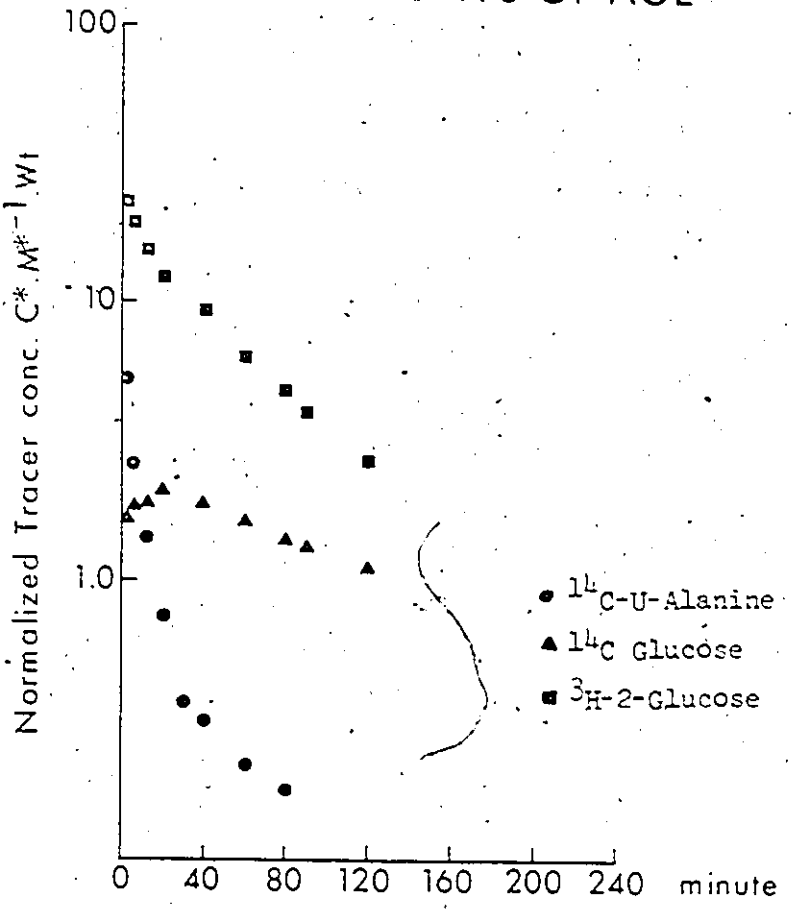


Fig. 6

RESPONSE TO A SIMULTANEOUS INJECTION OF ^{14}C-U-ALANINE AND ^{3}H-2-GLUCOSE IN ADULT DOGS

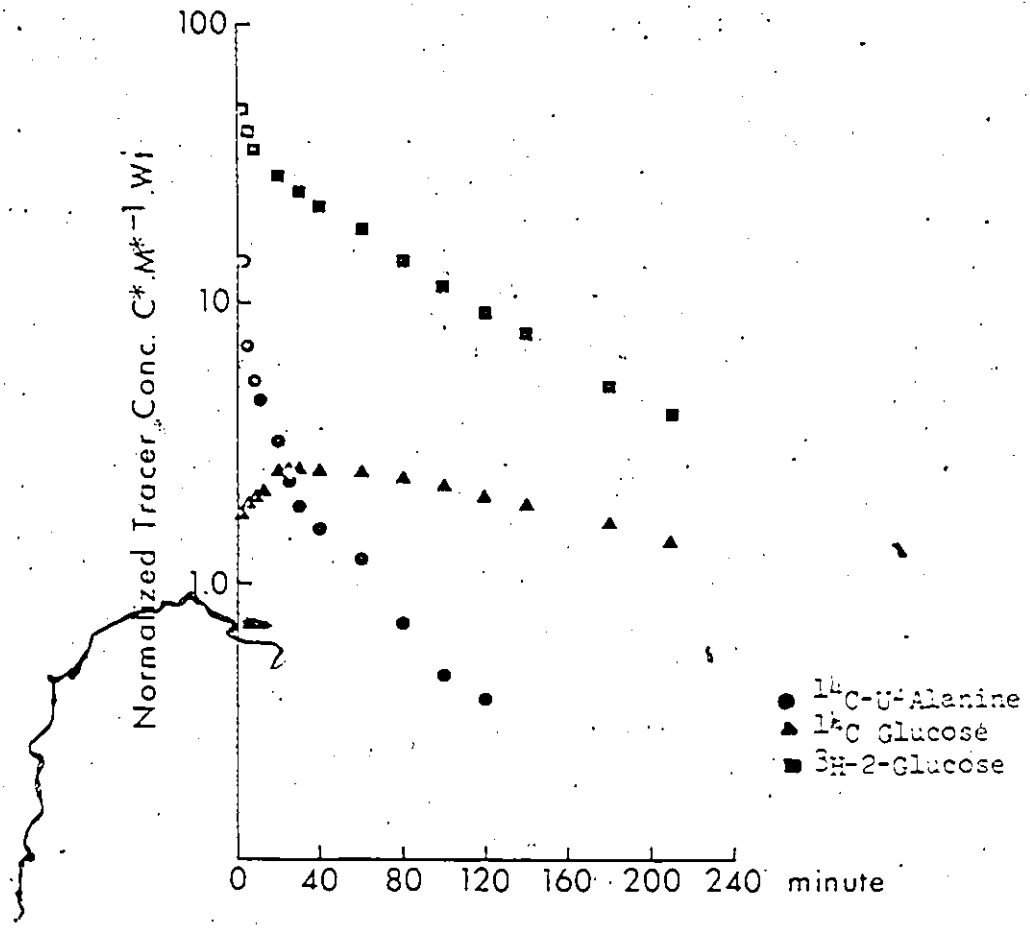
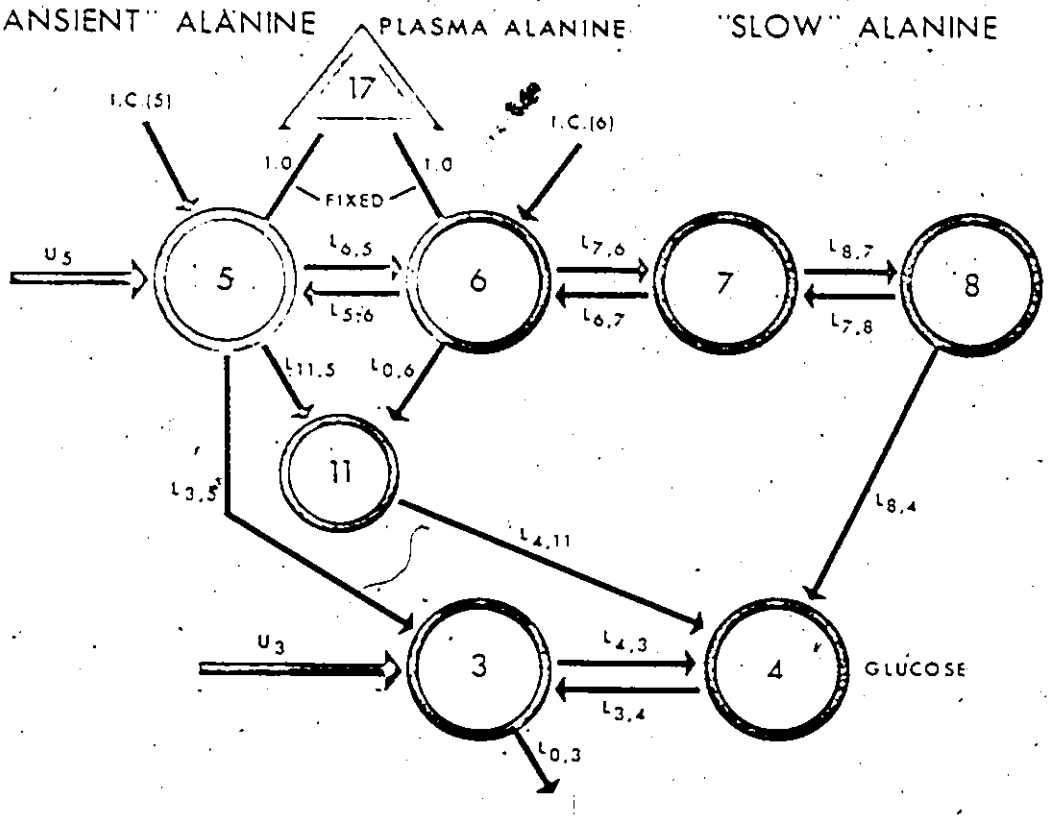


Fig. 7

MODEL OF THE ALANINE: GLUCOSE SYSTEM



Using the SAAM 26 computer modeling program an attempt was made to formulate a compartmental model of the alanine-C:glucose-C system using the normalized tracer concentration data (Figs. 5 and 6) and the steady state plasma concentrations of alanine and glucose. Calculation of rates and percentage conversions derived from such a model have the advantage that they do not depend on extrapolation of the data as do the previously described methods of data analysis.

The model which best fits the data is shown in Fig. 7. A three compartment system was necessary for the alanine subsystem to fit the ^{14}C-U-alanine tracer concentration versus time data.

As in the glycerol:glucose system the glucose subsystem could be adequately described by two compartments.

When simultaneously fitting all three sets of tracer data it was immediately apparent that the time course of the disappearance of ^{14}C-U-alanine and the appearance of ^{14}C in glucose did not satisfy a classical precursor-product relationship (15). A proportion of ^{14}C injected as ^{14}C-U-alanine appeared extremely rapidly in glucose. To allow for this rapid "dumping" of ^{14}C label in glucose, it required that some of the alanine tracer be injected directly into a "transient" alanine compartment (compartment 5) from where the carbon was rapidly transferred into glucose via $L_{3,5}$.

The remainder of the ^{14}C-U-alanine injected was in compartment 6. Compartments 5 and 6 are however both components of plasma alanine viewed singly in [17].

The later portion of the ^{14}C glucose appearance curve required

that ^{14}C continue to appear by means of slower pathways.

A delay compartment (compartment 11) was introduced between compartments 5 and 4 which slowed down the appearance of ^{14}C in plasma glucose from the "transient" alanine component. This pathway together with $L_{11,5}$ and $L_{4,8}$ were necessary and sufficient to fit the data.

This model has been validated when tested with our data and tracer data from similar experiments in man (15). However further experimental validation is required before this model could be validly employed to calculate physiological parameters.

3. CALCULATION OF GLUCOSE TURNOVER IN PUPS

In seven pups the glucose RT was calculated by a single injection method using the form of the Stewart-Hamilton equation already described. In six however a primed infusion tracer method was used employing the equation derived by Steele et al (164, 165).

$$Ra = R^* - pM \frac{SA_2 - SA_1}{t_2 - t_1} \cdot \frac{2}{SA_1 + SA_2}$$

where R^* = rate of infusion of tracer dpm min^{-1}.

M = mass of glucose in the system calculated as a product of plasma glucose concentration and the size of the apparent distribution space of glucose. mg. ($M = V \cdot C$)

p = the pool fraction of the central compartment, taken to be 0.65 (30).

SA = specific activity of glucose dpm mg^{-1}.

t = time min.

The injection and infusion methods for the calculation of turnover rates are equivalent procedures and hence yield identical results. The validation of such methods has been exhaustively discussed by Hetenyi and Norwich elsewhere (77).

RESULTS

PART I

THE EFFECTS OF AGE AND FASTING ON GLUCONEOGENESIS FROM GLYCEROL

The response data in all animals which received a simultaneous single injection of ^{14}C-U-glycerol and ^{3}H 2-glucose are shown on Tables 1 - 21 inclusive. Results are expressed as fraction of the injected dose in one ml. plasma ($min^{-1} \times 10^{4}$). These data were further normalized and averaged within age groups as previously described (p 44) and incorporated into the model describing the steady state kinetics of the glycerol:glucose system. Table 22 shows the fraction of the dose injected as ^{14}C-U-glucose appearing in glycerol in one ml. plasma ($min^{-1} \times 10^{6}$). The latter data were used to resolve the existence of $L_{1,3}$ (Fig. 3)

The steady state average concentration of glucose and glycerol for pups of different age groups and for adults in the postabsorptive state and fasted are shown on Table 23. In pups in the postabsorptive state the plasma level of glycerol decreased with age, reaching its lowest values in adults. Apart from the low glucose level in the youngest pups the concentration of glucose in plasma did not change with age. Fasting decreased both the plasma glycerol ($t = 3.12$ $p < 0.025$) and glucose levels in the youngest pups ($t = 3.55$, $p < 0.01$), but increased glycerol levels in older animals. The plasma glycerol level in pups 5-19 days of age was not significantly below that observed

in the postabsorptive state in pups 5-11 days old ($t = 2.06$, $p > 0.05$) and those in the 15-30 day old group ($t = 1.87$, $p > 0.05$).

Table 24 gives the calculated rate constants ($L_{i,j}$) expressed as min^{-1} for the model of the glycerol:glucose system shown on Fig. 3. Values for all pups and adult dogs are given for the respective age groups.

TABLE 1

Fraction of initial tracer injection of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in pups 0-3 days old in the Postabsorptive state

Experiment	Sex	Age days	Weight Kg.	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^4						
					2	4	7	12	20	30	40
3BG1	F	0.8	0.275	36.94	26.96	13.31	4.81	1.73	0.56	0.23	0.12
2BG1	F	0.3	0.241	56.94	43.62	20.63	13.3	8.68	3.52	1.84	1.16
2BG3	M	2.5	0.19	55.5	33.76	15.53	9.63	4.12	2.24	1.03	0.52
1BG2	F	2.5	0.375	56.0	25.22	13.58	5.69	3.05	1.38	0.6	0.37
3BG2	M	1.8	0.278	56.94	23.53	9.86	5.62	2.37	1.05	0.77	0.29
3BG3	M	2.8	0.283	56.94	23.89	5.93	2.16	0.18	0.16	0.15	0.14
1BG1	F	0.6	0.29	56.0	36.86	19.47	9.43	7.43	4.97	2.44	1.47

TABLE 2

Fraction of ^{14}C injected as glycerol present in glucose / ml plasma in pups 0-3 days old

Experiment	Fraction of dose $ml^{-1} \times 10^4$						
	2'	4	7	20	30	40	60
3BG1	25.68	26.93	25.6	18.94	16.48	13.94	10.52
1BG1	26.5	27.32	26.95	25.54	23.12	21.95	18.76
3BG3	41.41	25.26	27.93	20.96	13.8	13.7	11.54
3BG2	44.65	32.78	27.36	22.51	22.97	19.82	15.5
1BG2	42.32	36.68	32.83	26.03	22.62	19.22	15.56
2BG3	46.68	46.03	43.99	40.12	34.92	35.27	26.97
2BG1	28.3	27.57	25.14	21.63	19.14	17.33	11.26

TABLE 3

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in pups 0-3 days old in the postabsorptive state

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}						
		2	4	7	20	30	40	60
3BG1	30.0	92.7	77.85	69.69	47.05	37.71	31.85	22.45
2BG3	30.0	76.61	69.92	52.58	45.86	34.77	35.53	23.93
2BG1	30.0	118.91	107.14	84.85	54.0	47.17	37.14	23.47
3BG3	30.0	117.77	67.48	70.42	50.31	30.64	28.53	20.54
3BG2	30.0	118.31	79.45	70.96	47.3	43.07	36.27	25.56

TABLE 4

Fraction of initial tracer injection of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in pups 7-11 days old in the postabsorptive state

Experiment	Sex	Age days	Weight Kg.	Dose dpm x 10^{-6}	Fraction of Dose ml^{-1} x 10^4								
					2	4	6	10	15	20	25	30	40
6BG1	M	8	0.605	75.83	11.41	5.58	3.53	1.7	0.78	0.45	0.28	0.22	0.1
2BG4	F	9	0.468	55.6	11.02	4.31	2.47	1.11	0.47	0.34	0.27	0.13	0.11
5BG1	M	9	0.564	63.5	13.73	6.67	3.82	2.56	1.55	0.77	0.73	0.73	0.47
3BG4	M	7.5	0.547	86.9	14.14	5.95	3.34	1.54	0.76	0.32	0.19	0.16	0.08
3BG5	M	11	0.624	56.94	33.05	5.97	3.22	1.55	0.75	0.36	0.18	0.12	0.05
1BG3	M	7.5	0.67	56.0	11.48	7.42	4.79	2.94	1.55	1.00	0.84	0.70	0.54

TABLE 5

Fraction of ^{14}C injected as ^{14}C-U-Glycerol present in glucose / ml plasma at each sampling time in pups 7-11 days old in the postabsorptive state

Experiment	Fraction of dose $ml^{-1} \times 10^4$							
	2	6	10	20	25	40	50	60
6BG1	19.4	17.93	16.52	14.98	13.92	11.86	10.36	9.18
5BG1	18.63	18.66	16.3	13.44	14.3	12.48	10.11	10.1
1BG3	17.47	17.28	16.3	15.14	14.36	12.81	12.03	10.97
3BG5	11.73	9.77	9.15	8.16	7.78	6.74	5.41	5.54
3BG4	15.41	13.78	13.04	10.9	9.48	9.07	7.8	7.07
2BG4	29.23	24.47	24.0	20.88	19.26	15.41	13.25	11.1

TABLE 6

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in pups 7-11 days old in the postabsorptive state

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}							
		2	6	10	20	25	40	50	60
6BG1	34.2	49.01	34.7	29.75	24.86	24.91	15.67	11.61	11.36
3BG4	30.0	79.11	57.31	51.33	28.15	23.1	19.0	15.48	14.23
3BG5	30.0	63.21	31.95	26.04	21.89	22.74	17.2	13.13	12.36
5BG1	42.57	51.82	37.88	30.61	23.65	24.4	18.38	14.68	13.42
2BG4	30.0	64.88	46.11	39.08	31.95	28.71	21.6	17.49	13.39

TABLE 7

Fraction of initial tracer concentration of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in pups 15-30 days old in the postabsorptive state

Experiment	Sex	Age days	Weight Kg.	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^4								
					2	4	6	10	15	20	25	30	40
6BG2	F	15	0.788	128.9	12.23	5.09	2.88	2.09	1.48	0.81	0.51	0.31	0.24
3BG6	M	15	0.787	68.33	10.09	3.99	2.49	1.21	0.45	0.28	0.13	0.12	0.06
1BG4	F	15	0.965	66.7	24.14	11.18	4.85	2.04	1.04	0.68	0.43	0.22	0.1
3BG7	F	18	0.735	68.5	12.0	6.08	3.55	1.76	0.81	0.44	0.29	0.21	0.11
1BG6	F	21	1.085	66.7	15.13	3.54	2.07	0.99	0.51	0.31	-	0.09	0.03
5BG2	M	30	1.415	236.9	8.13	4.27	2.75	1.5	0.83	0.47	0.39	0.31	0.19
2BG5	M	16	0.396	56.9	9.34	2.87	1.38	0.55	0.33	0.14	0.12	0.1	0.07

TABLE 8

Fraction of ^{14}C injected as ^{14}C-U-Glycerol present in glucose /, ml plasma at each sampling time in pups 15-30 days old in the postabsorptive state

Experiment	Fraction of dose $ml^{-1} \times 10^4$							
	2	6	8	15	20	30	40	60
6BG2	13.76	12.26	11.54	10.77	10.06	9.7	8.48	6.82
2BG5	37.01	23.75	22.38	18.37	15.59	12.94	10.84	8.4
1BG4	8.61	8.32	8.17	8.03	7.46	6.79	6.27	4.71
3BG7	16.76	14.18	13.82	12.42	12.58	11.81	10.62	9.77
1BG6	12.8	12.55	12.42	11.13	10.48	9.16	8.4	6.97
5BG2	7.94	8.46	8.37	7.76	7.39	6.81	6.18	5.33
3BG6	9.59	8.52	8.41	8.1	7.22	7.26	6.09	3.75

TABLE 9

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in pups 15-30 days old in the postabsorptive state

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}							
		2	6	8	15	20	30	40	60
6BG2	45.5	37.01	24.2	23.62	21.58	20.38	16.07	13.32	10.27
2BG5	30.0	90.29	52.32	48.5	40.05	34.59	28.21	21.94	13.98
1BG6	44.4	35.74	21.28	20.56	18.03	16.84	14.44	12.24	8.2
5BG2	101.4	21.92	15.39	15.16	11.93	11.26	9.57	9.1	7.66
3BG6	36.0	28.85	18.16	17.7	16.21	13.33	12.25	9.45	5.95

TABLE 10

Fraction of initial dose of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in adult dogs in the postabsorptive state

Experiment	Sex	Weight Kg.	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^4									
				2	4	6	8	10	12	15	20	30	40
5G	F	10.9	447.76	1.84	0.82	0.52	0.32	0.29	0.2	0.13	0.08	0.07	0.06
2G	F	11.2	431.3	2.06	0.95	0.76	0.56	0.45	0.3	0.18	0.12	0.07	0.05
3BGM	F	9.0	373.3	1.96	0.7	0.46	0.3	0.2	0.15	0.09	0.06	0.03	0.02
2BGM	F	8.2	371.5	2.2	1.22	0.71	0.47	0.32	0.24	0.15	0.08	0.03	0.02
4BGM	F	9.5	364.9	1.78	0.94	0.5	0.36	0.23	0.19	0.12	0.07	0.03	0.02
1PG	M	5.5	334.3	2.5	1.06	0.98	0.38	0.22	0.15	0.13	0.08	0.04	0.02
4G	F	7.3	433.3	3.12	1.33	0.69	0.5	0.35	0.27	0.19	0.13	0.06	0.04
6G	F	10.8	400.6	1.19	0.51	0.31	0.24	0.17	0.13	0.09	0.06	0.04	0.03
3G	F	7.0	357.7	1.17	0.81	0.5	0.43	0.31	0.28	0.22	0.20	0.09	0.05
1BGM	F	10.2	222.0	1.09	0.52	0.21	0.12	0.11	0.099	0.06	0.023	0.006	0.005
4BGMU	F	9.8	348.3	1.22	0.5	0.24	0.18	0.122	0.096	0.06	0.03	0.017	0.007

TABLE 11

Fraction of ^{14}C injected as ^{14}C-U-Glycerol present in glucose / ml plasma at each sampling time in adult dogs in the postabsorptive state

Experiment	Fraction of dose $ml^{-1} \times 10^4$										
	2	4	8	10	15	20	30	40	50	60	90
3G	1.45	2.45	2.65	2.26	2.63	2.63	2.48	2.29	2.18	2.05	1.55
6G	1.41	1.62	1.42	1.40	1.31	1.3	1.24	1.13	1.06	0.94	0.71
4G	0.87	1.51	1.63	1.54	1.47	1.41	1.33	1.21	1.13	1.02	0.81
1PG	3.4	3.45	3.38	3.6	3.11	2.95	2.77	2.53	2.45	2.21	1.86
4BGM	1.58	2.49	2.61	2.61	2.57	2.45	2.36	2.00	2.17	2.06	1.67
2BGM	1.05	1.7	1.85	1.74	1.74	1.64	1.53	1.4	1.32	1.19	0.87
3BGM	1.34	1.75	1.89	1.81	1.71	1.59	1.45	1.38	1.29	1.15	0.96
2G	1.38	1.65	1.64	1.65	1.58	1.45	1.44	1.3	1.27	1.17	1.01
5G	0.81	1.29	1.52	1.5	1.46	1.45	1.41	1.31	1.26	1.21	1.01
1BGM	2.78	3.23	3.28	3.17	3.09	2.79	2.57	2.29	2.19	1.97	1.3
4BGMU	0.918	1.15	1.25	1.04	0.97	0.887	0.776	0.694	0.613	0.058	0.47

TABLE 12

Fraction of initial tracer injection of ^{3}H-2-glucose in 1 ml plasma at each sampling time in adult dogs in the postabsorptive state

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1}										
		2	4	8	10	15	20	30	40	50	60	90
5G	136.8	7.99	6.38	5.13	4.79	4.55	4.25	3.63	3.36	3.29	3.11	2.35
2G	235.8	5.67	4.53	3.83	3.01	3.27	3.16	2.81	2.49	2.33	2.17	1.71
3BGM	156.2	5.48	4.33	3.52	3.48	2.97	2.85	2.52	2.08	2.17	1.82	1.39
2BGM	156.2	4.98	4.12	3.43	3.32	2.98	2.82	2.47	2.22	1.9	1.66	0.97
4BGM	156.5	3.68	3.16	2.70	2.48	2.26	2.05	1.83	1.61	1.68	1.51	1.22
1PG	156.6	13.08	10.41	8.59	8.01	6.85	5.95	5.21	5.01	4.39	4.13	2.79
4G	136.8	9.97	8.68	7.52	7.02	6.18	5.55	4.59	4.01	3.83	3.49	2.38
6G	122.4	7.12	5.37	4.84	4.14	3.83	3.3	2.88	2.61	2.37	2.08	1.56
3G	215.2	7.48	6.17	5.33	5.29	4.67	4.21	3.94	3.42	3.02	3.0	1.97
1BGM	133.2	10.13	7.8	6.4	5.93	5.53	5.04	4.47	4.18	3.32	2.89	1.72
4BGMU	156.6	5.13	4.29	3.14	2.95	2.63	2.3	1.95	1.71	1.45	1.28	0.96

TABLE 13

Fraction of initial tracer injection of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in fasted pups 0-4 days old.

Experiment	Days days	Sex	Weight Kg	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}						
					2	4	7	12	20	30	40
3FG1	4	F	0.255	83.3	16.86	8.24	3.78	1.88	1.11	0.70	0.54
3FG2	4	F	0.298	83.3	24.31	12.33	6.17	3.02	1.32	0.90	0.70
2FG1	2	F	0.320	83.3	7.75	4.09	2.21	1.09	0.44	0.22	0.13
2FG2	3	F	0.295	83.3	21.07	10.59	5.22	2.44	1.38	0.74	0.69
4FG1	2	M	0.343	79.7	27.32	12.55	7.14	3.91	2.21	1.42	0.95
5FG1	1	F	0,268	73.4	35.77	13.87	8.27	4.35	2.41	1.51	1.04

TABLE 14

Fraction of ^{14}C injected as ^{14}C-U-Glycerol present in glucose / ml plasma at each sampling time in fasted pups 0-4 days old

Experiment	Fraction of dose $ml^{-1} \times 10^4$							
	2	4	7	20	30	40	50	60
2FG1	12.3	12.41	12.09	9.94	8.24	6.24	5.77	5.4
2FG2	21.72	18.18	15.31	9.58	6.64	6.2	6.27	5.68
3FG1	35.35	25.17	21.61	13.92	10.49	7.96	7.10	5.78
3FG2	30.47	26.55	23.33	18.81	16.32	13.36	11.6	9.78
4FG1	10.91	11.32	10.65	8.36	7.04	6.38	5.54	5.92
5FG1	12.71	11.47	10.9	8.44	6.96	6.14	5.03	4.81

TABLE 15

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in fasted pups 0-4 days old

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}							
		2	4	7	20	30	40	50	60
3FG1	34.0	124.04	79.15	71.43	39.7	23.33	14.87	11.27	8.9
3FG2	34.0	114.4	88.36	75.96	52.09	44.97	35.72	27.91	22.52
4FG1	34.0	83.69	64.51	52.97	31.44	23.02	17.24	14.17	12.5
2FG1	34.0	82.75	64.26	54.79	35.64	26.52	19.59	15.27	11.01
2FG2	34.0	81.36	63.15	51.43	27.01	16.16	11.64	8.57	7.12
5FG1	34.0	131.14	87.45	73.00	49.06	39.7	32.51	24.24	20.17

TABLE 16

Fraction of initial tracer injection of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in fasted pups 5-19 days old

Experiment	Age days	Sex	Weight Kg	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}						
					2	4	7	12	20	30	40
2FG3	5	M	0.460	83.3	19.15	10.81	6.42	3.07	1.48	0.83	0.63
2FG4	11	M	0.850	111.1	10.84	5.32	3.06	0.99	0.63	0.47	0.36
1FG1	8	F	0.639	80.7	21.48	6.60	3.80	1.95	1.08	0.73	0.54
4FG4	19	F	0.890	99.0	9.5	3.84	2.57	1.27	0.61	0.39	0.28
1FG2	10	F	0.619	80.7	15.36	9.14	6.18	3.03	1.43	0.52	0.35

TABLE 17

Fraction of ^{14}C injected as ^{14}C-U-Glycerol present in glucose / ml plasma at each sampling time in fasted pups 5-19 days old

Experiment	Fraction of dose $ml^{-1} \times 10^4$								
	2	6	10	20	25	40	50	60	80
1FG1	19.72	15.25	13.07	10.8	9.97	8.46	8.03	7.69	6.79
1FG2	13.32	13.26	12.72	12.87	12.14	12.1	12.06	11.21	10.22
2FG3	16.1	15.79	14.97	13.63	13.34	12.82	12.60	11.60	-
2FG4	13.7	12.00	11.13	10.41	10.64	9.66	9.10	8.86	-
4FG4	13.87	11.05	9.99	8.80	8.71	8.43	7.95	7.63	-

TABLE 18

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in fasted pups 5-19 days old

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}								
		2	6	10	20	25	40	50	60	80
1FG1	34.2	64.98	33.86	28.19	21.84	21.32	15.7	12.48	12.19	8.64
1FG2	34.2	53.12	38.46	33.29	27.26	26.91	19.89	19.34	18.21	13.92
2FG3	45.4	73.67	53.95	46.37	39.07	35.23	26.56	22.31	19.54	-
2FG4	45.4	52.22	34.98	28.72	25.44	22.38	18.86	18.74	16.23	-
4FG4	45.4	50.95	30.86	26.91	20.47	20.02	17.09	15.84	14.05	-

TABLE 19

Fraction of initial tracer injection of ^{14}C-U-Glycerol in 1 ml plasma at each sampling time in fasted adult dogs

Experiment	Weight Kg	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}									
			2	4	6	8	10	12	15	20	30	40
1F	7.5	392.5	1.79	0.88	0.60	0.38	0.29	0.23	0.16	0.10	0.06	0.03
2F	10.4	391.6	1.6	0.68	0.42	0.29	0.21	0.16	0.11	0.07	0.05	0.03

TABLE 20

Fraction of ^{14}C injected as ^{14}C-U-Glycerol present in glucose / ml plasma at each sampling time in fasted adult dogs

Experiment	Fraction of dose $ml^{-1} \times 10^4$								
	2	4	8	20	30	40	50	60	90
1F	0.89	1.28	1.41	1.41	1.41	1.38	1.30	1.28	1.45
2F	0.779	1.188	1.42	1.48	1.44	1.42	1.38	1.302	1.14

TABLE 21

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in fasted adult dogs

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}								
		2	4	8	20	30	40	50	60	90
1F	136.1	9.98	8.72	7.5	6.52	6.13	5.27	4.89	4.64	3.87
2F	136.1	6.21	5.47	4.89	4.18	3.73	3.64	3.2	2.84	2.13

TABLE 22

Fraction of dose injected as ^{14}C-U-Glucose appearing in Glycerol in 1 ml. of plasma at each sampling time

Experiment	Age Days	Fraction of dose ml^{-1} X 10^6											
		2	4	6	8	10	15	20	30	40	60	80	90
2GRCA	Adult	-0.6	-0.8	-0.8		-0.9	-0.7		-0.7		-0.6		-0.6
1GRC1	3	-2.0	-2.0		-0.2			-0.3		-1.0	-2.0	-3.0	
1GRC2	7	6.0	4.0		10.0			9.0	1.0	4.0			
1GRC3	8	3.0	1.0					0.5		-3.0		+2.0	
1GRC4	28	-1.0	-0.7		-0.2		-0.9	-0.3		0.01	-0.6	-0.8	-1.0

TABLE 23

Steady state concentration of glycerol and glucose in plasma. (Mean values ± s.e.m.)

Age (day)	Number of Animals	Glycerol mg/ml	Glucose mg/ml
A. Postabsorptive:			
0-4	6	0.0152 ± 0.0034	0.798 ± 0.062
7-11	5	0.00681 ± 0.0013	1.065 ± 0.079
12-30	5	0.00599 ± 0.00074	1.142 ± 0.118
Adult	10	0.00410 ± 0.00046	1.017 ± 0.030
B. Fasted			
0-4	6	0.00410 ± 0.0008	0.451 ± 0.058
5-19	5	0.00935 ± 0.0016	0.866 ± 0.044
Adult	2	0.00688 ± 0.0022	0.973 ± 0.040

TABLE 24

Rate constants (min^{-1}) ± S.D. of the Glycerol: Glucose System as shown on Fig. 3.

Age (days)	$L_{1,2}$	$L_{2,1}$	$L_{0,1}$	$L_{3,1}$	$L_{3,4}$	$L_{4,3}$	$L_{0,3}$
A. Postabsorptive:							
0-4	0.110 ±0.008	0.152 ±0.020	0.151 ±0.0136	0.132 ±0.0143	0.203 ±0.0195	0.971 ±0.165	0.103 ±0.018
7-11	0.071 ±0.009	0.085 ±0.012	0.136 ±0.016	0.131 ±0.016	0.256 ±0.044	0.915 ±0.327	0.082 ±0.025
12-30	0.108 ±0.007	0.103 ±0.012	0.163 ±0.013	0.164 ±0.0147	0.235 ±0.023	0.721 ±0.190	0.059 ±0.013
Adult	0.079 ±0.006	0.078 ±0.007	0.121 ±0.006	0.114 ±0.005	0.141 ±0.019	0.144 ±0.029	0.017 ±0.001
B. Fasted:							
0-4	0.069 ±0.008	0.115 ±0.014	0.164 ±0.015	0.048 ±0.005	0.168 ±0.017	0.011 ±0.16	0.215 ±0.039
5-19	0.079 ±0.009	0.123 ±0.017	0.131 ±0.013	0.097 ±0.011	0.180 ±0.018	0.805 ±0.179	0.067 ±0.015
Adult	0.078 ±0.008	0.086 ±0.009	0.158 ±0.011	0.068 ±0.004	0.153 ±0.071	0.069 ±0.046	0.009 ±0.001

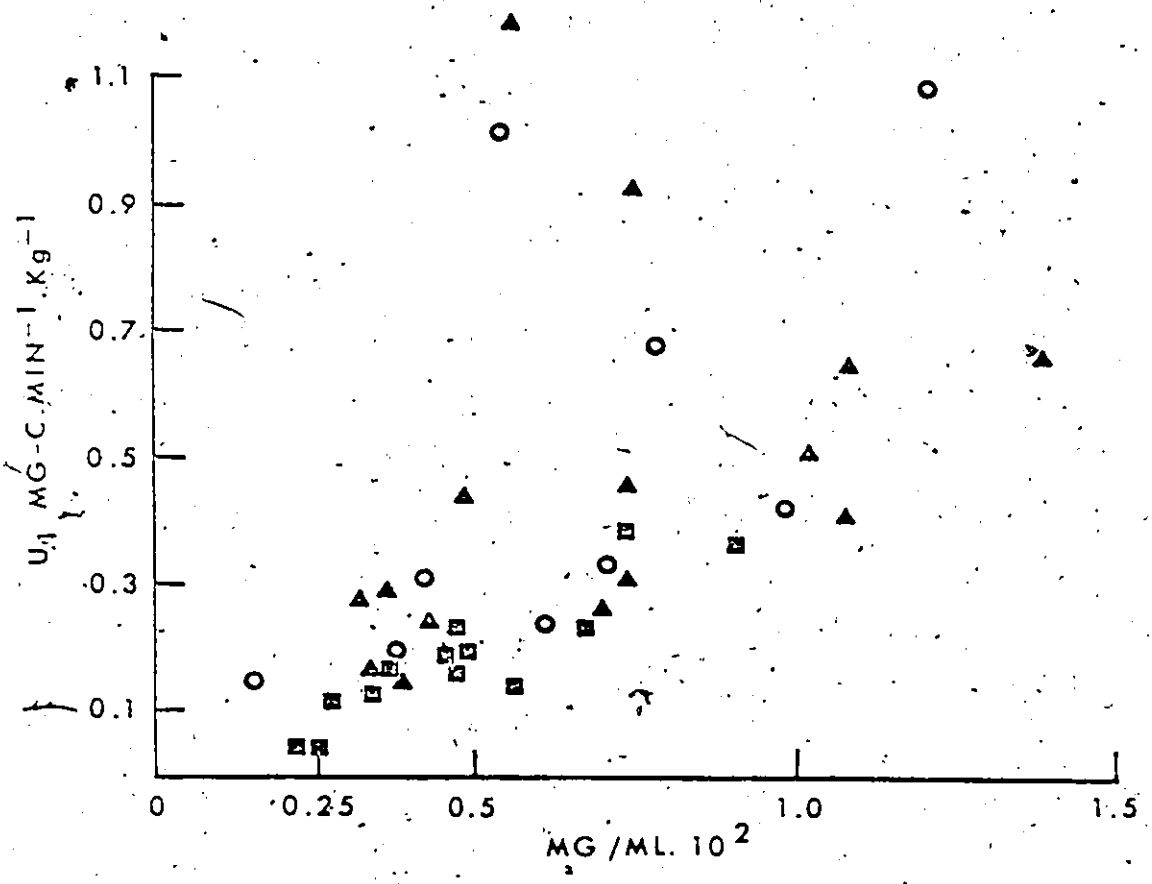
Total plasma equivalent volumes for the glycerol subsystem ($V_1 + V_2$) and the glucose subsystem ($V_3 + V_4$) together with compartmental carbon masses (M_1) and transports (U_1 or $R_{i,j}$) are shown on Table 25. All rates have been expressed per kg. body weight. Comparing the respective groups of fasted with non fasted pups, it is evident that the plasma equivalent volume of the glycerol subsystem was increased by fasting. No such difference was found in adult dogs.

As shown on Table 25, U_1, the irreversible disposal rate of glycerol decreases with age. Fasting increases U_1 except in the youngest pups in which the average U_1 was decreased to about one-third of the value calculated in the postabsorptive state. The conversion of glycerol to glucose defined as the transfer of C-atoms from the glycerol to the glucose subsystem, $R_{3,1}$ was increased by fasting in adult dogs but significantly decreased in pups less than 4 days of age. The turnover rate of glucose was decreased by fasting in all age groups.

The relationship between the concentration of glycerol in plasma and U_1 for individual animals in all age groups and both nutritional states is shown on Fig. 8. The linear equation $U_1 = 72.45.\bar{C} - 0.088$ ($r = 0.88$) was fitted to the data. The spread around the calculated regression line is larger at plasma concentrations above 0.5 mg/100 ml. This is due entirely to some values obtained in pups. Fitting the best linear equation to only the data from adult dogs $U_1 = 45.2C - 0.033$ ($r = 0.90$). The intercept of neither equation differed significantly from zero.

Fig. 8

The irreversible disposal rate of Glycerol U_1(mgC·Min^{-1}Kg^{-1}) plotted against the plasma concentration of Glycerol



o pups less than 3 days old; ▲ 7-30 days old.
▪ Adults.

The percentage of glucose-C derived from glycerol and the percentage of glycerol-C converted to glucose are given in Table 26. The percentage of glucose-C derived from glycerol was calculated as $(R_{3.1}/U_1).100$. In the postabsorptive state a higher percentage of glucose-C originates from glycerol in the youngest pups than at any later age. Fasting decreases this percentage in the youngest group, increases it in older animals. About one half of the glycerol-C atoms was converted into glucose in the postabsorptive state in all groups. In fasting this percentage decreased in adults and the youngest pups but was maintained by the intermediate age group.

Table 27 shows some of the parameters which were calculated for the only unanaesthetized dog 4BGMU. Both U_1 and $R_{0,3}$ were in the upper part of the range for the anaesthetized dogs. The percentage of glucose-C arising from glycerol was very close to the mean value calculated for the ten anaesthetized dogs in the postabsorptive state.

Table 28 shows the values of the physiologically most important parameters of the glycerol:glucose system as calculated from the averaged data for each age group and as the average of the values calculated for the individual animal. The former gives the better estimate of the mean for the group and the latter the better estimate of the mean of the scatter within the parameter. The corresponding magnitudes are expected to differ. However, in most instances the discrepancy is small.

TABLE 25

Steady state values for the parameters of the glycerol:glucose system as shown on Fig. 2.

Age days	V_1+V_2 Plasma equivalent volumes as a % of body weight	V_3+V_4	M_1+M_2 Compartmental masses mgC Kg^{-1}	M_3+M_4	U_1 Irreversible Disposal Rate of glycerol	U_3 Glucose-C from sources other than glycerol	$U_{3,1}$ Rate of transfer of glycerol to glucose	$R_{0,1}$ Glycerol-C to sources other than glucose	$R_{0,3}$ Rate of glucose turnover
					mg $Cmin^{-1}$ Kg^{-1}				
0-4	131.2	40.8	7.76	135.6	0.92	2.68	0.44	0.48	3.12
7-11	146.3	37.2	3.88	158.0	0.48	3.66	0.23	0.24	3.68
12-30	79.0	41.0	1.88	186.0	0.32	3.38	0.156	0.16	3.54
Adult	66.8	22.6	1.1	89.6	0.13	0.96	0.064	0.068	1.02
B. Fasted									
0-4	235.4	31.9	3.83	57.68	0.31	1.97	0.07	0.238	2.04
5-19	223.0	34.9	5.4	120.9	0.48	1.72	0.196	0.268	1.92
Adult	77.8	18.5	2.36	71.3	0.25	0.5	0.076	0.176	0.58

TABLE 26

Percentage of glucose-C derived from glycerol and percentage conversion of glycerol-C to glucose. Percentages calculated from averaged data of each age group.

Age (days)	Number of Animals	Glucose-C from glycerol %	Glycerol-C to glucose %
A. Postabsorptive			
0-4	6	13.8	46.9
7-11	5	6.2	49.3
12-30	5	4.5	50.1
Adult	10	6.1	48.8
B. Fasted			
0-4	6	3.4	22.6
5-19	5	10.3	43.1
Adult	2	13.3	30.1

TABLE 27

Concentrations, Rates of transfer of Carbon atoms and percentage interconversions for one ananaesthetized dog

Experiment	Concentration mg % X 1		Rates mg C min^{-1} kg^{-1}			% Conversions	
	Glucose	Glycerol	U_1	$R_{0,3}$	$R_{3,1}$	Glucose-C from Glycerol	Glycerol-C to Glucose
4BGMJ	127.2	0.734	0.39	2.12	0.135	6.37	34.6

TABLE 28

Rates of turnover and conversion of carbon in the glucose:glycerol system calculated from averaged data for each age group and as the average ±s.e.m. of individual experiments.

Age (days)	Number of animals	U_1=irreversible disposal rate of glycerol		$R_{0,3}$=rate of utilization of glucose		$R_{3,1}$=Rate of transfer glycerol to glucose-C		Glucose-C from glycerol %		Glycerol-C from glucose %	
		Aver.	Individ.	Aver.	Individ.	Aver.	Individ.	Aver.	Individ.	Aver.	Individ.
A. Postabsorptive											
0-4	6	0.92	0.96±0.27	3.12	3.16±0.72	0.440	0.545±0.290	13.8	16.4±3.3	46.9	41.2±9.8
7-11	5	0.48	0.47±0.14	3.68	3.41±0.36	0.230	0.231±0.081	6.2	6.4±1.7	49.3	44.6±5.7
12-30	5	0.32	0.44±0.18	3.54	3.94±0.77	0.156	0.198±0.063	4.8	6.7±3.3	50.1	50.4±3.9
Adult	10	0.13	0.15±0.02	1.02	1.09±0.12	0.064	0.086±0.018	6.1	7.7±1.5	48.8	51.5±6.2
B. Fasted											
0.4	6	0.31	0.42±0.17	2.04	1.94±0.14	0.070	0.096±0.038	3.4	4.7±1.5	22.6	29.2±8.0
5-19	5	0.48	0.43±0.07	1.92	1.86±0.13	0.196	0.188±0.033	10.3	10.5±2.0	43.1	43.6±1.7
Adult	2	0.25	0.25±0.09	0.58	0.58±0.10	0.076	0.071±0.011	13.3	15.6±7.0	30.1	30.1±7.4

All rates are expressed as $mgC.kg^{-1}.min^{-1}$

PART II

THE EFFECT OF AGE ON THE ALANINE:GLUCOSE SYSTEM

The response data from all animals which received a single simultaneous injection of ^{14}C-U-alanine and ^{3}H-2-glucose are shown on Tables 29 - 40 inclusive. This is the basic data from which all parameters were calculated and the model of the alanine:glucose system constructed.

Table 41 shows the rate constants ($L_{i,j}$) expressed as min^{-1} calculated for the model Fig. 7. However this model, requiring further validation, has not been used to calculate any of the physiological parameters given in this section.

Steady state plasma concentrations of alanine and glucose, together with the alanine and glucose turnover rates and the percentage interconversions are given in Table 42.

Both rates of turnover are highest in the youngest age group and decline to the lowest level in adults. In all pups the concentration of alanine in plasma was slightly lower than in adults, ($t = 1.97$, $0.1 > p > 0.05$) no difference being found between older and younger pups. In these animals there was no change in plasma glucose concentration with age.

The percentage of glucose carbon originating from plasma alanine was highest in the youngest pups, older pups deriving the same percentage of glucose-C from alanine as adult dogs. These differences

were probably not statistically significant because the calculation method employed relied upon extrapolation of the data (SA vs time).

In both the youngest pups and adult dogs the same percentage of alanine-C as calculated by a deconvolution was transferred to circulating glucose.

TABLE 29

Fraction of the initial tracer injection of ^{14}C-U-Alanine in 1 ml plasma at each sampling time in pups 0-5 days old

Experiment	Sex	Age days	Weight Kg.	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^4							
					3	6	12	20	40	60	80	90
3BA3	F	4	0.418	121.82	14.8	9.9	5.38	1.89	0.87	0.61	0.4	0.36
3BA1	M	1	0.344	125.7	15.19	5.85	2.88	1.94	1.03	0.87	0.76	0.72
3BA2	F	1	0.378	125.7	13.17	6.92	3.53	1.98	1.00	0.65	0.64	0.7
4BA1	M	2	0.326	106.9	24.07	8.7	4.7	3.13	0.94	0.64	0.46	0.45
4BA2	F	2	0.315	106.9	14.13	7.32	4.66	2.2	0.86	0.64	0.5	0.49
2BA2	M	5	0.266	128.6	16.59	6.98	3.69	2.08	0.86	0.45	0.35	0.38

TABLE 30

Fraction of the ^{14}C injected as alanine present in Glucose / ml plasma in pups 0-5 days old

Experiment	Fraction of the dose $ml^{-1} \times 10^4$								
	3	6	12	20	40	60	80	90	120
3BA2	4.69	5.12	5.29	5.51	4.88	3.42	2.75	2.42	1.94
4BA1	4.4	5.53	6.69	5.32	6.17	5.31	4.55	4.19	3.15
4BA2	4.81	4.66	5.0	7.15	5.14	5.2	4.41	4.06	4.33
3BA1	6.25	6.32	6.53	6.83	6.55	6.44	5.9	5.65	4.91
3BA3	3.35	3.75	4.54	5.61	5.59	5.09	4.67	4.46	3.83
2BA2	5.61	7.72	6.59	6.94	5.78	4.01	3.35	3.02	2.13

TABLE 31

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in pups 0-5 days old

Experiment	Dose dpm x 10^{-6}	Fraction of the dose ml^{-1} x 10^{4}								
		3	6	12	20	40	60	80	90	120
2BA2	41.7	74.22	63.1	40.07	33.95	18.24	10.52	6.91	5.44	2.32
4BA2	42.9	72.45	59.97	42.09	38.37	25.69	21.39	15.08	13.8	9.96
4BA1	42.6	63.24	61.4	50.66	39.5	29.59	22.54	15.5	11.49	7.49
3BA3	45.1	65.88	59.85	48.66	38.43	29.49	22.74	18.99	16.28	12.96
3BA2	49.9	63.6	49.7	43.3	34.83	22.29	11.91	7.92	5.77	2.98
3BA1	49.9	64.22	46.07	41.47	35.17	24.61	22.22	20.83	16.41	10.88

TABLE 32

Fraction of initial tracer injection of ^{14}C-U-Alanine in 1 ml plasma at each sampling time in pups 6-11 days old

Experiment	Sex	Age days	Dose dpm x 10^{-6}	Weight Kg.	Fraction of dose ml^{-1} x 10^4					
					3	6	12	20	40	60
2BA3	M	11	130.2	0.48	10:63	3.94	1.81	1.42	0.65	0.50
3BA4	M	10	162.4	0.815	4.79	2.82	1.52	1.08	0.42	0.39
5BA1	F	6	245.2	0.425	8.27	2.99	1.16	0.79	0.34	0.31
5BA2	M	7	245.2	0.45	17.65	6.16	3.81	2.06	0.68	0.41

TABLE 33

Fraction of ^{14}C injected as ^{14}C-U-Alanine present in glucose / ml plasma in pups 6-11 days old

Experiment	Fraction of dose $ml^{-1} \times 10^4$								
	3	6	12	20	40	60	90	110	120
2BA3	4.21	4.68	4.04	4.05	3.41	3.08	2.51	2.14	2.07
3BA4	3.73	4.18	3.91	4.01	3.23	3.04	2.57	2.35	2.24
5BA1	2.78	3.51	3.34	2.85	2.56	2.04	1.62	1.45	1.38

TABLE 34

Fraction of the initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in pups 6-11 days old

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}								
		3	6	12	20	40	60	90	110	120
2BA3	41.7	51.03	41.9	31.59	29.2	23.02	16.75	11.68	9.57	8.14
3BA4	60.2	30.65	25.83	20.6	19.18	12.91	10.02	6.25	5.74	5.49
5BA1	63.1	40.4	35.75	28.18	19.79	14.18	8.74	5.81	4.63	4.13

TABLE 35

Fraction of initial tracer injection of ^{14}C-U-Alanine in 1 ml plasma at each sampling time in pups 12-60 days old

Experiment	Sex	Age days	Weight Kg.	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}						
					3	6	12	20	30	40	60
3BA5	M	23	1.785	192.7	8.83	2.95	1.59	0.88	0.62	0.47	0.25
1MA3	M	60	2.85	238.8	2.28	1.31	0.74	0.43	0.29	0.14	0.12
3BA6	M	36	1.94	218.4	2.83	2.29	1.38	0.79	0.58	0.42	0.1
1BA2	M	13	0.494	128.6	13.99	8.19	5.41	3.24	2.18	1.12	0.63

TABLE 36

Fraction of ^{14}C injected as ^{14}C-U-Alanine present in glucose / ml plasma at each sampling time in pups 12-60 days old

Experiment	Fraction of dose $ml^{-1} \times 10^4$											
	3	6	9	12	20	30	40	60	80	100	120	140
3BA5	0.886	0.969	1.13	1.22	1.40	1.50	1.49	1.29	1.18	0.92	1.03	0.82
3BA6	0.74	0.898	1.05	1.21	1.51	1.55	1.57	1.55	1.38	1.17	1.06	0.96
1BA2	1.78	2.42	3.00	3.57	4.22	4.22	4.23	4.21	3.96	3.03	2.59	2.23
1MA3	0.725	0.795	0.87	0.945	0.989	0.946	0.903	0.884	0.77	0.659	0.572	0.479

TABLE 37

Fraction of initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in pups 12-60 days old

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}										
		3	6	9	12	30	40	60	80	100	120	140
1BA2	49.1	22.78	20.41	18.19	17.85	15.56	13.28	16.21	11.68	8.82	7.38	6.17
3BA6	103.4	15.79	14.3	11.3	10.56	8.96	7.36	5.54	3.84	2.86	2.02	1.7
1MA3	111.3	14.98	11.88	10.43	8.4	6.89	5.37	4.04	2.58	1.54	1.31	0.77
3BA5	94.4	22.64	18.21	13.05	11.2	10.06	9.2	6.64	4.94	4.76	3.17	2.52

TABLE 38

Fraction of the initial tracer injection of ^{14}C-U-Alanine in 1 ml plasma at each sampling time in adult dogs.

Experiment	Weight Kg.	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}											
			3	6	9	12	20	25	30	40	60	80	100	120
3BAM	10.6	372.8	1.49	0.80	0.49	0.45	0.31	0.24	0.19	0.15	0.11	0.07	0.05	0.04
1BAM	9.75	428.8	1.41	0.47	0.37	0.40	0.28	0.25	0.21	0.16	0.13	0.05	0.03	0.01
1MAM	7.4	385.4	3.19	1.78	1.47	1.16	0.93	0.56	0.44	0.37	0.32	0.21	0.12	0.13
4BAM	8.2	520.4	1.07	0.55	0.44	0.33	0.2	0.15	0.13	0.09	0.07	0.05	0.03	0.01
2BAM	8.8	406.0	0.98	0.54	0.37	0.29	0.19	0.16	0.13	0.11	0.09	0.06	0.05	0.03

TABLE 39

Fraction of ^{14}C injected as ^{14}C-U-Alanine present in glucose / ml plasma at each sampling time in adult dogs

Experiment	Fraction of dose $ml^{-1} \times 10^4$														
	3	6	9	12	20	25	30	40	60	80	100	120	140	180	210
1MAM	0.231	0.246	0.259	0.286	0.357	0.383	0.409	0.395	0.416	0.42	0.391	0.323	0.318	0.273	0.259
1BAM	0.115	0.164	0.171	0.177	0.22	0.206	0.192	0.205	0.207	0.205	-	0.187	0.172	0.155	0.142
2BAM	0.226	0.174	0.189	0.204	0.245	0.248	0.252	0.242	0.239	0.224	0.204	0.198	0.172	0.148	0.112
4BAM	0.182	0.194	0.206	0.208	0.213	0.199	0.185	0.171	0.143	-	0.106	0.09	0.075	0.069	0.066
3BAM	0.298	0.299	0.301	0.318	0.363	0.378	0.393	0.39	0.393	0.361	0.363	0.352	0.327	0.277	0.224

TABLE 40

Fraction of the initial tracer injection of ^{3}H-2-Glucose in 1 ml plasma at each sampling time in adult dogs

Experiment	Dose dpm x 10^{-6}	Fraction of dose ml^{-1} x 10^{4}												
		3	6	9	20	30	40	60	80	100	120	140	180	210
2BAM	180.5	5.01	3.9	3.12	2.73	2.47	2.1	1.8	1.52	1.25	1.0	0.87	0.54	0.45
4BAM	231.3	3.94	3.42	2.89	2.29	1.97	1.6	1.28	0.91	0.71	0.54	0.46	0.33	0.22
1MAM	165.1	8.51	7.72	6.93	5.28	4.84	4.4	3.63	2.92	2.25	1.72	1.44	0.81	0.69
1BAM	156.6	4.26	3.40	3.03	2.55	1.95	1.84	1.53	1.21	0.87	0.78	0.59	0.36	0.25
3BAM	178.2	5.98	5.04	4.10	3.55	3.16	2.76	2.33	1.88	1.47	1.22	1.07	0.84	0.66

TABLE 41

Rate Constants (min^{-1}) of the Alanine:Glucose System and Initial Conditions (IC) in the Alanine System

	Rate Constants min^{-1}											
Age Days	$L_{11,5}$	$L_{4,11}$	$L_{4,8}$	$L_{3,5}$	$L_{3,4}$	$L_{4,3}$	$L_{0,3}$	$L_{0,6}$	$L_{6,7}$	$L_{7,6}$	$L_{7,8}$	$L_{8,7}$
0-5	0.55	0.358	0.00256	0.25	0.166	0.628	0.0848	0.146	0.492	0.760	0.0172	0.0996
6-11	0.192	0.17	0.00148	0.42	0.0784	0.046	0.0209	0.149	0.12	0.332	0.0193	0.0554
12-60	0.272	0.0719	-	0.162	0.163	0.103	0.0248	0.00924	0.0719	0.277	0.0030	0.0642
Adult	0.632	0.2	0.00338	0.433	0.104	0.698	0.0179	0.0541	0.0532	0.0928	0.00566	0.00919

Age Days	Fraction of dose injected into compartments 5 and 6	
	IC_5	IC_6
0-5	0.114	0.886
6-11	0.159	0.841
12-60	0.147	0.853
Adult	0.086	0.914

TABLE 42

Parameters Calculated for the Alanine:Glucose System in Adult Dogs and Pups

Mean ± S.E.M.

Age Days	0-5 (n=7)	6-60 (n=9)	Adult (n=5)
Alanine (RT mg C min^{-1} kg^{-1})	1.49 ±0.25	1.0 ±0.08	0.61 ±0.13
Glucose RT mg C min^{-1} kg^{-1}	3.36 ±0.25	2.92 ±0.36	1.2 ±0.16
Alanine plasma concentration mg%	2.36 ±0.38	2.41 ±0.44	3.5 ±0.22
Glucose plasma concentration mg%	95.2 ±10.34	108.6 ±9.1	104.1 ±1.89
% of Glucose C originating from plasma Alanine	18.46 ±2.3	10.8 ±2.6	10.77 ±3.3
% of Alanine C transferred to Glucose (calculated from averaged data)	30.1	-	28.7

PART III

THE EFFECT OF INSULIN-INDUCED HYPOGLYCAEMIA ON PLASMA ALANINE AND BLOOD AMINO-N CONCENTRATION

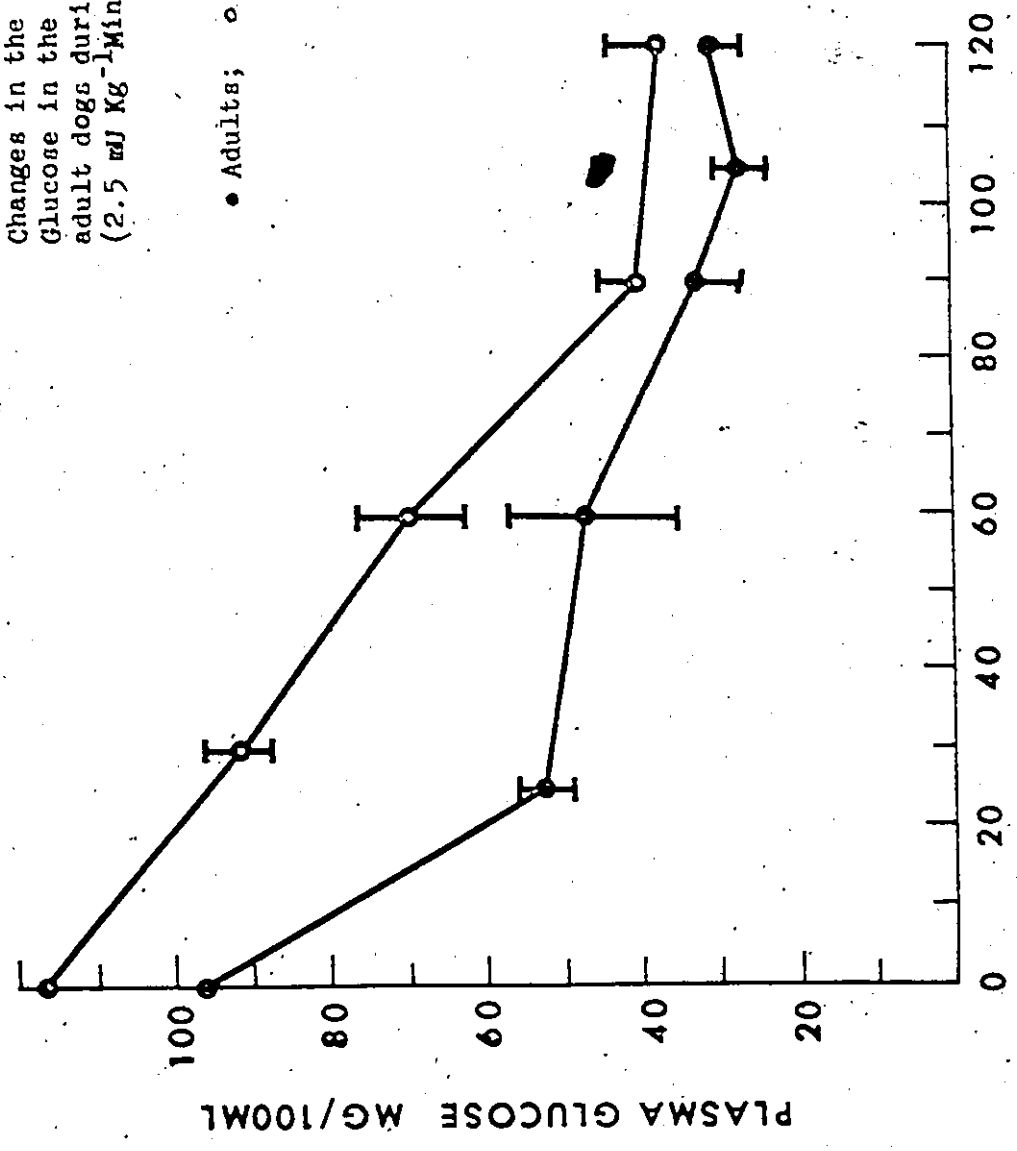
Figure 9 shows the changes in the concentration of glucose in plasma during the infusion of insulin. Although the initial fall is less in pups than it is in adult after 60 minutes, the same low level is reached in both pups and adults. No changes in plasma glucose were observed in control animals.

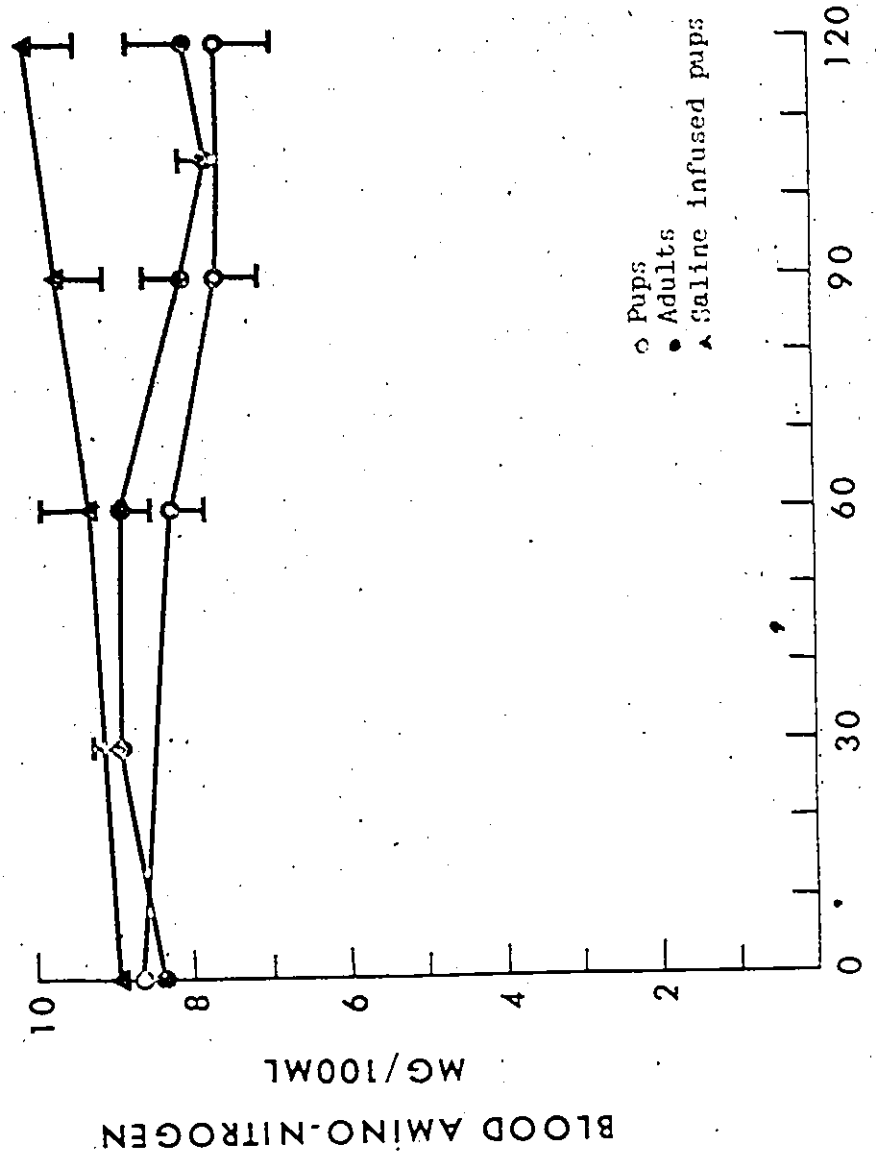
As shown on Figure 10 the amino-N concentration decreased during the infusion of insulin in both pups and adult dogs. A slight increase during the infusion of saline was observed in pups. Analysis of variance revealed both the rise ($p<0.025$) and the decrease ($p<0.01$) to be significant. The slight decrease seen in adult dogs was also found to be significant ($p< 0.05$). No amino-N determinations were carried out on control adults.

Figure 11 shows the percentage changes in the concentration of plasma alanine during the infusion of insulin. This presentation was chosen to focus on any changes with age and to eliminate the variations in concentration between animals. Analysis of variance carried out with the original data, i.e. the concentrations in plasma, showed that in pups both the interanimal variation and the decrease of concentration is highly significant ($p< 0.01$). In adult dogs only the interanimal variation was significant ($p< 0.01$) but not the decrease in concentration ($p<0.05$). No trend in the concentration of plasma, alanine with time was observed in control animals.

Fig. 9

Changes in the concentration of Glucose in the plasma of pups and adult dogs during insulin infusion ($2.5\ mU\ Kg^{-1}Min^{-1}$)

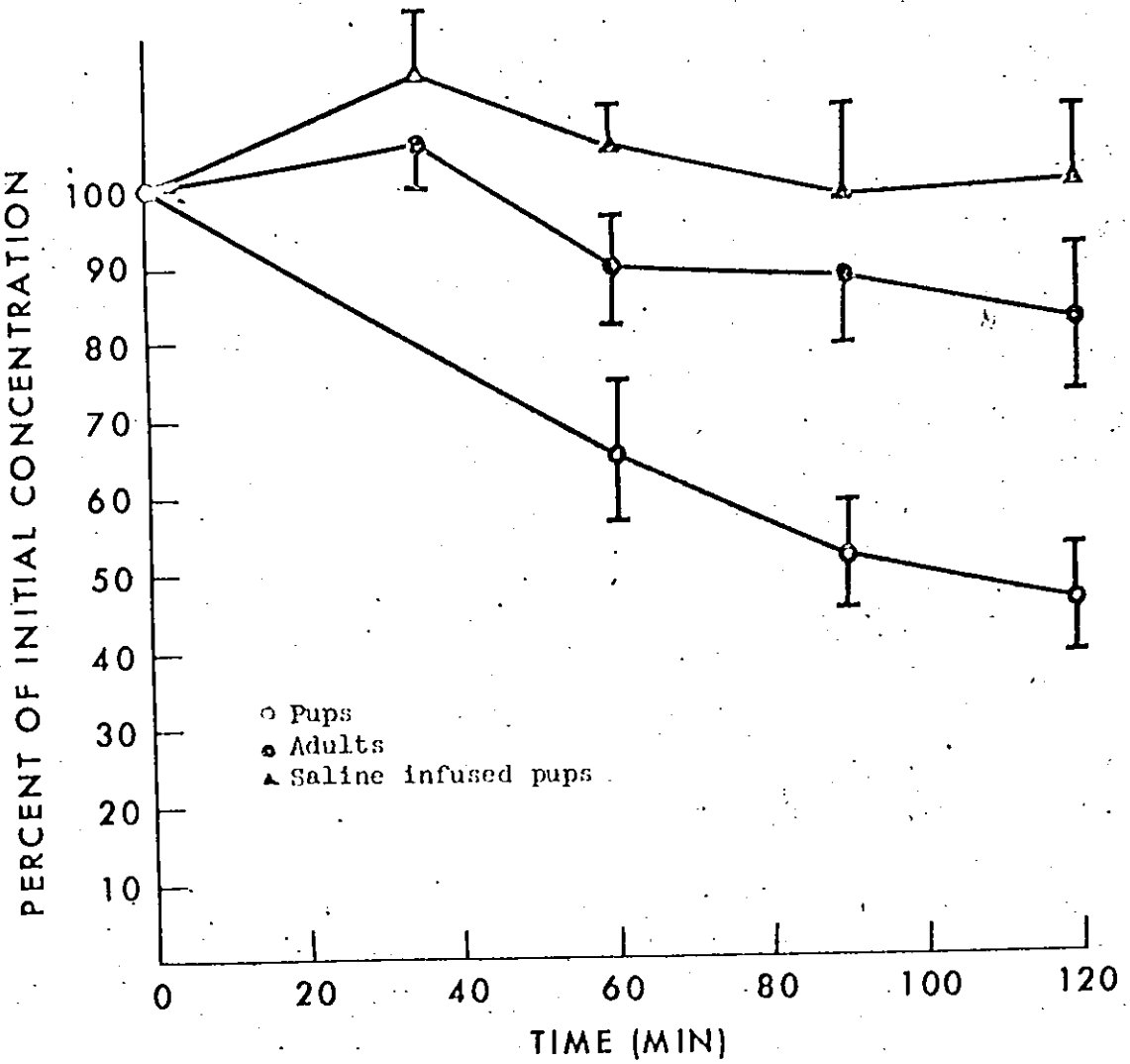




Changes in the concentration of Amino-N in the blood of pups and adult dogs

Fig. 10

Fig. 11 Changes in the concentration of alanine in plasma of pups and adult dogs.



PART IV

RE-INCORPORATION OF CARBON ATOMS INTO PLASMA GLUCOSE IN NEWBORN DOGS

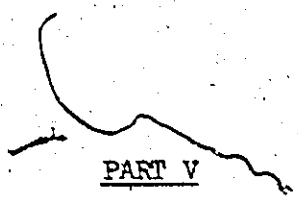
Table 43 gives the glucose turnover rate and the irreversible disposal rate calculated for each pup. The pups have been divided into three groups to show any differences which might occur in the first two days of life, between three and eight days of age, or in older pups. Mean values ± S.E.M. are shown for each age group. The differences between age groups were not statistically significant despite the low mean plasma glucose concentration in the youngest pups. The overall ratio for all groups was 1.35 with a standard deviation of ± 0.28 and a standard error of the mean of 0.08.

Plasma glucose concentrations are also shown on Table 43. Those pups less than 2 days old had considerably lower plasma glucose concentrations than either of the older groups.

Table 43

Glucose Turnover (RT) in pups calculated using ^{3}H-2-Glucose and ^{14}C-U-Glucose as tracers. Mean ± S.E.M.

Experiment	Age days	Plasma Glucose Concentration mg%	RT(^{3}H) mg min^{-1} kg^{-1}	RT(^{14}C) mg min^{-1} kg^{-1}	Ratio
1SP1	1	24	7.6	6.3	1.21
2SP1	1	37	8.4	6.3	1.33
1SP2	1	77	6.3	6.2	1.02
2SP2	1	49	8.0	6.1	1.28
2SP3	2	55	7.7	6.1	1.26
Mean		48.4	7.6	6.2	1.22
S.E.M.±		±8.9	±0.35	±0.044	±0.05
1GRC1	3	107	8.2	5.5	1.49
1SP4	4	109	6.3	5.9	1.07
1RC1	4	185	11.4	10.3	1.11
2GRC2	7	110	7.7	3.7	2.08
1GRC3	8	121	7.7	6.0	1.28
Mean		126.4	8.26	6.28	1.40
S.E.M.±		±14.9	±0.85	±1.1	±0.18
1ARC1	22	103	8.8	5.8	1.52
1GRC4	28	139	6.6	5.3	1.25
1RC2	33	143	6.8	4.2	1.62
Mean		128.3	7.4	5.1	1.46
S.E.M.±		±12.72	±0.702	±0.47	±0.11
			Overall Mean ± S.E.M.		1.35 ±0.08



PART V

THE EFFECTS OF AGE AND FASTING ON PLASMA GLUCAGON CONCENTRATION

Table 44A gives the plasma glucagon ($pg.ml^{-1}$) and glucose (mg/100 ml) concentration for pups in the postabsorptive state. High glucagon concentrations were found in pups less than 4 days old. In older pups the mean concentration has practically reached that found in adult dogs (188). The mean plasma glucose concentration was lower in the youngest age group.

The concentrations found in fasted pups and adult dogs are shown in Table 44B. Lower glucagon concentrations were found in fasted pups, less than four days old than in their counterparts in the post-absorptive state ($t = 2.5$ $p < 0.05 > 0.02$). Significant differences were also found in glucagon and glucose concentration between fasted pups, less than four days old and older fasted pups.

The relationship between the concentrations of glucagon and glucose in plasma for individual animals in both nutritional states is shown on Figure 12. A multiple linear regression $IRG = -4.95.Age - 1.497.Glucose\ \bar{C} + 495.17$ was fitted to the data for the pups in the postabsorptive state. The linear regression coefficients were not significant but the variables were significantly correlated. r (age:$\bar{C}$ glucose) = 0.72 $p < 0.01$, r (age:IRG) = -0.569 $p < 0.01$ r ($\bar{C}$ glucose: glucagon) = -0.581 $p < 0.01$. Fitting the best linear equation to only

the data from fasted pups glucagon $\bar{C}$ = 1.27.glucose C + 249.8 (r = 0.71).

On Table 45 glucagon concentrations (mean ± S.E.M.) are given for pups in the postabsorptive state and those with spontaneous hypoglycaemia, insulin-induced hypoglycaemia and fasting-induced hypoglycaemia. Basal values are given for all pups with glucose concentrations above 66 mg/100 ml. plasma. The values in pups can be compared to the values obtained in the same pups before insulin treatment, the difference not being significant. Pups with spontaneous hypoglycaemia had higher glucagon levels than their counterparts in the postabsorptive state ($t = 1.7$, $p < 0.1$). The difference between glucagon levels in pups with fasting-induced hypoglycaemia and those in spontaneous hypoglycaemia was highly significant ($t = 3.9$, $p < 0.01$).

TABLE 44

Plasma Glucagon and Glucose Concentration in pups in the postabsorptive state and pups and adult dogs during fasting. Mean ± S.E.M.

Age	Glucagon (I.R.G.) mg ml^{-1}	Glucose mg %
A: Postabsorptive		
0-4 (n = 13)	394 ± 48.4 ↑ t = 3.25, $p < 0.01$ ↓	69.3 ± 9.1
older pups (n = 10)	198 ± 26.8	132.2 ± 2.7
B: Fasting		
0-4 (n = 6)	209.5 ± 12.2 ↑ t = 4.8, $p < 0.01$ ↓	38.8 ± 4.2
older pups (n = 5)	131.4 ± 10.2	84.4 ± 6.9
Adult 1	75	109
2	103	117

Normal value for adult dogs in the postabsorptive state is 134 ± 33 pg ml^{-1} (188).

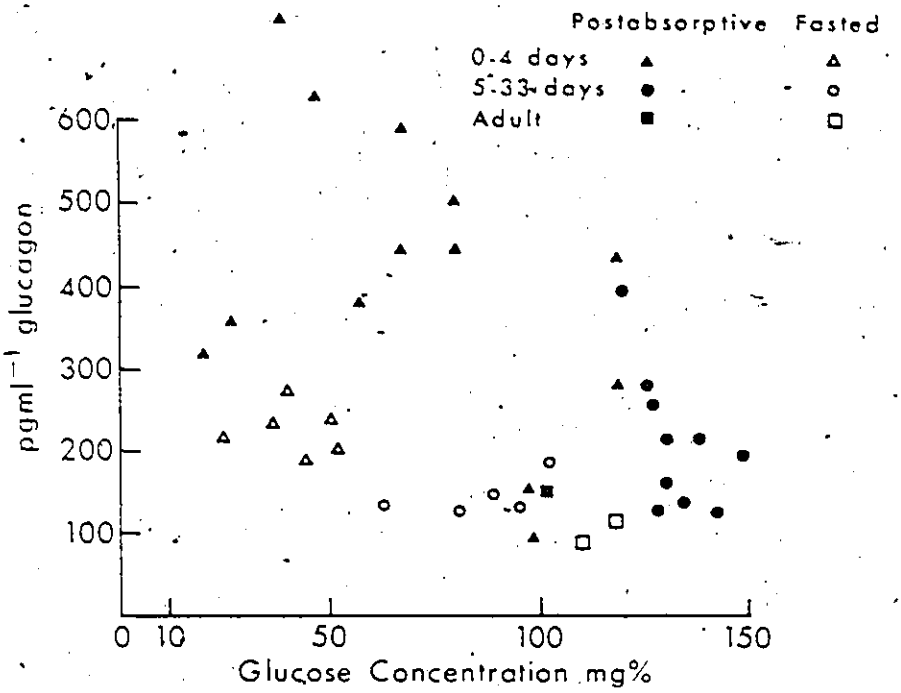
TABLE 45

Glucagon levels in pups 0-4 days old in Spontaneous and Insulin-induced Hypoglycaemia and Hypoglycaemia induced by fasting

	Basal levels (plasma glucose >66 mg %) n = 7	Basal levels before insulin n = 4	Insulin-induced (plasma glucose < 60 mg %) n = 4	Spontaneous (plasma glucose < 66 mg %) n = 5	Fasted (plasma glucose < 50 mg %) n = 6
Mean ± S.E.M.	323.7 ± 60.9	376.5 ± 100	405 ± 96.6	476 ± 67	209.5 ± 12.2

Fig. 12

PLASMA GLUCOSE CONCENTRATION
AND ITS RELATIONSHIP TO
PLASMA GLUCAGON CONCENTRATION



DISCUSSION

GLYCEROL METABOLISM

Glycerol Subsystem

In adult dogs the total plasma equivalent volume of distribution for glycerol was 66.8% of body weight or 668 ml kg^{-1}, a figure suggesting even distribution of glycerol, throughout total body water. This is extremely close to the value of 650 ml kg^{-1}, calculated originally by Larsen in cats (104) and which has since been employed by others in their calculation of glycerol turnover in dogs (71, 196).

Puppies under 11 days of age had plasma equivalent glycerol volumes at least twice that of the adults (Table 25). Obviously in these puppies glycerol capable of exchanging with the tracer must be more concentrated in some region(s) other than plasma.

The neonates of most mammalian species possess significant amounts of brown adipose tissue which in a temperate climate diminishes with age (108). This tissue contains glycerokinase and is therefore capable of glycerol uptake as well as its release into the circulation. Rapid exchange of glycerol with the glycerol stored in brown adipose tissue could therefore account for the increased glycerol mass and larger volume of distribution in the younger animals. By the time the puppies reach the 12-30 day age range, the plasma equivalent volume for glycerol has almost declined to the adult value.

Glycerol Metabolism in the Postabsorptive State and During Fasting

In the early neonatal period (0-4 days) the high rate of

appearance of glycerol in the plasma together with the elevated plasma glycerol concentration is indicative of rapid breakdown of gestationally deposited triglyceride; whether the site of this increased lipolysis is located in white or brown adipose tissue is unknown.

Lipolysis in brown adipose tissue might be initiated by the temperature drop experienced by the neonate at birth (70). The regulation of lipolysis in neonatal white adipose tissue is poorly understood but it does appear to be different from that in adults (135).

Apparently the rate of glycerol turnover U_1 is related linearly to the concentration of glycerol in plasma within 0.0015 and 0.014 mg ml^{-1}. Therefore within these limits the metabolic clearance rate of glycerol (U_1/C) remains constant. Such a relationship has been reported to exist in the grown dog (166, 197), rabbit (79) and man (18). At relatively high levels of glycerol concentration however the relationship deviates from linearity because of a few high rates of U_1 observed in some pups. This might suggest that other mechanisms of glycerol release are operative in these particular animals.

In prematures (143) and possibly those small for gestational age the percentage of brown fat to total fat is greater than in those born at term. Any rate of glycerol turnover in this tissue would not result in the expected, i.e. those predicted from Fig. 8, levels of glycerol in plasma due to utilization of glycerol in the tissue itself. Exposure to cold (34) or hypoxia (108) would accelerate glycerol turn-

over in brown adipose tissue. Despite the fact that measures were taken to prevent both conditions, some sensitive animals may have responded to minor deviations.

After a seven day fast the mean rate of glycerol release in adult dogs rose from 0.13 mg C min^{-1} kg^{-1} to 0.25 mg C min^{-1} kg^{-1}. A similar percentage increase in glycerol turnover in lean men, subjected to a lengthy period of fasting has been reported by Bortz et al (18). In contrast pups less than 4 days old, not only were incapable by maintaining their already high glycerol turnover but exhibited a considerable decline both in U_1 and in the plasma concentration of glycerol in response to a 1 - 2 day fast. The concentration of FFA in plasma was not followed in our studies. However experiments carried out on newborn pups fasted in a warm environment (195) show a marked decline in plasma FFA concentration. A similar decrease was also found in fasted newborn rabbits (72), rats (69), and pigs (170). Human babies less than 36 hours old also fail to respond to fasting with an elevation of plasma FFA or ketonemia as do older infants (93).

The most likely explanation for this decrease in both plasma glycerol and FFA is a reduction in lipolysis. Accumulation of mono or diglycerides in adipose tissue might also account for this observation (152). However without knowing the plasma glycerol:FFA ratio one cannot differentiate between the two. Nevertheless, it appears that during the first few days of life, in many mammalian species concentrations of both glycerol and FFA are reduced by fasting.

Pups older than 4 days did not increase glycerol turnover in

response to fasting but maintained it at the rate observed in the postabsorptive state. The difference between these two age groups cannot be accounted for by the small amount of fat in the newborn beagle, and its rapid increase within the first day of life. According to Shen and Huggins (157), in a beagle weighing 250 gm. 4.65% of body weight, i.e. 11.6 gm. can be accounted for by lipids. In a pup of 500 gm. the lipids account for 7.1% of body weight, corresponding to 35.5 gm. So in a pup of 250 gm., the amount of fat catabolized during 1 day of fasting at the rate calculated for the postabsorptive state is estimated to amount to 8.45 gm. or at the rate calculated for the end of the fasting period to 2.85 gm. (assuming 10% of neutral fat is released as glycerol). The calculated amounts may be regarded as maximal or minimal amounts of fat mobilized. Thus overall availability of substrate does not appear to be the rate limiting step in lipolysis at this age.

In older pups weighing 500 gm., the amount of fat utilized would be 17.1 gm. during a 2 day fast: about one half of the estimated fat content of the body at that weight. Again the absence of an increase in the rate of glycerol release cannot be due to a lack of substrate.

In these experiments approximately 50% of glycerol-C was converted to glucose in the postabsorptive state independent of age. In the postabsorptive state at glycerol concentrations between 0.03 and 0.3 mM Basso et al reported that a maximum of 70% of glycerol is extracted by the liver (11). In the youngest pups if hepatic blood is estimated to be one-

third of the cardiac output (5), i.e. 22 ml.min^{-1} then 22 X 0.015 = 0.33 mg of glycerol is coming to the liver every minute. A 70% extraction would lead to 0.7 X 0.33 = 0.23 mg carbon extracted from the circulation every minute. In an average 300 gm. pup the value calculated from the 50% conversion of glycerol at the lower rates of glycerol turnover in these animals is 0.5 X 0.3 X 1.7 = 0.26. Thus it is possible that all glycerol extracted by the liver is converted to glucose. Even at higher rates of hepatic glycerol extraction, quoted by others (197) very little glycerol will be available in the liver for direct oxidation, conversion to triglyceride or incorporation into lipoprotein.

Evidently, gluconeogenesis from glycerol is determined by substrate (glycerol) availability, regulation being exerted at the level of lipolysis. It can be stated that in all adult dogs and most pups the concentration of glycerol in the plasma is a good index of the extent of gluconeogenesis from glycerol.

During fasting, adult dogs converted about the same amount of glycerol-C to glucose-C per minute per kg. body weight ($R_{3,1}$). However a larger fraction of glycerol-C was utilized by other processes, presumably including increased oxidation to CO_2.

Pups 5-19 days old continued to convert approximately half of the available glycerol-C to glucose.

Under 4 days only 22.6% of glycerol-C appeared as glucose and the fraction utilized by other processes increased considerably.

Apparently in the early neonatal period, energy production for the continuance of basal activities takes precedence over maintenance of the plasma glucose concentration which is more precisely regulated in the adult (76).

ALANINE METABOLISM

Alanine Subsystem

The model shown on Fig. 7 is of heuristic value only highlighting some anomalies in the classical precursor product relationship between alanine-C and glucose-C. It draws attention to the possibility that the three Carbon atoms of alanine are not equally distributed between the three compartments of the alanine subsystem. Further development and elucidation of the system will necessitate experiments with different specifically labelled types of alanine together with a knowledge of differential labelling of the resultant glucose molecule

Alanine Metabolism in the postabsorptive state

Judged by the plasma level and turnover rate of alanine, during the neonatal period there appears to be no shortage of alanine available for gluconeogenesis. The theoretical difficulty in comparing metabolic rates between newborn and grown animals is well known (46). However, as with glycerol or glucose the turnover rate on a body weight basis is highest in the youngest pups and the percentage difference between pups and adults is nearly equal to that of the glucose RT.

The mean value of 0.61 $\pm$ 0.13 mg.C kg^{-1} min^{-1} turned over by

adults is in reasonable agreement with the only other known study in dogs where values from 0.25 - 0.4 mg.C kg^{-1} were reported using ^{14}C alanine as tracer (16). Similar values within the range of these results were obtained using ^{3}H-2-3 alanine and deuterated alanine (ALA - D_4). The turnover rate of alanine will probably be closely approximated using ^{3}H-3-alanine as tracer because the ^{3}H label attached to the third C-atom is expected to be lost in the reverse Embden Meyerhof pathway (39) thus obviating any component of the alanine:glucose cycle.

In both the youngest pups and adult dogs one-third of the plasma alanine carbon appears in glucose. Most, if not all the conversion of alanine-C to glucose will have taken place in liver, renal blood flow in pups being extremely low (87). Thus in an average pup less than four days old with one-third of the cardiac output of 72.5 ml (5) going to the liver 72.5 X 0.3 X 0.0096 = 0.21 mg alanine-C are presented to the liver each minute. Actual glucose-C production is 1.02 mg C min^{-1}, so 20.5% of glucose-C produced was originally plasma alanine-C. This value is in agreement with that calculated by the ratio of integrals. Thus it appears that all alanine-C extracted by the liver is converted into glucose. However, approximately 0.5 mg alanine-C are being turned over per min. in a 300 gm. pup of which 0.21 mg is extracted by the liver. Therefore in a steady state some

organs must be releasing more alanine into the circulation than they are removing from it. This is in keeping with the observation that negative arterovenous differences are consistently found across skeletal muscle both in man (49) and other species (150).

The Effects of Insulin-induced Hypoglycaemia

The level of alanine in adult animals is known to remain unchanged or even increase during insulin administration (54). In our grown dogs no significant decrease in alanine concentration was observed during infusion of insulin. The pups however became markedly hypoalaninaemic under the influence of insulin.

Alanine is continuously released into and removed from the circulation. If the rate of release equals the rate of removal the extracellular concentration of alanine remains steady, irrespective of the magnitude of these rates. An unchanging net rate of release or uptake will not reveal the magnitude of any equal simultaneous change in the release and uptake of alanine by the tissue. The decrease in plasma alanine concentration in pups clearly proves that during the infusion of insulin more alanine was removed from, than released into, the plasma, assuming a constant alanine "space".

Hypoglycaemia induced by insulin has been shown to increase the rate of hepatic glucose production in adult animals (76). This increase is probably due to glycogen breakdown, and to some extent to gluconeogenesis at the expense of protein (147), effects possibly mediated by glucagon which has been claimed to preferentially increase hepatic alanine uptake (118). Thus glucose-C becomes available for

translocation into muscle where it is converted to alanine-C, in which form it re-enters the circulation. Therefore in adult animals the unchanged plasma alanine level is probably a reflection of parallely increased rates of removal and release of alanine.

As opposed to adults, newborn dogs do not react to hypoglycaemia with an increased rate of hepatic glucose release (75). They are also less sensitive to insulin as judged by the magnitude of the increase in the metabolic clearance of glucose (182). Therefore no increase in alanine turnover is expected at low blood sugar levels induced by insulin.

In the experiments reported the concentration of total amino-N in blood decreased during hypoglycaemia by the same modest extent in pups and adult dogs. Obviously the net effect of insulin at any age is to promote the removal of amino acids from the blood; thus the insensitivity to insulin of the glycaemic response is not extended into the area of protein metabolism in the neonate. This lack of synchronization of development of the metabolic effects of insulin is teleologically advantageous, permitting more of the circulating glucose to be utilized by non insulin-dependent tissues such as the rapidly developing brain, while at the same time facilitating amino acid uptake for protein systnesis and consequent growth.

GLUCOSE METABOLISM

Glucose Subsystem

In the model used to analyze the glycerol:glucose subsystem, the glucose subsystem was adequately represented by two interchanging

compartments ($V_3 + V_4$) yielding a total plasma equivalent volume in adult dogs of 22% of body weight. The total mass of exchangeable glucose-C ($M_3 + M_4$) and the plasma equivalent volume of glucose per kg. body weight were about twice as much in pups as in adults. This may result from a larger proportion of body weight being represented in neonates by the liver (70), an organ known to contain glucose at a high concentration together with glucose-6-P and glycogen, all of which contribute to the exchangeable mass of glucose-C. A larger relative plasma volume and total body water content in the newborn beagle (156) might also contribute to the higher plasma equivalent volumes and total glucose values, observed in pups.

Sources of Circulating Glucose

Re-incorporation of Carbon Atoms into Plasma Glucose

In dogs (83), rats (74, 91), rabbits (91), and man (180), the steady state turnover of glucose calculated using ^{3}H-2-glucose as tracer has been found to be considerably higher than that calculated using ^{14}C-U-glucose or ^{14}C-1-glucose. In dogs the ratio of the calculated apparent turnover rates amounted to 1.3 - 1.35 (83). The difference has been attributed to the re-incorporation of the ^{14}C label into newly formed glucose via the Cori cycle and other cyclical pathways. Tritium, attached to the second carbon atom of glucose is not re-incorporated into newly synthesized glucose to be relaesed by the liver; since in the isomerization between glucose-6-P and fructose-6-P the ^{3}H label is detached and converted to water (74). So the turnover rate calculated

with ^{3}H-2-glucose as the tracer very closely approximates the total rate of glucose released by the liver regardless of whether the glucose arises from glycogen, gluconeogenesis or "futile" cycling.

In pups the ratio of the two rates was 1.35 ± 0.08, very similar to the value reported by Issekutz in grown dogs (83). The extent of recycling of C through tricarbon intermediaries apparently does not vary with age despite the variation in plasma glucose level. At any age, under basal conditions, approximately 65% of glucose-C released into the circulation has not passed through the glucose pool before. Presumably in the youngest pups a larger proportion of these C-atoms will have been supplied by gluconeogenesis, an increasing proportion being supplied by glycogen as the hepatic glycogen stores regain their adult level.

Contribution From Glycerol in the Postabsorptive State and During Fasting

It is possible that the proportion of glucose-C derived from glycerol might be underestimated by the exchange of C^{12} for ^{14}C in any glycerol arriving in glucose after passage through lactate pyruvate and alanine (197). However in the youngest animals in the postabsorptive state and in older puppies and adults during fasting, the expected high rate of oxidation of FFA should ensure that flux through the Embden-Meyerhof pathway is towards glucose (132).

In the postabsorptive state the contribution of glycerol-C to glucose accounts for 6.1% of total glucose production. The value calculated for the single unanaesthetized adult dog in these studies was 6.37%. It was, therefore, very close to the mean value and it

does not appear that gluconeogenesis from glycerol is affected by the Nembutal anaesthesia. A similar mean value in grown dogs was also reported by Winkler et al (197). However we found that this percentage varied over a wider range from 1.4% to 15.7%. In man the variation appears to cover the same range, higher values being found in obese individuals (18). In the first 4 days after birth, irrespective of the source or the stimulus for glycerol release, it supplies 13.8% of glucose-C in the postabsorptive state, which is more than twice the percentage of glucose arising from glycerol in the adult animal. Thus it appears that the relative contribution from other glucogenic precursors will be less at this time than later in life. Certainly the rapid depletion of hepatic glycogen at birth (154), will severly curtail the contribution from this source.

All older pups relied on glycerol-C to provide the same fraction of newly formed glucose as the adult dogs. At about 4 days of age the hepatic glycogen concentration in the dog has practically reached the adult value (154) and becomes available to form a greater proportion of the glucose produced.

In all age groups the glucose turnover rate fell by about the same extent during fasting. In adult dogs it fell to 57% of the rate measured in the postabsorptive state. Cowan et al found a decrease to 65% of basal in dogs after a 7 day fast (29). Similarly in man, after a 5 - 6 week fast the rate of glucose production falls to 60%, a reduction attributed to a decreased availability of glucogenic amino acids (54).

In the adult dogs the percentage of glucose-C arising from glycerol rose to a mean of 13.3%. This increase was accomplished by increased glycerol production and by the conversion of more glycerol to glucose together with the reduction in the total glucose production. In the intermediate age group the contribution of glycerol-C to glucose, was not increased after a 2 day fast but it now accounted for 10.3% of the diminished glucose production.

Because of the severe reduction in lipolysis and consequent lack of circulating glycerol-C in pups less than 4 days old, after a 1 - 2 day fast only 3.4% of glucose-C can be accounted for by transport of glycerol.

This reduction in available glycerol for gluconeogenesis and the concomittant lack of FFA and ketone bodies (195) will severely handicap the newborn in its ability to withstand more than a relatively short period without feeding.

Contribution from Alanine

An important feature bearing on the interpretation of the tracer data is that oxaloacetate is an intermediary common not only to the gluconeogenetic (reverse glycolysis) pathway, but also to the pathway of respiration (194).

The oxaloacetate pool from which both C-atoms of carbon dioxide are generated during one turn of the Krebs cycle becomes labelled when a gluconeogenic substrate such as alanine is labelled. Equilibration of isotope via the dicarboxylic acid shuttle occurs about

four times as fast as does direct phosphorylation of pyruvate (172). Hence alanine carbon atoms can partake in the Krebs cycle and supply carbon atoms of respiration although on balance all alanine transaminated to pyruvate may be converted into glucose and all respiratory carbon dioxide may be derived from oxaloactate.

So there is what has been termed by Krebs (101) a "crossing over" of carbon atoms from alanine, pyruvate and lactate to carbon dioxide in exchange for carbon atoms from respiratory substrates such as acetoacetate. Indeed Lorber et al (112) estimated from the distribution of isotope in glycogen, resulting from the administration of labelled lactate, that less than one sixth of the lactate entered glycogen directly by reversal of the reactions of glycolysis. They postulated that the bulk of lactate had passed through reactions of the Krebs cycle resulting in randomization of isotope in alpha and beta carbon atoms before being incorporated into glycogen.

Because of this crossing over and subsequent randomization of the ^{14}C label it is impossible from the tracer data to distinguish between true synthesis of glucose, resulting in a net gain of glucose-C for the animal and metabolic incorporation (101) of the isotope.

In adult dogs and pups older than four days, 10.8% of glucose-C turned over had originated from plasma alanine carbon. Although in pups less than four days old this percentage might be slightly higher. However it appears that in the immediate neonatal period pups are as capable as their mothers in deriving glucose carbon from alanine.

Glucagon and its Role in Neonatal Metabolism

High plasma glucagon levels were found in this investigation in pups; the mean value in the 0-4 day group being at least five times that measured in normal adult dogs (188). Similar high glucagon levels have been observed in newborn human babies (82) and lambs (57). However these high values have been reported incidentally, and no comment has been made as to their possible significance in neonatal metabolism.

In these studies plasma glucose and glucagon concentrations were found to be inversely related (Fig. 12). Glucose concentrations of around 83 mg/100 ml. have been reported in cord blood of human infants (140) and Villeé found a mean value ± S.E.M. of 110 ± 5.4 mg/100 ml. plasma in the human foetus when the maternal plasma glucose concentration was 117 ± 4.4 mg/100 ml. (185). The stimulus, therefore for these exceptionally high neonatal glucagon levels is probably the abrupt withdrawal of the maternal glucose supply at birth. As there is some evidence to suggest that neonates do not respond to amino acid infusion with increased glucagon production (57), continued high levels are probably maintained by the high dietary fat intake in addition to hypoglycaemia.

Injection of glucagon via an umbilical catheter to the human newborn, 3 hours after delivery, considerably raised the blood glucose concentration (82). Such a rise in the plasma glucose concentration has not been observed in adult dogs due to the balance of the opposing

effects of insulin and glucagon (25). A similar balance of the antagonistic effects of the two hormones is not likely to be achieved in the neonate whose sensitivity to insulin is low (182). The insulin: glucagon molar ratio (203) calculated for normal adult dogs from the data of Vranic et al (188) is 3.6 whereas that calculated for three pups three days old and less was 1.72 ± 0.74. However even if identical molar ratios of insulin and glucagon were reached in the newborn the functional ratio would be shifted in favour of glucagon, the physiological effects of which would predominate.

In view of the lipolytic action of glucagon (106) it is tempting to speculate that it is this hormone which is the agent responsible, at least in part, for the high rates of lipolysis and gluconeogenesis observed in newborn pups in the first few days of life. During fasting in the youngest pups, the glucagon levels fell from 394 ± 48.4 pg ml^{-1} to 209.5 ± 12.2 pg ml^{-1}, probably contributing to the observed, severe hypoglycaemia and lack of gluconeogenesis from glycerol.

In spontaneous hypoglycaemia the glucagon concentration was raised whereas hypoglycaemia induced by insulin left it unchanged. The latter effect might be due to insulin facilitated entry of glucose into the α cell, suppressing glucagon release (136). Thus hypoglycaemia in itself is not responsbile for the low glucagon levels observed in fasted pups 0-4 days old. Unless the cost of glucagon production in terms of energy expenditure becomes too high in fasting, apparently some dietary component such as amino acids or fat, providing

a continuous "enteric signal" (37) is required in the early neonatal period to maintain basal glucagon levels. Furthermore the absence of such a signal would override the effects of hypoglycaemia.

The hypothesis that the control of glucagon release has not yet matured, in the youngest pups, to the adult norm is further strengthened by the observation that somatostatin, a hypothalamic peptide (20) capable of suppressing glucagon secretion both in vitro (64) and in vivo, in man (65) and diverse other species (95) including dogs (189) does not reduce glucagon levels in pups (190).

In adult dogs mean glucagon levels in the postabsorptive state, have been measured by Vranic et al (188). In fasting there is a slight but significant fall in plasma glucagon concentration. This is in contrast to the widely held assumption that glucagon levels are elevated in fasting. Due to the diminution of the rate of insulin secretion in fasting (22), long regarded as a major adaptation, the effects of glucagon predominate. Thus it appears that it is the ability to maintain plasma glucagon concentrations, close to the postabsorptive level which enables the mature animal to withstand relatively long periods of fasting. This ability is missing in the neonate, preventing it from maintaining glucose homeostasis during even a relatively short period of fasting.

SUMMARY AND CONCLUSIONS

The ratio of the total turnover rate of glucose, calculated using ^{3}H-2-glucose as tracer, and the irreversible disposal rate of glucose, calculated with ^{14}C-U-glucose tracer, in pups, in the postabsorptive state, was found to be 1.35 ± 0.08. Thus the same percentage of newly formed glucose-C released into the circulation has come from recycled C in both pups and adults.

However pups less than four days old rely to a greater extent than do older pups or adults, on gluconeogenesis from glycerol; 13.8% of newly formed glucose coming from this source as opposed to 6.1% in adults. Irrespective of age 50% of glycerol-C was converted to glucose.

Pups in the youngest age group are as capable as adult dogs in deriving glucose carbon from alanine and 30% of alanine-C appears rapidly in plasma glucose in both age groups. Whether this contribution to glucose from alanine represents net glucose synthesis or is due to metabolic incorporation of the tracer due to "crossing over" can not be resolved.

Under the influence of insulin Amino-N concentration declined by the same modest extent in both pups and adult dogs, indicating that the net effect of insulin at any age is to remove amino acids from the blood. Plasma alanine concentration in adult dogs did not decline, presumably due to an increased rate of operation of the glucose:alanine

cycle. Before this can be stated definitively however, actual alanine turnover rates must be calculated. The lack of glucoregulatory processes, involving gluconeogenesis, in response to insulin-induced hypoglycaemia in pups was reflected by a continually declining plasma alanine concentration during an insulin infusion. Thus the absence of such regulatory mechanisms leaves the effect of insulin on protein synthesis unopposed.

During fasting adult dogs maintained glucose homeostasis by reducing glucose turnover and increasing lipolysis. Consequently glycerol became quantitatively a more important glucose precursor, 13.3% of glucose-C coming from this source. Older pups showed some hypoglycaemia when fasted. They too, reduced the rate of glucose turnover so that although glycerol turnover was not increased carbon from glycerol formed 10.3% of the newly formed glucose.

Pups of four days old or less also reduced glucose turnover when fasted, but because glycerol turnover was markedly reduced, gluconeogenesis was severely curtailed and they were totally unable to maintain glucose homeostasis.

High glucagon levels were found, in pups in the postabsorptive state, which declined with age. During fasting pups exhibited a marked decline in plasma glucagon levels, despite hypoglycaemia. It appears therefore from this and other discussed evidence that the control of glucagon release is different in the neonate from the adult. It is suggested that the effects of glucagon predominate in the postabsorptive state in the neonatal period and that glucagon

might be the agent responsible for the high rates of lipolysis and gluconeogenesis observed in pups in the postabsorptive state. Moreover, the lack of maintainance of basal glucagon levels during fasting explains the inability of the newborn to withstand even a relatively short period of fasting.

Concentrations of Gluconeogenetic Precursors and their Contribution to Circulating Glucose

Parameter	0-5 days		6-11 days		12-60 days	Adult	
	Post-absorptive	Fasted	Post-absorptive	Fasted 5-19 days	Post-absorptive	Post-absorptive	Fasted
SUBSTRATE CONCENTRATION mg %							
1. Glucose	79.8 ± 6.2	45.1 ± 5.8	106.5 ± 7.9	86.6 ± 4.4	114.2 ± 11.8	101.7 ± 3.0	97.3 ± 4.0
2. Glycerol	1.52 ± 0.34	0.41 ± 0.08	0.681 ± 0.13	0.935 ± 016	0.599 ± 0.074	0.41 ± 0.046	0.688 ± 0.22
3. Alanine	2.36 ± 0.38	-	2.41 ± 0.44	-	2.41 ± 0.44	3.5 ± 0.22	-
R.T. mgC $min^{-1}kg^{-1}$							
1. Glucose	3.12 ± 0.72	2.04 ± 0.14	3.68 ± 0.36	1.92 ± 0.13	3.54 ± 0.77	1.02 ± 0.12	0.58 ± 0.1
2. Glycerol.	0.92 ± 0.27	0.31 ± 0.17	0.48 ± 0.14	0.48 ± 0.07	0.32 ± 0.02	0.13 ± 0.02	0.25 ± 0.09
3. Alanine	1.49 ± 0.25	-	1.0 ± 0.08	-	1.0 ± 0.08	0.61 ± 0.13	-
% OF GLUCOSE-C ARISING from:							
1. Glycerol	13.8 ± 3.3	3.4 ± 1.5	6.2 ± 1.7	10.3 ± 2.0	4.5 ± 3.3	6.1 ± 1.5	13.3 ± 7.0
2. Alanine	18.6 ± 2.3	-	10.8 ± 2.6	-	10.8 ± 2.6	10.77 ± 3.3	-
3. Recirculation	22 ± 5.0	-	40 ± 18	-	46 ± 11	35 (83)	-
% of glycerol to glucose	46.9 ± 9.8	22.6 ± 8.0	49.3 ± 5.7	43.1 ± 1.7	50.1 ± 3.9	48.8 ± 6.2	30.1 ± 7.4
% of alanine to glucose	30.1	-	-	-	-	28.7	-

APPENDIX

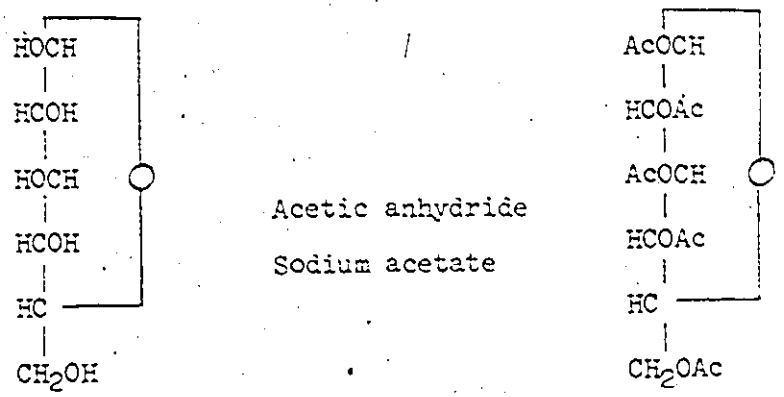
VALIDATION OF METHODS

VALIDATION OF METHODS

Experiments designed to determine the extent of gluconeogenesis from either glycerol or alanine necessitated the efficient separation of the precursors both from glucose and other compounds in which the radioactive label would be incorporated. Similarly it was required that glucose be isolated.

1. Isolation of glucose

Glucose was isolated by preparation of glucose pentaacetate (G.P.A.) (85) a white crystalline compound, soluble in Brays solution with negligible quenching. The basic reaction is



Glucose — Glucose Pentaacetate

i Separation from glycerol

An aliquot of ^{14}C-U-glycerol solution containing approximately 555 X 10^3 dpm was added to 1 ml. of 10% glucose solution and the pentaacetate prepared according to the method (85). The recovery of ^{14}C in the G.P.A. was determined and corrected to allow for total recovery of the 100 mg. carrier glucose to G.P.A. A second series of

experiments was also carried out with thorough washing of the filtered G.P.A. crystals with cold distilled water, together with one recrystallization, followed by further washing.

The dpm ^{14}C recovered in the crystals corrected for total conversion to G.P.A. were expressed as a percentage of the total number of ^{14}C dpm originally added. The effects of recrystallization on the weight of G.P.A. formed was also investigated. The results are shown in Table I.

ii Separation from Alanine and Lactate

Two series of experiments were carried out simultaneously. G.P.A. was prepared with thorough washing and one recrystallization from 1 ml. 10% glucose solution (100 mg. glucose) and;

1. 0.02 ml ^{14}C-U-alanine approximately equivalent to 111 X 10^3 dpm
2. 0.02 ml ^{14}C-U-Na. Lactate approximately equivalent to 111 X 10^3 dpm.

The results shown in Table II are expressed as the % of the original number of dpm ^{14}C recovered from G.P.A. corrected to 100% conversion of the carrier glucose.

It was concluded from the above experiments in sections (i) and (ii) that:-

1. Recrystallization of G.P.A. reduced the yield by at least 50%.
2. Contamination of G.P.A. by ^{14}C-U-glycerol was so slight as to be negligible.
3. Contamination of G.P.A. both by alanine and lactate although only approximately 3% would be significant when corrected to 100% recovery of carrier glucose.

TABLE I

Separation of Glucose from Glycerol-U-^{14}C

Experiment #	Sample #	^{14}C-U-Glycerol Added dpm x 10^{-3}	Wt. G.P.A. formed mg.		% of dpm in glycerol recovered and corrected for 100% conversion of glucose to G.P.A.	
			1st preparation	Recrystallized	1st preparation	Recrystallized
1	1	555	117.4		1.007	
	2	555	123.1		1.44	
	3	555	132.7	77.9		0.18
	4	555	120.2	80.8		0.2
2	1	1128.3	140.3		1.21	
	2	1128.3	121.9		0.995	
	3	1128.3	125.1		1.09	
	4	1128.3	120.3		0.883	
	5	1128.3	128.8	85.6		0.185
	6	1128.3	118.0	54.4		0.179
3	1	564.1	109.0		1.51	
	2	564.1	121.8		1.42	
	3	564.1	123.4	78.3		0.208
	4	564.1	107.3	73.5		0.174
4	1	564	121.5		1.07	
	2	564	86.2		1.18	
	3	564	115.0	68.1		0.271
	4	564	108.7	56.3		0.289
5	1	555	96.5		1.4	
	2	555	not measured	63.0		0.17
		Mean S.E.M. ±	117.74 ± 2.84	70.87 ± 3.68	1.20 ± 0.064	0.206 ± 0.0145

TABLE II

Separation of Glucose from Alanine and Lactate

Experiment #	Sample #	^{14}C added dpm x 10^{-3}	Wt. G.P.A. mg	^{14}C dpm in GPA
1	1	79.8 ^{14}C-U-Alanine	91.5	3.91
	2		90.0	3.69
	3		84.0	3.21
	4		82.0	3.10
	5		82.5	3.77
2	1	84.4 ^{14}C-U-Alanine.	73.5	2.36
	2		80.7	2.68
	3		73.0	2.55
	4		88.1	2.71
	5		92.7	2.16
	6		90.9	3.26
			MEAN ± SEM	3.13 ± 0.16
3	1	104.4 ^{14}C-U-Na Lactate	64.3	3.18
	2		74.5	3.8
	3		66.0	3.03
	4		68.7	2.86
	5		63.3	3.09
	6		74.6	3.68
			MEAN ± SEM	3.27 ± 0.15

It was therefore decided in all *in vivo* experiments where ^{14}C-U-alanine was administered that a correction would be made in the calculation of specific activity (dpm mg^{-1}) of ^{14}C glucose for each sample to allow for this contamination. Further recrystallization of G.P.A. would reduce the yield and thus lower the statistical accuracy during the counting procedure.

2. Isolation of Glycerol

Glycerol was isolated by a modification of the method of De Freitas (36). Instead of eluting ^{14}C glycerol from the chromatogram the glycerol containing strip was cut out and oxidized to $^{14}CO_2$ in a Packard Sample Oxidizer.

i Recovery of ^{14}C from oxidizing ^{14}C-U-glycerol in a Packard Sample Oxidizer.

Approximately 222 X 10^3 dpm ^{14}C-U-glycerol dissolved in water were applied to Whatman No. 1 chromatography paper. These pieces of paper were then made into a pellet in a pellet press and burned in the sample oxidizer.

The effects of paper size were assessed by making the pellets from differing sizes of paper. A maximum of 515 mg was used because preliminary experiments indicated that at weights exceeding this there was an impairment of counting efficiency.

The results expressed as percentage of the original counts recovered as $^{14}CO_2$ are shown in Tables III and IV.

From these experiments it was concluded that:

1) The recovery of $^{14}CO_2$ from ^{14}C-U-glycerol was independent of paper weight in the range investigated.

2) Any loss of ^{14}C during the burning procedure was negligible.

ii Recovery of ^{14}C-U-glycerol from the chromatogram

(a) A preliminary experiment was performed whereby 0.05 ml of a solution of glycerol in plasma (4 mg ml^{-1}), containing at least four times the physiological plasma glycerol concentration was run in

TABLE III

The effect of the weight of filter paper on the recovery of $^{14}CO_2$ from ^{14}C-U-Glycerol

Sample #	^{14}C-U-Glycerol dpm x 10^{-3}	Weight of paper mg	% recovery of ^{14}C
1	229	515	101.4
2	229	515	91.7
3	229	515	98.5
4	229	129	97.3
5	229	258	98.1
6	229	386	97.9

TABLE IV

Recovery of ^{14}C as $^{14}CO_2$ from oxidizing ^{14}C-U-Glycerol and ^{14}C-U-Glucose in a Packard Sample Oxidizer

Experiment #	Sample #	Radioactivity Applied dpm x 10^{-3}	% Recovery of $^{14}CO_2$
1	1	47.4 ^{14}C-U-Glycerol in plasma	102.4
	2		96.5
	3		105.3
	4		100.9
	5		103.7
2	1	47.0 ^{14}C-U-Glycerol in plasma	103.8
	2		99.7
	3		99.0
	4		102.3
	5		100.4
	6		98.5
	7		103.1
	8		99.5
	9		98.2
	10		102.0
	11		99.99
	12		101.6
3	1	2.29 ^{14}C-U-Glucose in aqueous solution	91.7
	2		98.5
	3		97.3
	4		98.1
	5		97.9
4	1	516.6 ^{14}C-U-Glycerol in aqueous solution	99.77
5	1	111 ^{14}C-U-Glycerol in aqueous solution	106.3
	2		99.0
	3		104.3
		MEAN	100.375
		S.E.M. ±	3.13

the solvent as described in the method (36). After drying, the whole chromatogram was sprayed with ammoniacal silver nitrate and the glycerol detected. Fig. I shows a photograph of this chromatogram after spraying.

From this it was determined that the glycerol containing strip extended 1.1" towards the origin and 0.9" away from it from the middle of the guide spot, and 0.5" either side of the origin.

(b) 0.05 ml of a ^{14}C-U-glycerol plasma solution was applied to the origin in a 3" streak.

Guide spots on either side of the chromatogram were each 0.005 ml (4 mg ml^{-1}) glycerol solution.

The chromatograms were run by descending chromatography according to the method and the glycerol containing strip was cut out and burned in the Packard Sample Oxidizer. The results are shown in Table V.

It was concluded from this experiment (b) that only 88.9 ± 1.4% of the radioactivity was recovered from the chromatogram and a correction factor would have to be introduced to allow for this loss in the final calculation of the specific activity of plasma glycerol.

iii Separation of glycerol from Glucose and Lactate

(a) A preliminary experiment designed to determine the R.F. values of glycerol and glucose was performed.

0.01 ml spots of plasma containing glycerol (4 mg ml^{-1}) were applied at the origin of the chromatogram, which was then run in the usual manner. After drying, the whole chromatogram was sprayed with

Fig. 1

Separation of Glucose and Glycerol by Paper Chromatography

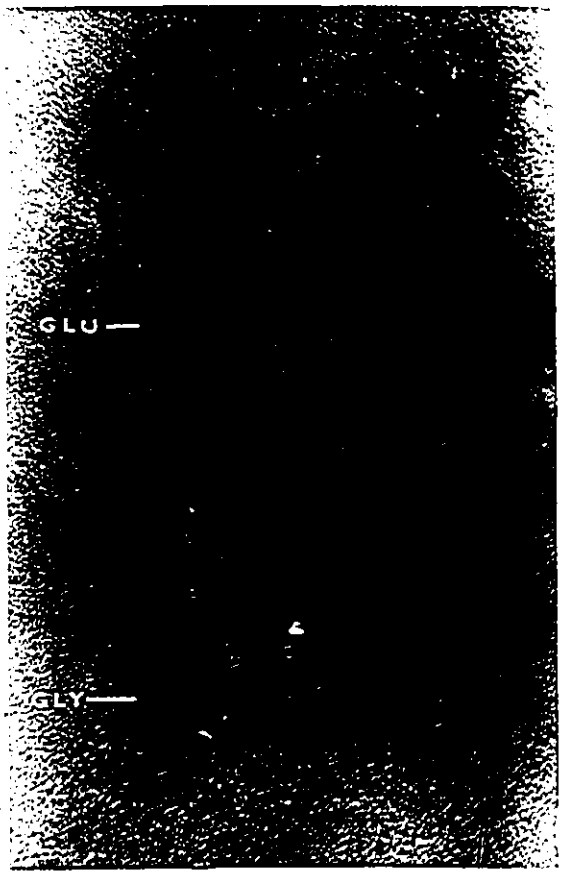


TABLE V

Recovery of ^{14}C-U-Glycerol from the chromatogram

Experiment #	Sample #	^{14}C-U-Glycerol at origin dpm 10^{-3}	% recovery
1	1 2	86.2	92.5 88.38
2	1 2 3	66.5	86.8 92.7 90.8
3	1 2 3 4 5 6	45.4	92.5 85.5 83.0 88.9 91.7 85.0
		MEAN S.E.M. ±	88.89 ± 1.04

ammoniacal silver nitrate. This solution was prepared by adding ammonium chloride to 2 M silver nitrate carefully until the addition of 1 drop dissolved the precipitate of silver chloride. The individual glucose and glycerol spots were clearly delineated and the R.F. calculated. The results are shown in Table VI.

This experiment indicated that there was sufficient distance (13 cm) between the glycerol and glucose spots that contamination should be minimal.

(b) Experiments designed to show the amount of glucose and lactate present in the glycerol strip were carried out in two series.

1. 0.05 ml of ^{14}C-U-glucose solution containing known amount of radioactivity was applied at the origin.
2. 0.05 ml of ^{14}C-U-lactate solution containing a known amount of radioactivity was applied at the origin.

In both series, guide spots 0.005 ml glycerol solution (4 mg ml^{-1}) were used to identify the glycerol containing strip. This strip was cut out, after running and drying the chromatogram, and was oxidized in the sample oxidizer.

The radioactivity in this strip was determined and expressed as a percentage of the original amount at the origin. The results appear in Table VII.

It was concluded from the experiments in section 2 that

1. Contamination of glycerol by glucose was negligible.
2. Contamination of glycerol by lactate was negligible.

However, it was realized that if in an <u>in vivo</u> experiment ^{14}C glucose was administered in an attempt to determine the ^{14}C appearing in glycerol

then the contamination of the glycerol strip could become appreciable and a correction factor would be required.

TABLE VI

R.F. values of Glucose and Glycerol

No.	Distance from origin cm		Distance of solvent front from origin c.m.	R.F.	
	Glucose	Glycerol		Glucose	Glycerol
1	11.3	24.4	43.6	0.26	0.56
2	12.2	25.4	45.2	0.27	0.56
3	10.8	23.8	43.3	0.25	0.55
4	10.9	24.0	42.9	0.25	0.56
			MEAN S.E.M. ±	0.26 ± 0.004	0.557 ± 0.0025

TABLE VII

Separation of glycerol from lactate and glucose

Experiment	Sample	^{14}C at origin dpm x 10^{-3}	% recovery of ^{14}C in glycerol strip
1	1	42.2 ^{14}C-U-Na Lactate	1.66
2	1	36.6 ^{14}C-U-Lactate	1.08
		MEAN S.E.M. ±	1.37 ± 0.29
3	1 2 3 4 5 6	59.0 ^{14}C-U-glucose	0.3807 0.4024 0.409 0.431 0.537 0.295
		MEAN S.E.M. ±	0.409 ± 0.032

3. Isolation of Alanine

It was decided to isolate alanine by ion exchange chromatography on a sulphonated polystyrene resin. The resin employed was Dowex 50 Na^+ 200-400 mesh, prepared by the method of Stein and Moore (128) Because all determinations had to be from a maximum of 0.1 ml plasma the method had to be modified considerably.

Glass columns internal diameter 0.7 I.D. X 23 cm were used. These were calibrated so that 2 ml of buffer could be added to the top of the 14 cm resin column.

Preliminary experiments were carried out to determine whether alanine could be retained by and eluted from the column and efficiently separated from glucose, sodium lactate, and sodium pyruvate.

The design and results of these experiments are summarized on Table VIII and IX and Figs. II A - G and Fig. III.

Using a pH 4.4 buffer, amino acids other than alanine would also be eluted from the column. A further process separating alanine from other amino acids was therefore required. It was decided to convert alanine to pyruvate, using the enzyme glutamate pyruvate transaminase (G.P.T.) and convert this pyruvate to its phenlyhydrazine derivative by a modification of the method of Jorfeldt (86). The validation of this method appears in section 3 (iii).

However the column eluate at pH 4.4 would be too low for the determination of alanine by the enzymic process and so the eluting buffer was changed to M/15 phosphate buffer: pH 7.2 (141). All subsequent experiments were carried out using this buffer.

TABLE VIII

Preliminary experiments to establish the recovery of Alanine and its separation from Glucose, Pyruvate and Lactate

Experiment	Aim	Design	Results
1	To retain and elute alanine from the resin column.	0.2 ml ^{14}C-U-Alanine in pH 2.2 citrate buffer (128) on column. Eluted with 14 ml pH 3.46 citrate buffer (128). Actual number of dpm unknown.	Fig. II A
2	Separation of ^{14}C-U-alanine from ^{3}H-2-glucose.	0.2 ml of ^{14}C-U-alanine and 0.2 ml of ^{3}H-2-glucose in pH 2.2 citrate buffer were applied to the origin of two separate resin columns. Exact original number of dpm unknown. Results expressed as counts per minute (cpm).	Fig. II B
3	Separation of ^{14}C-U-alanine from ^{3}H-2-glucose.	0.1 ml of ^{14}C-U-alanine and 0.1 ml of ^{3}H-2-glucose were applied at the origin of one column. 9 ml of pH 2.2 citrate buffer was run through the column and than exchanged for pH 4.4 buffer. (+.05 ml Brij/100 ml) to elute alanine.	Fig. II C
4	Separation of ^{14}C-U-alanine from ^{3}H-2-glucose and ^{14}C-U-Na Lactate.	0.1 ml of ^{3}H-2-glucose (273.2 x 10^3 dpm) and 0.1 ml of ^{14}C-U-alanine (45.7 x 10^3 dpm) were applied to the top of one column. 0.2 ml of ^{14}C-U-Lactate pH 2.2 was applied to another column. 6 ml pH 2.2 buffer was run through. The buffer was then changed to pH 4.4. Results expressed dpm X 10^3 recovered.	Fig. II D and E
5	Separation of ^{14}C-U-alanine from ^{3}H-2-glucose and ^{14}C-U-Na Lactate.	Column 1. 0.1 ml ^{14}C-U-alanine (30.4 x 10^3 dpm) + 0.1 ml ^{3}H-2-glucose (91.1 x 10^3 dpm), pH 2.2. Column 2. 0.2 ml ^{14}C-U-Lactate, pH 2.2. The buffer was changed to 7.2 PO_4 buffer. Results expressed as % recovery of original amount of radioactivity.	Fig. II G

TABLE IX

Experiment	Aim	Design	Results
6	Separation of ^{14}C-U-alanine from ^{3}H-2-glucose and ^{14}C-U-Na pyruvate.	Column 1. 0.1 ml alanine-U-^{14}C (25.0×10^3 dpm) + 0.1 ml ^{3}H-2-glucose (136.6×10^3 dpm) all pH 2.2. Column 2. 0.1 ml ^{14}C-U-Na pyruvate. Procedure as in 4 and 5.	Fig. II F
7.	Recovery from column using 5 ml sample.	5 ml ^{14}C alanine (270.1×10^3 dpm) pH 2.2 at origin. 9 ml of pH 2.2 buffer run through. 10 ml of pH 7.2 buffer (5) run through and each ml collected and counted. Results expressed as % of radioactivity at the origin.	Fig. III

Fig. II Alanine Separation by Ion Exchange Chromatography

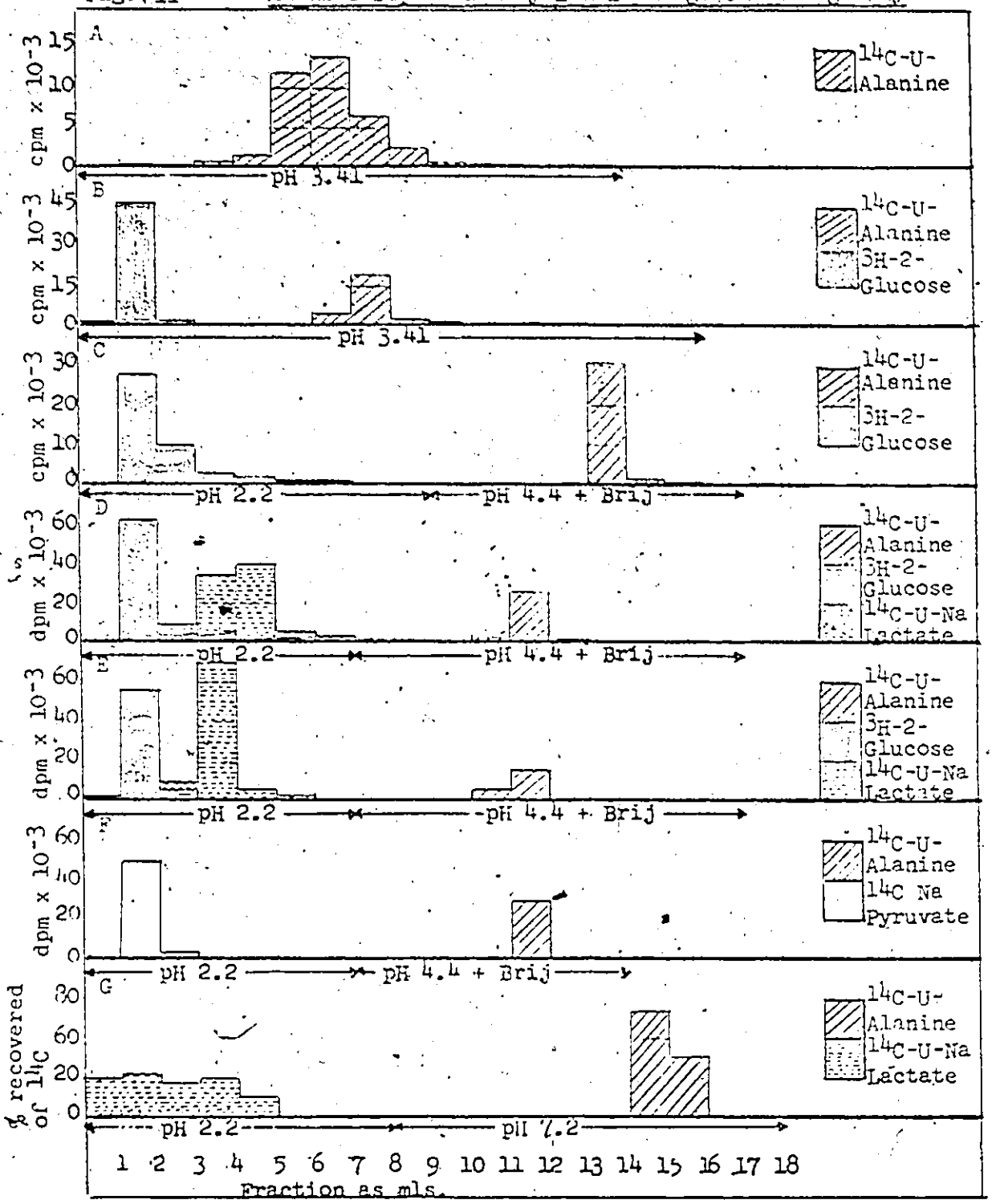
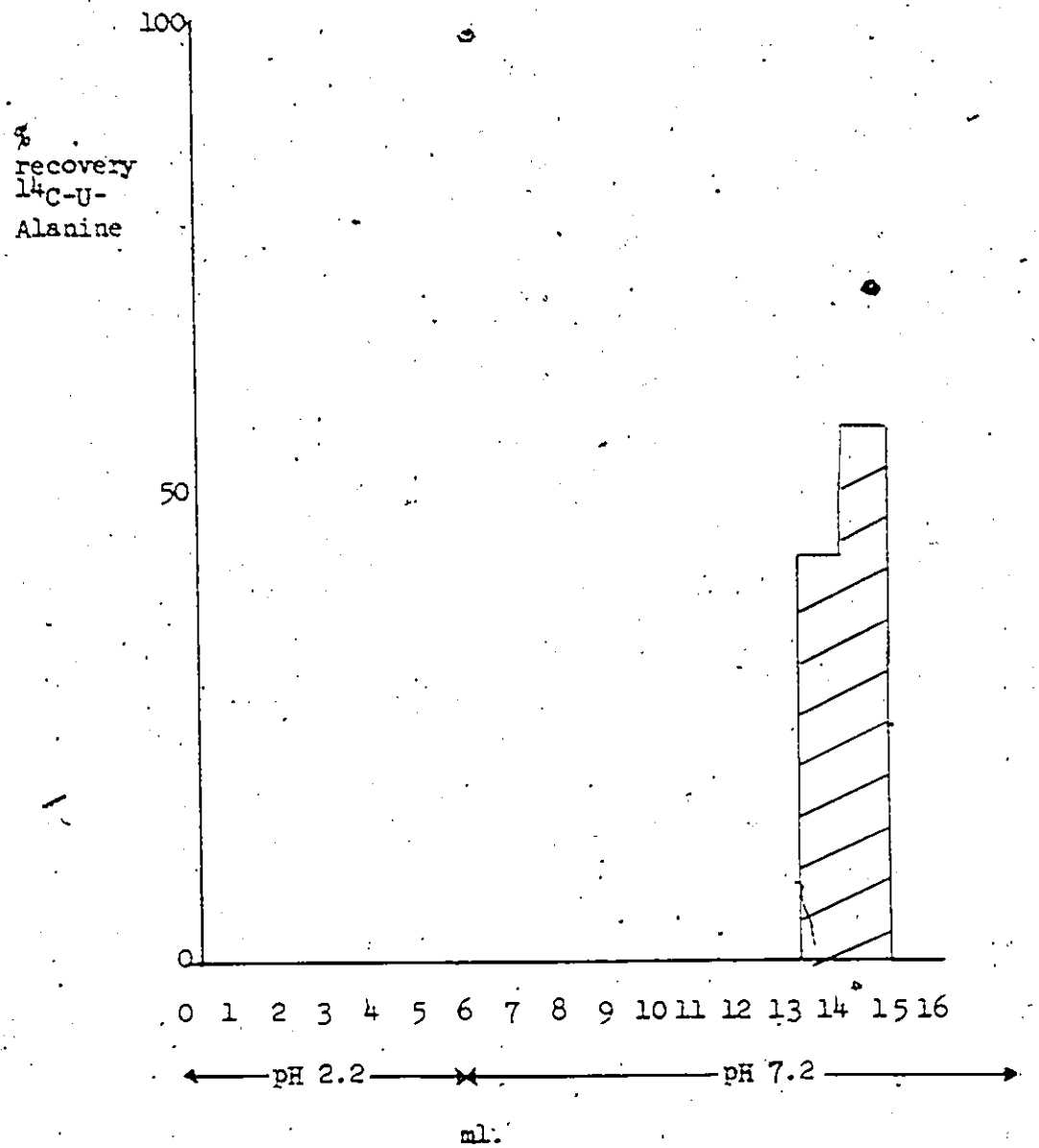


Fig. III

Recovery of ^{14}C-U-Alanine by Ion Exchange Chromatography



i Recovery of ^{14}C-U-alanine in a 3 ml fraction of column eluate

0.2 ml of ^{14}C-U-alanine solution containing a known amount of radioactivity was added to the resin column and was adsorbed. This solution was made from a 6% perchloric acid deproteinized filtrate neutralized with potassium Hydroxide, to remove perchlorate ions and adjusted with normal HCl to pH 2.2.

7 ml of pH 2.2 citrate buffer(128) was added in 1 ml aliquots to the top of the column and the eluate discarded.

6 ml of pH 7.2 phosphate buffer was then put on the column and the eluate collected.

The amount of radioactivity recovered in this fraction was calculated as a percentage of the amount originally at the origin. The results of these experiments are given in Table X.

It was concluded from these experiments

1. Retention of alanine and its elution using this resin and combination of buffers was efficient and reproducible.
2. Recovery was slightly variable for each new batch of Dowex Na^+ so it was decided to determine recovery from each new batch before experiments.

ii Separation of alanine from glucose and pyruvate

These experiments were conducted in two series.

1. 0.2 ml deproteinized plasma pH 2.2 containing a known amount of ^{14}C-U-glucose at the origin.
2. 0.2 ml deproteinized plasma containing a known amount of ^{14}C-U-Na pyruvate at the origin.

The columns were operated as in the previous experiments (i) and the

TABLE X

Recovery of ^{14}C-U-alanine in a 3 ml fraction by ion exchange chromatography

Experiment #	Sample #	^{14}C-U-alanine/0.2 ml at origin, dpm x 10^{-3}	% Recovery of ^{14}C in a 3 ml fraction
1	1	18.1	93.93
	2	"	94.05
	3	"	97.13
2	1	189.6	97.66
	2	"	97.18
3	1	119.4	100.6
	2	"	104.13
	3	"	104.59
4	1	137.4	102.53
	2	"	99.56
5	1	96.9	116.65
	2	"	113.1
	3	"	110.96
	4	"	113.93
	5	"	117.28
6	1	18.45	109.29
	2	"	98.5
	3	"	108.9
	4	"	105.88
7	1	120.4	94.74
	2	"	94.59
	3	"	92.67
	4	"	95.49
	5	"	93.18
	6	"	92.96
8	1	38.9	97.18
	2	"	94.11
	3	"	97.1
	4	"	95.2
9	1	153.9	98.63
	2	"	100.81
	3	"	97.944
	4	"	101.23
		MEAN	100.94
		S.E.M. ±	± 1.27

3 ml fraction previously found to contain all the alanine was collected and the radioactivity in it determined.

The results are shown in Table XI.

It was concluded from these experiments that the alanine fraction was not significantly contaminated by either glucose or pyruvate.

iii Separation of alanine from other amino acids

Alanine was converted to pyruvate by G.P.T. and pyruvate converted to its phenylhydrazine derivative (86). This method has been used on a macro scale by Kreisberger et al (102) but it required considerable modification for micro quantities.

A series of preliminary experiments was carried out to determine:-

1) Optimum volumes of reagents resulting in sufficient weight of phenylhydrazone to be weighed reliably. Because pyruvate phenylhydrazine is yellow, excessively large amounts would result in excessive quenching and statistical inaccuracy in the counting procedure.

2) Recovery by weight and of radioactivity by conversion of carrier Na pyruvate + ^{14}C-U-Na pyruvate after conversion to the phenylhydrazine derivative.

3) Recovery of ^{14}C in the phenylhydrazone after conversion of ^{14}C-U-alanine + carrier alanine.

4) Recovery of ^{3}H in the phenylhydrazone by conversion of ^{3}H-2-3 alanine + carrier alanine.

In all experiments involving liquid scintillation counting a blank sample was prepared containing no radioactivity.

TABLE XI

Separation of Alanine from Pyruvate and Glucose

Experiment #	Sample #	Radioactivity in 0.2 ml at origin dpm x 10^{-3}	% of radioactivity in 3 ml "alanine" fraction
1	1	258	0.22
	2	^{14}C-U-Glucose	0.13
	3		0.15
	4		0.13
		MEAN	0.158
		S.E.M. ±	± 0.021
2	1	390.6	0.095
	2	^{14}C-U-Na pyruvate	0.118
		MEAN	0.107
		S.E.M. ±	± 0.011

These experiments are summarized in Tables XII and XV.

It was concluded from these experiments:-

1) The optimum volumes of reagents were:

0.1 ml Na pyruvate

0.6 ml phenylhydrazine hydrochloride

5 ml phosphate buffer pH 7.2 (141) containing ∝ ketoglutarate (146 mg/100 ml)

25 units G.P.T.

The blank contained 8 ml buffer to allow for 3 ml column eluate in the samples.

2) Although the recovery of ^{14}C from either alanine ^{14}C-U-alanine or ^{14}C-U-pyruvate was consistent within batches, there could be considerable variation between batches. Therefore the individual recovery in each sample was determined by the use of a second tracer, ^{3}H-2-3 alanine.

3) It appeared that half ^{3}H was lost from ^{3}H-2-3 alanine during conversion.

iv Estimation of recovery of ^{14}C from ^{14}C-U-alanine, converted to the pyruvate phenylhydrazine derivative

The basic reactions are:

1. $CH_2NH_2CH_2COOH + CH_2 \cdot CO \cdot COOH \cdot CH_2COOH \xrightarrow{G.P.T.} CH_2 \cdot CHNH_2COOH \cdot CH_2COOH +$

Alanine ∝ Ketoglutaric acid Glutamic acid

$CH_3CO \cdot COOH$

Pyruvic acid

TABLE XII

Preparation of the phenylhydrazine derivative of Pyruvate

Experiment	Sample #	Vol 1M pyruvate ml	Vol phenylhydrazine HCL ml	Radioactivity added	Units GPT	Incubation time	Wt. ppt mg	dpm recovered	S.A. dpm mg^{-1}	% recovery radioactivity	MEAN ±S.E.M.
1	1	0.1	0.6	0.01 ml of ^{14}C-Na pyruvate containing 6.33 x 10^3 dpm.	-	-	13.38	4615	344.9	72.95	
	2	0.1	0.6				14.18	4916	346.7	77.7	75.04
	3	0.1	0.6				13.98	4663	333.5	73.71	± .933
	4	0.1	1.2				14.3	4744	331.7	74.99	
	5	0.1	1.2				13.9	4539	326.5	71.75	
	6	0.1	1.2				13.77	4513	327.7	71.33	
	7	0.2	1.2				28.41	4912	172.9	77.64	
	8	0.2	1.2				28.17	4815	170.9	76.11	
	9	0.2	1.2				29.03	5009	172.5	79.17	
2	1	0.1	0.6	29.45 x 10^3 dpm alanine-U-^{14}C.	50	1 hr	12.3	-	-	61.69	
	2	0.1	0.6		50	"	10.64			53.9	52.87
	3	0.1	0.6		50	"	11.57			55.7	±2.42
	4	0.1	0.6	10.19 x 10^3 dpm Na pyruvate U ^{14}C.	-	-	11.37			44.85	
	5	0.1	0.6		-	-	11.91			47.87	
	6	0.1	0.6		-	-	11.55			52.9	

TABLE XIII

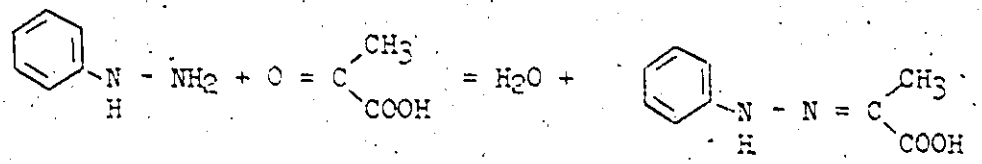
Experiment	Sample #	Vol 1M pyruvate ml	Vol phenylhydrazine HCL ml	Radioactivity added	Units GPT	Incubation time	Wt. ppt mg	dpm recovered	S.A. dpm mg^{-1}	recovery radioactivity	MEAN ±S.E.M.
3	1	0.1	0.6	0.01 ml of ^{14}C-U-Na pyruvate containing 10.61 x 10^3 dpm	-	-	12.5	2938.6	235.1	27.68	28.164 ±0.93
	2	0.1	0.6		-	-	11.94	2854.5	239.1	26.88	
	3	0.1	0.6		-	-	11.27	2839.4	251.9	26.75	
	4	0.1	0.6		-	-	8.87	3370.8	380.0	31.75	
	5	0.1	0.6		-	-	8.22	2978.6	362.4	28.06	
	6	0.1	0.6	1.5 ml carrier 4.6% alanine solution containing 47.38 x 10^3 dpm	50	1 hr	12.22	25966.2	2124.9	54.8	51.91 ±4.07
	7	0.1	0.6		50	1 hr	8.17	18838.9	2305.9	39.76	
	8	0.1	0.6		25	1 hr	12.27	26724.5	2178.0	56.4	
	9	0.1	0.6		25	1 hr	12.74	26858.6	2108.2	56.68	
	10	0.05	0.6		50	1 hr	1.31	4766.4	3638.5	10.06	
	11	0.05	0.6		50	1 hr	0.56	2092.7	3737.0	4.42	
4	1	0.1	0.6	1.5 ml carrier alanine solution containing 142.98 x 10^3 dpm	25	1 hr	13.36	100219.34	7501.4	58.0	56.4 ± .80
	2	0.1	0.6		25	1 hr	13.06	98760.96	7562.1	56.09	
	3	0.1	0.6		25	1 hr	13.23	99347.44	7509.3	57.36	
	4	0.1	0.6		25	1 hr	13.57	97280.308	7168.8	58.34	
	5	0.1	0.6		25	1 hr	12.93	93986.8	7268.9	53.07	
	6	0.1	0.6		25	1 hr	13.16	95602.31	7264.6	55.4	

TABLE XIV

Experiment	Sample #	Vol 1M pyruvate ml	Vol phenylhydrazine HCL ml	Radioactivity added	Units GPT	Incubation time	Wt. ppt mg	dpm recovered	S.A. dpm mg^{-1}	% recovery radioactivity	MEAN ±S.E.M.
5	1	0.1	0.6	1.5 ml	25	1 hr	8.27	62479.4	7554.95	38.67	
	2			4.6%	25		15.83	66684.3	42125.27	41.27	43.0
	3			carrier	25		12.49	74230.86	5943.22	45.94	±1.64
	4			alanine	25		16.77	64492.0	3845.68	39.9	
	5			solution	25		10.46	79753.5	7624.6	49.36	
	6			containing 161.57 x 103 dpm	25		18.29	69318.89	3789.988	42.9	
	7			3.0 ml	25		8.36	16009.17	1914.97	45.9	
	8			carrier	25		11.00	18985.78	1725.98	54.44	49.54
	9			solution	25		11.26	18157.47	1612.56	52.07	±1.63
	10			containing	25		9.19	16997.12	1849.5	48.74	
	11			34871.4 dpm	25		9.2	16249.22	1766.2	46.597	
	12			0.01 ml			11.82	9376.75	793.295	36.69	
	13			^{14}C-U-Na			12.1	9263.84	765.6	36.25	33.74
	14			pyruvate			9.49	8122.595	855.9	31.78	±2.88
	15			solution			11.03	8684.67	787.36	33.99	
	16			containing 25.55 x 103 dpm			10.76	7659.06	711.8	29.97	

TABLE XV

Experiment	Sample #	Vol 1M pyruvate ml	Vol phenylhydrazine HCL ml	Radioactivity added	Units GPT	Incubation time	Wt. ppt mg	dpm recovered	S.A. dpm mg^{-1}	% recovery radioactivity	MEAN $\pm$S.E.M.
6	1	0.1	0.6	3 ml carrier solution containing 175.03 x 10^3 dpm ^{3}H-2-3 alanine	25	1 hr	8.53	41037.19	4810.93	23.45	21.92 $\pm$ .868
	2						8.91	46570.15	5246.9	26.7	
	3						8.3	40875.68	4924.78	23.35	
	4						7.79	37583.55	4824.6	21.47	
	5						6.28	33977.99	5410.5	19.412	
	6						6.517	36882.5	5613.77	21.07	
	7						6.02	34853.08	5789.5	19.9	
	8						6.13	35078.0	5722.35	20.0	

2. 

Phenylhydrazine Pyruvic acid Pyruvate phenylhydrazone

During the conversion of alanine to pyruvate it would be expected that the ^{3}H on the second carbon atom would not appear in pyruvate or the eventual phenylhydrazine derivative.

Experiments designed to assess the recovery of ^{3}H from ^{3}H-2-3 alanine, compared with that of ^{14}C from ^{14}C-U-alanine were therefore carried out.

1 "Double labelled" phenylhydrazone was prepared from varying mixtures of ^{14}C-U-alanine and ^{3}H-2-3 alanine in pH 7.2 PO_4 buffer.

The results are shown in Tables XVI A and B.

2) Phenylhydrazone was prepared from ^{14}C-U-alanine and ^{3}H-2-3 alanine respectively.

The results are shown in Tables XVI A and B.

Because of the quenching by phenylhydrazone, radioactivity in precipitates containing both ^{3}H and ^{14}C was determined in the liquid scintillation counter (Nuclear Chicago Mark II) with the attenuator on channel B set to D250.

It was concluded from these experiments that 50% of ^{3}H from ^{3}H-2-3 alanine was lost during the conversion procedure.

TABLE XVI

Comparison of the recovery as pyruvate phenylhydrazone of ^{3}H from ^{3}H-2-3 alanine with the recovery of ^{14}C from ^{14}C-U-alanine

A:

Experiment #	Sample #	Radioactivity	Counting Efficiency ^{14}C	Counting Efficiency ^{3}H	Spill-over	% recovery radioactivity ^{14}C	% recovery radioactivity ^{3}H
1	1	3 ml containing ^{14}C-U-alanine	58.7			49.5	
	2		59.72			51.04	
	3		56.66			58.27	
	4	2 ml ^{14}C-U-alanine	56.07	19.5	1.12	56.39	26.14
	5	1 ml ^{3}H-2-3 alanine	57.43	21.42	1.075	55.6	25.25
	6	2 ml ^{3}H-2-3 alanine	58.57	21.98	1.023	50.65	23.72
	7	1 ml ^{14}C-U-alanine	60.77	22.12	0.98	46.93	23.36
	8	3 ml ^{3}H-2-3 alanine		19.07			27.63
	9			21.26			24.09
				MEAN ± S.E.M.		52.63 ±1.6	25.03 ±0.67

TABLE XVI

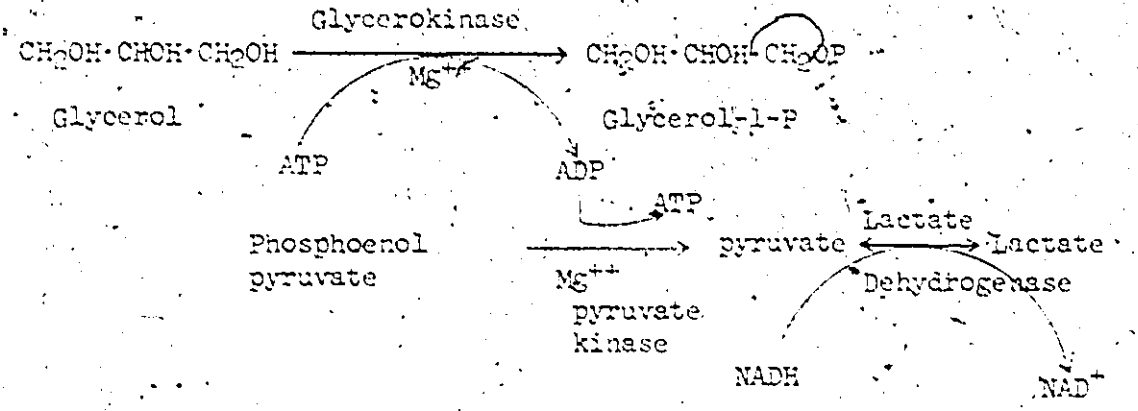
Comparison of the recovery as pyruvate phenylhydrazone of ^{3}H from ^{3}H-2-3 alanine with the recovery of ^{14}C from ^{14}C-U-alanine

B.

Experiment #	Sample #	Radioactivity	dpm recovered ^{14}C x 10^{-3}	dpm recovered ^{3}H x 10^{-3}	% recovery ^{14}C	% recovery ^{3}H
1	1	0.02 ml ^{14}C-	15.71		37.06	
	2	U-alanine	17.54		41.36	
	3	42.4 x 10^{3}	18.17		42.85	
	4	dpm in 3 ml PO_4 buffer pH 7.2	15.74		37.12	
	5	0.02 ml ^{3}H-2-3		28.1		15.17
	6	alanine 185 x		31.4		17.02
	7	10^{3} dpm/3 ml		33.2		17.96
	8	PO_4 buffer pH 7.2		33.8		18.27
				MEAN	39.59	17.1
				± S.E.M.	±1.46	±0.69
2	1	0.02 ml ^{14}C-	20.31		49.16	
	2	U-alanine	18.64		44.94	
	3	41.5 x 10^{3}	16.0		38.63	
	4	dpm/3 ml PO_4 buffer pH 7.2			44.44	
	5	0.02 ml ^{3}H-2-		31.5		15.35
	6	3 alanine		30.7		14.97
	7	205.1 x 10^{3}/		30.4		14.82
	8	3 ml PO_4 buffer pH 7.2		34.7		16.92
				MEAN	44.29	15.51
				± S.E.M.	±2.16	±0.48
			Overall MEAN Recovery S.E.M.± Table A&B		46.93	20.047
					±1.77	±1.26

Estimation of concentration of free Glycerol in plasma

The concentration of free glycerol in plasma was determined using a kit purchased from the Boerhinger Chemical Co. The reactions occurring are:



The change in optical density (O.D.) when $NADH \rightarrow NAD^+$ is measured in a spectrophotometer.

i Accuracy of estimation

Aqueous solutions of glycerol of known concentration were made up and assayed according to the method.

The results appear in Table XVII A.

ii Reproducibility

The glycerol concentration in 10, 0.05 ml aliquots of dog plasma was determined.

The results are given in Table XVII B.

iii The effect of freezing dog plasma on the free glycerol concentration

The concentration of glycerol in plasma was measured from 0.05 ml aliquots of fresh dog plasma. The concentration was measured in the same sample the next day after freezing overnight.

The results are shown in Table XVII C.

It was concluded from these experiments:-

1) The assay was reliable in terms of reproducibility.

2) Freezing raised the concentration of glycerol in plasma, probably due to breakdown of plasma triglyceride. It was decided in all in vivo experiments to determine the concentration of glycerol immediately from fresh plasma.

TABLE XVII

A. Determination of Accuracy of Glycerol Assay

Sample	Content	OD	OD -Blank	mg glycerol 100 ml
1	Blank	0.03		
2	0.2 ml 1 mg% standard	0.177	0.147	0.682
3	0.2 ml 3 mg%	0.509	0.479	2.874

TABLE XVII
Continued

B. Reproducibility of the Assay

Sample	glycerol concentration mg/100 ml	MEAN ± S.E.M.
1	0.372	
2	0.348	0.319
3	0.300	± 0.01
4	0.300	
5	0.300	
6	0.348	
7	0.276	
8	0.276	
9	0.324	
10	0.348	

TABLE XVII.
Continued

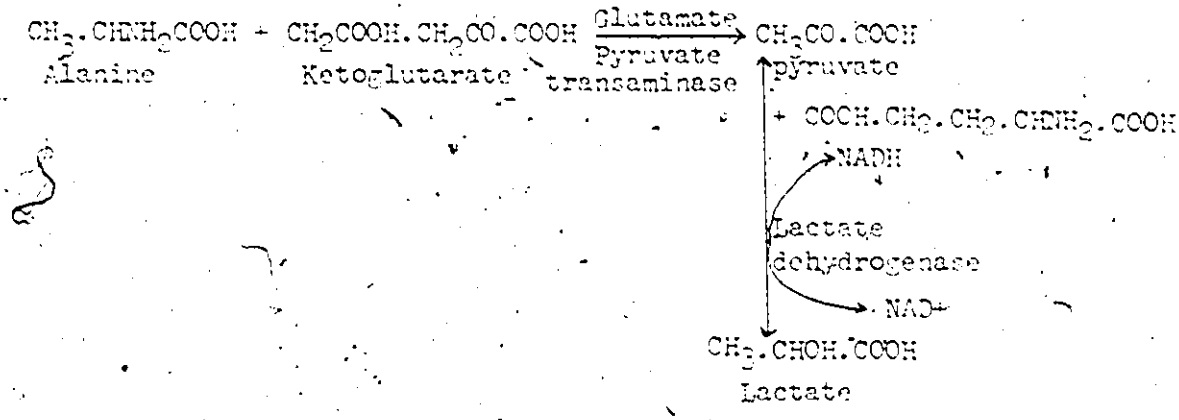
C. The effects of freezing plasma on the concentration of free Glycerol

Sample #	Concentration glycerol mg/100 ml	
	Before freezing	After freezing
1	0.348	1.072
2	0.403	0.977
3	0.271	1.44
4	0.373	1.32
5	0.360	1.22

Estimation of the Concentration of Alanine in Plasma

The concentration of alanine in plasma is determined using the technique of Pfleiderer (141) with a Tris: HCl buffer pH 8.1 (114).

The reactions occurring are:



Alanine is transaminated to its corresponding keto acid, pyruvate which is reduced to lactate. The change in O.D. when NADH is oxidized to NAD+ is measured in a spectrophotometer.

The change in optical density resulting from aliquots of an alanine "standard" solution (50 Ug ml^{-1}) equivalent to 1, 2, and 3 Ug alanine was measured before each assay. A standard curve of O.D. vs alanine concentration was constructed and used to calculate the concentration of alanine in the plasma sample. Blank samples were prepared by substituting a volume of distilled water equal to the sample volume.

Effects of sample volume on measured alanine concentration

The concentration of alanine was measured in three 0.1 ml

aliquots of plasma and compared with the concentration measured in 0.05 ml aliquots of plasma + 0.05 mls water.

The results are shown in Table XVIII A.

ii Reproducibility

The alanine concentration in 9, 0.1 ml and 9, 0.05 ml aliquots of dog plasma was determined.

The results are shown in Table XVIII B.

From these experiments it was concluded:

1) That plasma alanine concentration could be determined reliably by this technique from either 0.05 or 0.1 ml plasma.

2) The assay was reliable in terms of reproducibility.

TABLE XVIII

A Effects of Sample Volume on Measured alanine concentration

Sample #	Ug alanine standard	Volume of Plasma ml	Δ OD - Blank	Ug alanine
	1	-	0.005	-
	2	-	0.0102	-
	3	-	0.0157	-
1	-	0.05	0.01558	3.06
2	-	0.05	0.01560	3.1
3	-	0.1	0.03233	6.35
4	-	0.1	0.03233	6.35

TABLE XVIII
Continued

B Reproducibility of the Alanine Assay

Sample #	Ug alanine standard	Volume of plasma ml	Δ OD -Blank	mg/100 ml alanine
	1	-	0.0325	-
	2	-	0.055	-
	3	-	0.0775.	-
1	-	0.05	0.0395	3.08
2	-	0.05	0.0395	3.0
3	-	0.1	0.082	3.22
4	-	0.1	0.077	2.97
5	-	0.1	0.077	2.97
6	-	0.1	0.077	2.97
7	-	0.1	0.072	2.75
8	-	0.1	0.0725	2.78
9	-	0.1	0.084	3.3
10	-	0.1	0.076	2.94
11	-	0.1	0.078	3.02
			MEAN ± S.E.M.	3.0 ± 0.049

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