GLUCOSE TRACER KINETICS AND TURNOVER IN MONKEYS AND CHICKENS INFUSED WITH ETHANOL, 1,3-BUTANEDIOL, OR FRUCTOSE

By

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ABSTRACT

GLUCOSE TRACER KINETICS AND TURNOVER IN MONKEYS AND CHICKENS INFUSED WITH ETHANOL, 1,3-BUTANEDIOL, OR FRUCTOSE

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Mixtures of (2-^{3}H) and (U-^{14}C) or (6-^{3}H) and (U-^{14}C) glucose were injected as single doses into fasted cynomolgus monkeys to assess glucose tracer kinetics and obtain rates of turnover. Data were treated by stochastic and compartmental analyses and results from both analyses closely agreed. However, (2-^{3}H) data analyzed by the compartmental analysis required three pools to fit the glucose disappearance curve while (6-^{3}H) data fit a two or three pool model equally well. Turnover rates averaged 4.9, 4.0, and 3.0 mg/min x kg^{-1} body weight with (2-^{3}H), (6-^{3}H) and (U-^{14}C) glucose tracers, respectively. The data heuristically suggest that the slow turnover pool that was necessary to fit (2-^{3}H) glucose data is related to isotope discrimination.

Effects of four treatment solutions on (6-^{3}H) glucose metabolism in monkeys were examined. The solutions and their rates of infusion (umoles/min x kg^{-1}) were: 1) ethanol, 110; 2) 1,3-butanediol, 110; 3) fructose, 30; and 4) ethanol plus

fructose, 110 and 30, respectively. Glucose turnover rates markedly decreased during either ethanol or 1,3-butanediol infusions (2.6 and 3.1 mg/min x kg^{-1}, respectively versus control values of 5 mg/min x kg^{-1}). Since glucose clearance was not decreased by ethanol or 1,3-butanediol, both treatments caused hypoglycemia. Fructose infusions increased the glucose turnover rate and concentration by increasing the glucose production rate 20%. The glucose clearance rate was lowest during the ethanol plus fructose infusions.

Chickens were chosen for further study to simplify the ethanol infusion paradigm (oxalacetate conversion to phosphoenolpyruvate is exclusively mitochondrial in chickens, but not monkeys). Ethanol infusions (222 or 444 umoles/min x kg^{-1} body weight) in chickens (1500 g) fasted 64 hours did not cause hypoglycemia although the high dose slightly decreased the rate of glucose turnover 15% (14.0 versus 12.0 mg/min x kg^{-1}). It was further found that neither the hepatic cytosolic nor the mitochondrial redox state significantly changed in chickens infused with the high dose of ethanol. The unchanged hepatic metabolite ratios in chickens are consistent with their unusual resistance to ethanol-induced hypoglycemia.

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LIST OF ABBREVIATIONS

ADP.....adenosine diphosphate

AMP.....adenosine monophosphate

ATP.....adenosonine triphosphate

ATPase.....adenosine triphosphatase

BD.....1,3-butanediol

CoA.....coenzyme A

cm.....centimeter

dl.....deciliter

ETOH.....ethanol

f-6-p.....fructose-6-phosphate

FDPase.....fructose diphosphatase

g.....grams

G-6-Pase.....glucose-6-phosphatase

GK.....glucokinase

GTP.....guanosine triphosphate

Hr.....hour

kg.....kilogram

Km.....Michaelis constant

mg.....milligram

min.....minute

ml.....milliliter

mm.....millimoles

LIST OF ABBREVIATIONS (cont'd)

mM.....millimolar

N.....normal

NAD.....nicotinamide adeninine dinucleotide

NADH.....reduced nicotinamide adeninine dinucleotide

NADP.....nicotinamide adeninine dinucleotide phosphate

NADPH..reduced nicotinamide adeninine dinucleotide phosphate

PC.....pyruvate carboxylase

PEPCK.....phophoenolpyruvate carboxykinase

tm.....mean transit time

TT.....turnover time

uCi.....microcurie

umoles.....micromoles

Vmax.....maximum velocity

wt.....weight

w/v.....weight to volume

INTRODUCTION

Since the brain, nerves, red blood cells, kidney medulla and testes all have an absolute requirement for glucose, the maintainance of glucose homeostasis is essential for vertebrate survival. Normally, vertebrates are subject to wide variations in food intake and physical activity; therefore, when dietary sources of glucose are insufficient, de novo glucose synthesis must occur to prevent hypoglycemia and death.

Studies reveal factors controlling the de novo synthesis of glucose are important to understanding how glucose production relates to other aspects of metabolism. Furthermore, periods of normal metabolic stress (fasting and lactation) as well as profound metabolic stress are known to affect glucose kinetics--though to different degrees. For example, Long et al. (1978) reported that glucose kinetics in patients suffering extensive burns differ from those in patients suffering from severe skeletal trauma.

Much of what is known about gluconeogenesis has come from studies using rats; however, Hanson (1974) has cautioned readers to be wary of extrapolating rat data to humans and suggested that, relative to gluconeogenesis guinea pigs more closely resemble man. Hanson (1974) also

suggested that monkeys might be worthwhile models for studying glucose production in humans. However there is little information about glucose kinetics in monkeys.

The studies presented in this thesis were undertaken: 1) to establish basal values for the rate of glucose turnover in monkeys and elaborate on the tracer methodologies commonly used to measure glucose flux; 2) to study alterations in glucose flux rates when monkeys were infused with ethanol and fructose, both of which are known to affect glucose homeostasis in other species including man; and 3) to evaluate the effects of 1,3-butanediol on glucose metabolism. Butanediol is a relatively high energy compound that may be suitable for use in intravenous feeding.

In order to somewhat simplify the ethanol paradigm for further study, chickens were chosen as the animal model because oxalacetate is converted to phosphoenolpyruvate exclusively in the mitochondria.

PART 1

REVIEW OF LITERATURE

THE ORGANS OF GLUCOSE SYNTHESIS

The liver and the renal cortex are the only organs capable of synthesizing glucose from non-carbohydrate sources: amino acids, glycerol and lactate (Bhagavan, 1974). However, the renal contribution to the total glucose production is generally nil except during prolonged fasts: then it may contribute nearly 40% of the total glucose released into the bloodstream (Owen et al., 1976). Disregarding the differences in the gluconeogenic capacities of the two organs, the gluconeogenic pathways between them are identical.

GLUCONEOGENIC SUBSTRATES AND PATHWAYS

Quantitatively, lactate, glycerol and amino acids are the most important precursors for gluconeogenesis. But lactate is a product of glucose degradation in tissues that are functioning anaerobically such as the renal medulla, erythrocytes and exercising skeletal muscle. Therefore, gluconeogenesis from lactate does not represent a net synthesis of glucose for oxidation by tissues such as the brain (Newsholme and Start, 1974). The reactions that make

the reversal of glycolysis possible are shown below with their enzymes following in parentheses.

1) Pyruvate -> Oxalacetate
(Pyruvate carboxylase)

2) Oxalacetate -> phosphoenolpyruvate
(Phosphoenolpyruvate carboxykinase)

3) Glucose-6-phosphate -> Glucose
(Glucose-6-phosphatase)

The conversion of pyruvate to oxalacetate (reaction 1) is catalyzed by pyruvate carboxylase (PC), an enzyme that was thought to reside in the cytosol and mitochondria, but was later shown to exist only in the mitochondria (Barritt, Zander, and Utter, 1976; Hanson, 1974). PC is an allosteric enzyme that is activated by acetyl CoA and competitively inhibited by many CoA derivatives present in the mitochondria (Barritt et al., 1976). Besides allosteric modification, the metabolic flux through PC is also dependent on the pyruvate concentration. Normally, the concentration of pyruvate is lower than the Km reported for PC (isolated from chicken liver); a slight decrease in pyruvate could significantly affect the rate of flux from pyruvate to oxalacetate (Scrutton and Utter, 1965).

A decrease in the mitochondrial ATP/ADP ratio also strongly inhibits PC but this is apparently through competitive inhibition since Utter (1978) has shown that PC is not dependent on a phospho-dephosphorylation mechanism for activity.

PC requires the presence of monovalent and divalent cations for activity and K^{+} and Mg^{++} fulfill the requirement better than others. However, there is not good evidence demonstrating that metal ions regulate PC activity in vivo.

Phosphoenolpyruvate carboxykinase (PEPCK) is the regulatory enzyme that catalyzes the conversion of oxalacetate to phosphoenolpyruvate (reaction 2).

The cellular distribution of this enzyme varies among species and perhaps even among different tissues within a species. In fed rats, mice and hamsters, 90% or more of the enzyme is cytosolic while the remainder is mitochondrial. Most other species including rabbits, guinea pigs, swine, sheep, dogs, cows and humans have considerable amounts of PEPCK in both the cytosol and mitochondria (Soling and Kleineke, 1976). In all of these species PEPCK is adaptive, but in avians, PEPCK is non-adaptive and nearly all mitochondrial (Soling et al., 1973; Brady et al., 1978; Gevers, 1967).

Regulation of hepatic PEPCK is primarily affected by hormonal action, though the energy status of the cell (the concentration of GTP) is known to influence activity as well. Mn^{++} and Fe^{++} stabilize PEPCK in vitro and Bentle and Snoke (1974) have isolated a cytosolic protein that activites PEPCK in vitro by increasing its sensitivity to Fe^{++}. Whether this protein and Fe^{++} regulate PEPCK activity in vivo, remains to be seen.

Glucagon, as well as cyclic AMP has been shown to cause a three-fold increase in the hepatic activity of PEPCK within 4 hours of administration in vivo (Reshef and Hanson, 1972) whereas insulin is known to decrease the rate of hepatic PEPCK synthesis in vivo (Tilghman et al., 1974) and in vitro (Tilghman et al., 1975). The case for PEPCK regulation by glucocorticoids is not as clear since they have been shown to increase as well as decrease the PEPCK activity (Tilghman et al., 1976). Newsholme and Start (1974) have suggested that glucocorticoids play a permissive role by increasing the sensitivity of the PEPCK synthetic site to cyclic AMP.

PEPCK activity in the rat kidney, unlike that in the liver, is unaffected by cyclic AMP and more sensitive to depression by bicarbonate than to insulin. Conversely, synthesis of PEPCK is markedly increased by glucocorticoids and acidosis and these appear to be the major regulatory factors governing the rate of PEPCK synthesis in the kidney (Tilghman et al., 1976).

The third regulated reaction in the sequence leading to glucose production is catalyzed by fructose-1,6-diphosphatase (FDPase). In all species examined thus far, the FDPase activity is significantly higher than that of its antagonist, phosphofructokinase (Soling and Kleineke, 1976). FDPase is an allosteric enzyme that is strongly inhibited by AMP and stimulated by 3-phosphoglycerate, excess ATP, and

near physiological concentrations of citrate (Lehninger, 1975; Bhagavan, 1974).

FDPase requires divalent Mg^{++} or Mn^{++} and a metal chelate for optimal activity. Pontremoli et al. (1974) have evidence to suggest that physiological concentrations of histidine chelate divalent cations and optimal activity can be achieved in the presence of these histidine-metal chelates. Thus histidine may be an important in vivo modulator of FDPase activity.

Evidence of hormonal regulation of FDPase is limited and sometimes contradictory. Increased FDPase activity in the presence of glucagon is certainly an attractive possibility and experiments performed by Taunton et al. (1972) demonstrate such a relationship. However cyclic AMP, the presumed mediator of glucoagon stimulated gluconeogenesis, has been shown to decrease FDPase activity in the kidney (Holzer and Duntze, 1972).

Glucocorticoids might also be responsible for increasing FDPase activity by increasing hepatic, intracellular concentrations of histidine via their effects on lysozymes and proteolytic activity. However, to date, there is no evidence demonstrating such a mechanism.

Glucose-6-phosphatase (G-6-Pase) catalyzes the final reaction in the pathway to glucose production. Though the maximum enzyme activity is low, and the mass-action ratio of products/reactants is much lower than the apparent equilibrium constant of G-6-Pase, there is no experimental

evidence showing that this reaction is regulated by anything other than glucose-6-phosphate concentration in vitro or in vivo (Newsholme and Start, 1974).

Since G-6-Pase appears to be a constitutive enzyme, the regulation of glucose release from the liver has tentatively been assigned to glucokinase (GK). GK is the antagonist to G-6-Pase. Therefore, when glucose concentration is high, GK activity is increased through an insulinotropic effect on GK synthesis. When GK activity exceeds that of G-6-Pase a net uptake of glucose occurs. Conversely, as blood glucose and insulin levels decrease, GK activity falls; when it is less than G-6-Pase activity, a net release of glucose occurs (Newsholme and Start, 1974). Since both enzymes are simultaneously active, a glucose -> glucose-6-phosphate -> glucose cycle occurs. The presence of such a substrate cycle is supported by several reports (Issekutz, 1977; Katz et al., 1976; Armstrong, Romsos and Leveille, 1979).

THE REDOX STATE AND GLUCONEOGENESIS

The cellular $NADH/NAD^+$ ratio is known to be one of the determinants of flux rate through the gluconeogenic and glycolytic pathways. While a moderate increase in the cytosolic $NADH/NAD^+$ favors gluconeogenesis in all species studied thus far, the redox state in the mitochondria affects gluconeogenesis differently in various species

depending on the distribution of PEPCK between the mitochondria and the cytosol.

As much as 95% of the PEPCK in rat liver is cytosolic and while an increase in the cytosolic NADH/NAD^+ ratio might be expected to reduce the concentration of oxalacetate available for phosphoenolpyruvate formation, this occurs only to a limited extent (Krebs, 1968) because malate is transported into the mitochondria, reoxidized to oxalacetate, and transported back to the cytosol as aspartate. After deamination, the oxalacetate thus formed is once again reduced to malate and the cycle is repeated (Hanson, 1974). The mitochondrial NADH derived from this shuttle is oxidized by the electron transport chain or used to form beta-hydroxybutyrate from acetoacetate--the former ultimately released from the liver for utilization by peripheral tissues.

In species where the PEPCK is primarily mitochondrial or distributed equally between the mitochondria and cytosol, the transport of reducing equivalents and its effect on gluconeogenesis becomes more complex. In these species, the transport of reducing equivalents into the mitochondria and the concomittant increase in the mitochondrial NADH/NAD^+ ratio is able to reduce the mitochondrial concentration of oxalacetate if the reoxidation of NADH is unable to keep pace with NADH production. It is now beginning to appear (perhaps by coincidence) that species with mitochondrial PEPCK present to any significant extent also have low

beta-hydroxybutyrate dehydrogenase activities which preclude them from using acetoacetate as a metabolic sink for reducing equivalents. Evidence of low beta-hydroxybutyrate dehydrogenase has been reported for the chicken (Brady et al., 1978), guinea pig and man (Jomain-Baum et al., 1978). Despite the low enzyme activities in the chicken and guinea pig, fasting does not result in an increase in the mitochondrial $NADH/NAD^+$ as might be expected (Brady et al., 1978; Jomain-Baum et al., 1978). Fasting rats are known to have an increased $NADH/NAD^+$ ratio (Hanson, 1974). How do chickens and guinea pigs maintain a more oxidized mitochondria when mitochondrial NADH is being generated by beta-oxidation?

Jomain-Baum et al. (1978) have suggested that a relatively oxidized $NADH/NAD^+$ ratio can be maintained in species with intramitochondrial PEPCK because the consumption of GTP in the conversion of oxalacetate to phosphoenolpyruvate ensures a significant activation of the electron transport chain. They have further shown that adding physiological amounts of ammonium to solutions perfusing guinea pig livers diverts significant amounts of alpha-ketoglutarate and NADH to glutamate and aspartate via the glutamate dehydrogenase and aspartate transaminase reactions. Both of these products are released from the mitochondria for subsequent conversion to urea in the hepatic cytosol.

Since ethanol is known to alter hepatic $NADH/NAD^+$ ratios, it is a useful treatment for the study of gluconeogenesis.

THE SITES AND METABOLISM OF ETHANOL

Less than 10% of the total ethanolic load given to animals or humans can be excreted by the kidney and lungs: the rest is oxidized in the body, principally in the liver (Lieber, 1970). The main hepatic pathway for the oxidation of ethanol (ETOH) requires the cytosolic enzyme, alcohol dehydrogenase and the primarily mitochondrial enzyme, acetaldehyde dehydrogenase (Havre, Margolis and Abrams, 1976). Both enzymes are NAD^+ dependent and neither are known to be allosterically regulated, though alcohol dehydrogenase requires Zn^{++} as a cofactor (Weiner, 1969). The reactions are as follows.

1) Ethanol + NAD^+ -> Acetaldehyde + NADH

2) Acetaldehyde + NAD^+ -> Acetate + NADH

When alcohol is present in excessive amounts, this reaction sequence generates a surplus of reducing equivalents and is responsible for a variety of metabolic abnormalities (Lieber et al., 1975).

While the cytosolic oxidation of ETOH is the major pathway, Lieber et al., (1975) have shown that ETOH metabolism persists even when alcohol dehydrogenase is

specifically inhibited by pyrazole. Further study revealed that there is a secondary pathway for ETOH oxidation located in the hepatic microsomes. This pathway is dependedent on NADPH as a cofactor, characterized by a high Km for ETOH (10 mM) and adaptable to chronic ETOH administration (Lieber et al., 1975). Since alcohol dehydrogenase activity is high and its Km for ETOH is low, the microsomal system probably accounts for only a small fraction of the ETOH metabolism when the dose is low and acute.

RATE-LIMITING FACTORS IN ETHANOL METABOLISM

The precise factors which limit the rate of ETOH utilization are still not known, but it appears that the rate of oxidation may be controlled by several different factors--each of them rate-limiting under different physiological conditions.

Meijer et al. (1975) have shown that ETOH oxidation via alcohol dehydrogenase is limited by the reoxidation rate of NADH. Further, they have shown that the oxidation rate of NADH is limited by the mitochondrial shuttle activity in fasted rats and by the electron transport chain in fed rats.

Alcohol dehydrogenase activity has not been considered a rate-limiting factor in ETOH metabolism because the in vitro activity has been reported to be much higher than the rate of ETOH oxidation in vivo. But Crow, Cornell and Veech

(1977) have found that previously reported results are erroneously high due to methodological flaws in previously reported assay systems. In some cases, alcohol dehydrogenase activity was measured in the direction of acetaldehyde -> ETOH and in others, the reaction mix contained lactaldehyde which can accelerate the alcohol dehydrogenase catalyzed oxidation of ETOH by 50 to 100 fold. When hepatic alcohol dehydrogenase activity was assayed using a revised method the activity was found to be about 120% of the ETOH oxidation rate as measured in vivo (Crow et al., 1977).

THE EFFECTS OF ETHANOL ON GLUCOSE TURNOVER

Reports correlating the consumption of alcohol with hypoglycemia began to appear in the early 1940's (Dellon, Dyer and Smelo, 1940; Brown and Harvey, 1941; Tucker and Porter, 1942). As early as 1959 some investigators (Smith and Newman, 1959) were suggesting that hypoglycemia resulting from alcohol ingestion was linked to an increased, hepatic, $NADH/NAD^+$ ratio and impaired gluconeogenesis.

It is now well established that ETOH increases the hepatic cytoplasmic $NADH/NAD^+$ ratio. Krebs et al. (1969) found that pyruvate added to the solution perfusing livers from starved rats in either the absence or presence of ETOH caused a sharp increase in the hepatic lactate concentration in both groups with little effect on the rate of

gluconeogenesis. When the pyruvate was depleted, the livers perfused with the pyruvate solution were able to convert the lactate back to pyruvate and then to glucose. But livers perfused with pyruvate and ETOH were unable to convert the accumulated lactate back to pyruvate and then to glucose when the pyruvate in the perfusate was depleted. Apparently, ETOH increased the $NADH/NAD^{+}$ ratio and kept pyruvate trapped as lactate.

While the experiment of Krebs et al. (1969) provided some direct evidence for ETOH inhibition of gluconeogenesis, one cannot exclude the possibility that ETOH provokes hypoglycemia in vivo by increasing the peripheral rate of glucose utilization. Lochner, Wulff and Madison (1967) infused ETOH into anesthetized dogs and found that glucose production was inhibited by 65% while peripheral utilization was decreased by 25%. Searle et al. (1974) found that glucose removal rates in normal humans were unaffected while glucose production and plasma glucose concentrations were declining. However, when their patients reached a hypoglycemic nadir, and a steady state assumption was made for plasma glucose concentration, the removal rate was significantly less than it was during the control and post-ethanol, pre-steady state treatment periods.

Dittmar and Hatenyi (1978) did glucose turnover studies in dogs infused with ETOH. They found that glucose production rates decreased while metabolic clearance rates for glucose increased slightly but not significantly.

However, after 40 minutes of ETOH infusions the glucose production rate and plasma glucose concentration increased. Generally, it is agreed that ETOH infusions can decrease the rate of gluconeogenesis in fasted animals. However, whether or not ETOH infusions affect the metabolic clearance rate of glucose is still not clear.

GLUCONEOGENESIS FROM FRUCTOSE

The production of glucose from fructose occurs in the hepatic cytosol and does not require PC or PEPCK. However, the conversion of fructose to glucose does require three enzymes that are not required for gluconeogenesis from pyruvate or lactate--fructokinase, aldolase B, and triokinase (Veneziale, 1976). The reactions catalyzed by these enzymes are shown below.

1) Fructose + ATP -> Fructose-1-phosphate + ADP
(Fructokinase)

2) Fructose-1-phosphate -> Dihydroxyacetone phosphate + D-Glyceraldehyde
(Aldolase B)

3) D-glyceraldehyde + ATP -> D-glyceraldehyde-3-phosphate + ADP
(Triokinase)

The first reaction is catalyzed by fructokinase, a relatively unregulated enzyme with a high activity and a low Km for fructose (Lee, 1974). Since the liver is freely permeable to fructose, high doses of fructose can deplete

hepatic ATP stores (Raivio, Kekomaki and Maepaa, 1969) and cause fructose-1-phosphate to accumulate in the liver.

Scholz et al. (1975) have added fructose to the buffer perfusing livers from fasted rats and calculated the relative flux rates of fructose to glucose, glycerol, pyruvate and lactate. They observed that 52% of the fructose was recovered as glucose, 2% as glycerol and 46% as pyruvate and lactate. When both ETOH and fructose were in the buffer, 77% of the fructose was recovered as glucose, 11% as glycerol and 14% as pyruvate and lactate.

THE "FRUCTOSE EFFECT" ON ETHANOL METABOLISM

Stuhlfauth and Neumaier (1951) were the first to demonstrate that fructose infusions increased the rate of ETOH oxidation. Many explanations (some of them contradictory) have been advanced to explain this effect and the most viable of these can be classified as those that suggest a direct effect on the cytosolic redox state versus those that suggest fructose enhances ETOH oxidation by decreasing the hepatic energy level.

Since Thorell and Chance (1951) established that the dissociation of the alcohol dehydrogenase-NADH complex is the slowest step in the conversion of alcohol to acetaldehyde, and Holzer and Schneider (1955) have shown that glyceraldehyde can be converted to glycerol by the

alcohol-NADH complex, Tygstrup, Winkler and Lundquist (1965) have proposed that glyceraldehyde from fructose is converted to glycerol by the enzyme-NADH complex; thus, the dissociative step is eliminated and the alcohol dehydrogenase-NAD^+ complex is left intact to further react with ETOH. This now appears to be unlikely because the Km of the enzyme-NADH complex for glyceraldehyde is about 10 times higher than the concentrations of glyceraldehyde--even in livers perfused with 4.0 mM solutions of glyceraldehyde (Veneziale, 1976).

Tygstrup et al. (1965) also showed that at least part of the fructose effect can be attributed to the production of sorbitol via sorbitol dehydrogenase; however, only a small amount of the fructose effect can be accounted for by this pathway.

Tygstrup et al. (1965) disregarded the phosphorylation of glyceraldehyde to glyceraldehyde-3-phosphate via triose kinase as being relevant to the fructose effect because, "...this will give no possibility for explaining the increased ethanol oxidation." Here, they failed to consider the effects of ADP generated by this reaction.

Scholz et al. (1975) believe that fructose increases the rate of ETOH oxidation by decreasing the hepatic ATP/ADP ratio thus stimulating electron transport. This hypothesis is supported by their experiments showing a linear relationship between the number of ethanol-derived reducing equivalents passing through the electron transport chain and

the amount of ATP necessary to support glucose production from fructose added to the perfusion medium in 4 different concentrations. Their argument is convincing and supported by several other lines of evidence.

If one assumes that transport of reducing equivalents is not rate-limiting in the fructose perfused livers as Meijer et al. (1975) have shown to be true in fed rats, then both groups of investigators (Scholz et al., 1975; Meijer et al., 1975) agree that stimulating the electron transport chain--whether it be done by decreasing the ATP/ADP ratio, or by uncoupling electron transport with dinitrophenol--will result in increased rates of ETOH oxidation. Furthermore, rats fed ETOH chronically have an increased rate of ETOH metabolism that has been linked to an increase in the Na^+-K^+-ATPase activity (Lieber, 1976).

At present, the mechanism for the fructose effect proposed by Scholz et al. (1975) seems to fit best with what is known about rate-limiting factors in ETOH metabolism.

THE SITES AND METABOLISM OF 1,3-BUTANEDIOL

Butanediol (BD) is metabolized in the liver: first to 3-hydroxybutanal by alcohol dehydrogenase, and then to beta-hydroxybutyrate by an aldehyde reductase (Mehlman, Tobin and Mackerer, 1975). While the pathway is identical to that previously described for the oxidation of ETOH,

there is some evidence to suggest that alcohol dehydrogenase has a lower Vmax for BD than for ETOH (Tate, Mehlman and Tobin, 1971).

METABOLIC EFFECTS OF 1,3-BUTANEDIOL

Several groups of investigators have studied the metabolic effects of BD after feeding it to chickens and pigs (Romsos et al., 1975), rats (Romsos, Belo and Leveille, 1974; Romsos, Sasse and Leveille, 1974), cattle (Young, 1975) and humans (Tobin et al., 1975).

When BD was fed as 18% of the dietary energy to pigs and chickens, blood ketones significantly increased (Romsos et al., 1975). Similar results were obtained when cattle were fed BD as 4% of the dietary energy (Young, 1975). Blood ketones in humans fed BD as 5% of the dietary energy did not change (Tobin et al., 1975).

Romsos et al. (1975) reported that BD feeding caused plasma glucose to increase in pigs, but not chickens. Plasma glucose did not change in BD fed cattle (Young, 1975), but it was significantly decreased in humans (Tobin et al., 1975). Tobin et al. (1975) also reported that BD exerted a significant nitrogen sparing effect in humans. Similar results have been reported in dogs infused with BD (Kremer, Vitolina and Frank, 1970).

Kremer et al. (1970) and Vitolina, Gergensone and Kremer (1977) are the only ones known to have studied the effect of BD infusions. The first study demonstrated the nitrogen sparing effect and the second study showed that the elevated ketone bodies resulting from BD infusions can be reduced by fibrinosol or insulin. Both of these studies were done on dogs.

To date, there have been no studies showing how BD infusions affect glucose turnover.

PART 2

GLUCOSE TURNOVER IN FASTED CYNOMOLGUS MONKEYS (*MACACA FASCICULARIS*) AS MEASURED BY (2-^{3}H), (6-^{3}H), AND (U-^{14}C) GLUCOSE

INTRODUCTION

Rates of glucose utilization determined by injecting or infusing animals with a mixture of tritiated glucose and (^{14}C)-glucose have been reported for a number of species, including dogs (Issekutz, 1977; Belo, Romsos and Leveille, 1976), cows (Kronfeld, 1977), chickens (Brady, Romsos and Leveille, 1977), rats and rabbits (Katz et al., 1974b). Similar studies of monkeys are especially interesting because they are phylogenetically close to man. For example PEPCK, a major regulatory enzyme in gluconeogenesis, is known to differ among species relative to cellular distribution, adaptivity and total activity (Soling and Kleineke, 1976). If phylogenetic proximity is related to metabolic similarity, then the monkey may serve as a good model for the study of human metabolism.

The experiments in this study were undertaken to compare the in vivo glucose flux rates in Cynomolgus monkeys (Macaca fascicularis) as estimated by (2-^{3}H) or (6-^{3}H) glucose.

MATERIALS AND METHODS

Glucose turnover studies were performed on six adult, female Cynomolgus monkeys fed a commercially prepared biscuit-type diet (Purina Monkey Chow 25, Ralston Purina Co., St. Louis, MO) and fasted 64 hours prior to the experiments. The studies were generally conducted simultaneously on two monkeys per day. The monkeys were seated in primate restraining chairs to which they were accustomed. A 20-gauge around-needle catheter (Becton, Dickinson and Co., Rutherford, NJ) was inserted into an antebrachial vein without surgery or anesthesia. The monkeys were then allowed to sit for approximately 45 minutes prior to the injection of a single dose of 200 uCi of either $(2\text{-}^{3}H)$ or $(6\text{-}^{3}H)$ glucose (Amersham/Searle, Arlington Heights, IL) plus 29 uCi of $(U\text{-}^{14}C)$ glucose (ICN, Irvine, CA) dissolved in 2.5 ml of 0.9% NaCl. A 1 ml blood sample was withdrawn prior to the tracer injection and every 3 minutes after the tracer injection for the first 15 minutes. Samples were obtained at 5 minute intervals from 15 to 30 minutes and at 15 minutes intervals from 30 to 60 minutes. Additional samples were withdrawn at half-hour intervals from 60 to 300 minutes. Blood samples were collected in chilled tubes containing heparin and sodium fluoride. The samples were centrifuged as soon as possible and the plasma was removed and frozen pending further analysis. At the conclusion of the experiments the animals were anesthetized

(Sernylan, Bio-Ceutic Labs, Inc., St. Joseph, MO) and weighed.

Plasma samples were deproteinized with barium hydroxide and zinc sulphate (Somogyi, 1945). Aliquots of the supernatant fluid were used for the determination of plasma glucose (Glucostat, Worthington Biochemical, Freehold, NJ). Other aliquots of the samples were passed through 1 X 5 cm columns containing equal parts by weight of Dowex 1-x8, Cl^- and Dowex 50W-x8, H^+ (Bio-Rad Laboratories, Rockville Centre, NY). The glucose was eluted from the column by rinsing them with three, 5 ml portions of water. Glucose eluates were collected in scintillation vials, evaporated to dryness in a 37°C water bath under an airstream, and redissolved in 1 ml of water prior to the addtiion of scintillation cocktail (3a70, Research Products International Corp., Elk Grove Village, IL). Radioactivity was quantified with a liquid scintillation spectrophotometer adjusted to discriminate tritium emissions from those of ^{14}C. Counting efficiency was obtained by using an external radium standard, and counts appearing in the tritium channel were adjusted for spillover from the ^{14}C channel.

Parameters of glucose metabolism were calculated by stochastic analysis as described by Katz, Rostami and Dunn (1974a) except that the areas under the rate of utilization curve and transit time curve were obtained by employing the trapezoidal rule instead of graphical analysis. For the sake of comparison, general, three-term, exponential equations

for each tracer were derived by plotting the log of the specific activity versus time. The coefficients and rate constants were obtained by a curve-peeling process described by Riggs (1963). The specific equations used to calculate the rate of utilization, mean transit time and body mass of glucose from the derived equations are from Shipley and Clark (1972). The values obtained for the glucose parameters were tested for statistical significance by an analysis of variance.

RESULTS

During the 6 months of these experiments, neither the body weights of individual monkeys nor their average as a group (3.8 kg with a range of 2.7 to 5.3 kg) changed significantly (Table 1). The overall mean plasma glucose value obtained during the (2-^{3}H) glucose experiments did not differ significantly from the value obtained during the (6-^{3}H) glucose experiments (Table 1); however, plasma glucose values for time points within the (2-^{3}H) experiments were found to differ significantly (Figure 1).

Estimates of glucose utilization as calculated from the (2-^{3}H) glucose disappearance curves were significantly higher than estimates calculated from either the (6-^{3}H) or the (U-^{14}C) glucose data. The glucose utilization calculated from the (6-^{3}H) glucose data was significantly higher than

Table 1. Body Weights and glucose parameters of monkeys injected with either (2-^{3}H) or (6-^{3}H) and (U-^{14}C) glucose.

	(2-^{3}H) and (U-^{14}C)		(6-^{3}H) and (U-^{14}C)	
Weight (kg)	$3.7 \pm 0.3^{a\dagger}$		3.8 ± 0.3^{a}	
Plasma glucose (mg/dl)	67 ± 7^{a}		49 ± 8^{a}	
Rate of utilization	4.8 ± 0.3^{a}	3.3 ± 0.3^{c}	4.0 ± 0.3^{b}	3.0 ± 0.3^{c}
(mg/min per kg)	$(5.0 \pm 0.3^{a})^{\ddagger}$	(3.0 ± 0.3^{c})	(4.2 ± 0.3^{b})	(2.8 ± 0.3^{c})
% ^{14}C recycling	----------		25 ± 5	
			(33)	
% Glucose (-) F-6-P	18		----------	
cycling	(18)		----------	
Mean transit time (min)*	56 ± 3^{b}	69 ± 4^{a}	43 ± 4^{a}	65 ± 3^{a}
Body mass glucose (mg/kg)	276 ± 24^{a}	$223 \pm 25^{a,b}$	170 ± 18^{b}	$198 \pm 20^{a,b}$

* Mean transit time was calculated according to Katz et al. (1974a).

† Values are mean ± S.E.M. for 12 experiments performed in six monkeys. Numbers in the same row with different superscripts are significantly different ($p<0.05$) as determined by analysis of variance.

‡ Values in parentheses are calculated from three-term, exponential equations and are not significantly different from the values directly above them as determined by Student's t-test ($p>0.05$).

Fig. 1. Plasma glucose values in (2-^{3}H) and (6-^{3}H) glucose experiments plotted as a function of time. Each point represents the mean $\pm$ S.E.M. of 12 values obtained from six monkeys. Plasma glucose values in the (2-^{3}H) experiments, but not in the (6-^{3}H) experiments, decreased ($p<0.05$) with time.

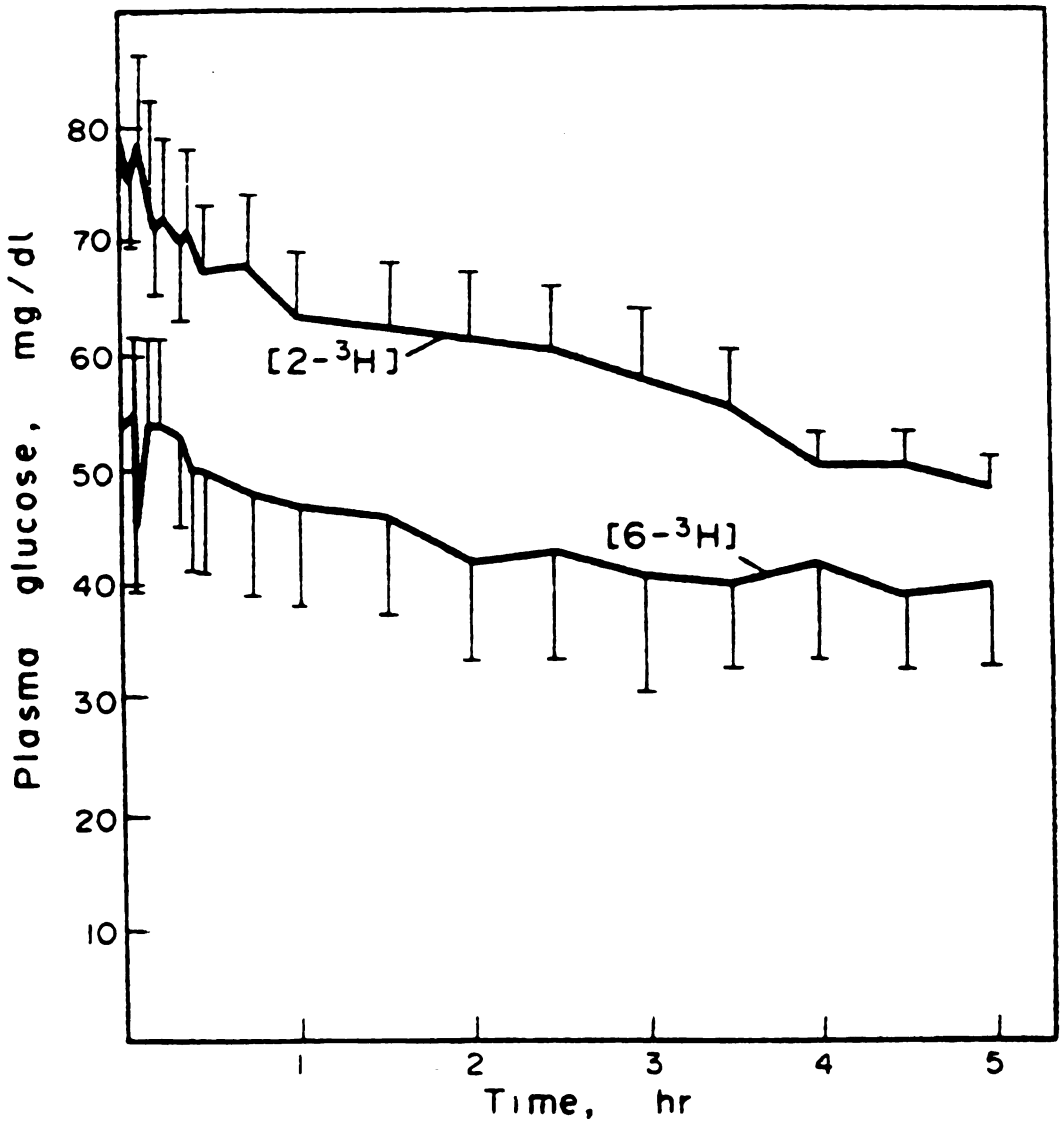

Plasma glucose, mg/dl
80
70
60
50
40
30
20
10
[2-3H]
[6-3H]
1
2
3
4
5
Time, hr

Fig. 1.

that calculated from the (U-^{14}C) glucose data (Table 1). As expected, the rates calculated from the (U-^{14}C) data were statistically the same regardless of the isotopic form of tritiated glucose that was simultaneously injected with the (U-^{14}C) glucose (Table 1).

The percentage of the radioactive carbon from glucose which was recycled back to glucose is shown in Table 1. This calculation was only done for those experiments using (6-^{3}H) and (U-^{14}C) glucose because (6-^{3}H) yields a better estimate of glycolytic flux than does (2-^{3}H) glucose (Katz et al, 1976). The recycling of ^{14}C has been used to approximate the amount of three-carbon substrates converted back to glucose (Katz et al., 1976); however, the estimate is only qualitative because the intracellular specific activity of gluconeogenic substrates was not determined.

The percentage of glucose <-> fructose-6-phosphate substrate cycling was 18.4. This value was obtained by subtracting the (6-^{3}H) rate from the (2-^{3}H) rate and dividing by the (2-^{3}H) rate of glucose utilization (Issekutz, 1977).

The mean transit time, the average length of time a molecule remains in the sampling pool, was shortest when calculated from the (6-^{3}H) data and longest when calculated from the (U-^{14}C) data (Table 1). Mean transit times calculated from (6-^{3}H), (2-^{3}H) and (U-^{14}C) glucose tracers differed significantly from each other.

The estimates of body glucose mass ranged from 170 to 276 mg/kg body weight when obtained with (6-^{3}H) and (2-^{3}H) glucose, respectively. Estimates of body glucose mass calculated from (U-^{14}C) glucose were intermediate between these two values.

The number in parenthesis in Table 1 shows the rates of glucose utilization and percentage of both ^{14}C recycling and glucose <-> fructose-6-phosphate substrate cycling obtained when general, three-term, exponential equations were derived from the data (Figure 2). Difficulties arising from differences in pool size were circumvented by integrating the equations and applying the appropriate formulae for a stochastic analysis (Katz et al., 1974a). The average rates of glucose utilization for each tracer did not differ significantly whether results were calculated by the trapezoidal rule or by integrating the derived equations.

DISCUSSION

The results reported for glucose utilization are qualitatively consistent with those reported by other investigators using (2-^{3}H) or (6-^{3}H) combined with (U-^{14}C) glucose. However, a notable exception to the conclusion that (2-^{3}H) glucose yields higher estimates of glucose flux than (6-^{3}H) glucose has been reported in sheep by Judson and Leng (1972).

Fig. 2. Specific activities of (2-^{3}H), (6-^{3}H), and (U-^{14}C) glucose plotted as a function of time. Each point represents a mean of 12 values obtained from six monkeys at sampling time. o, data from (2-^{3}H); •, data from (6-^{3}H) experiments. ———, tritium data; -----, (U-^{14}C) data. Each curve was separated into three component pools (0-20, 0-120, and 0-300 minutes). The derived equations are:

$$2\text{-}^{3}H,\ SA = 12.28e^{-0.1200t} + 22.89e^{-0.0383t} + 14.88e^{-0.0170t}$$

$$U\text{-}^{14}C,\ SA = 65.52e^{-0.1967t} + 33.36e^{-0.0271t} + 13.88e^{-0.0065t}$$

$$6\text{-}^{3}H,\ SA = 140.0e^{-0.2626t} + 18.47e^{-0.0287t} + 15.49e^{-0.0124t}$$

$$U\text{-}^{14}C,\ SA = 173.7e^{-0.3104t} + 39.36e^{-0.0303t} + 12.75e^{-0.0059t}$$

The dose for all monkeys was normalized to 10,000 distintegrations per min per animal.

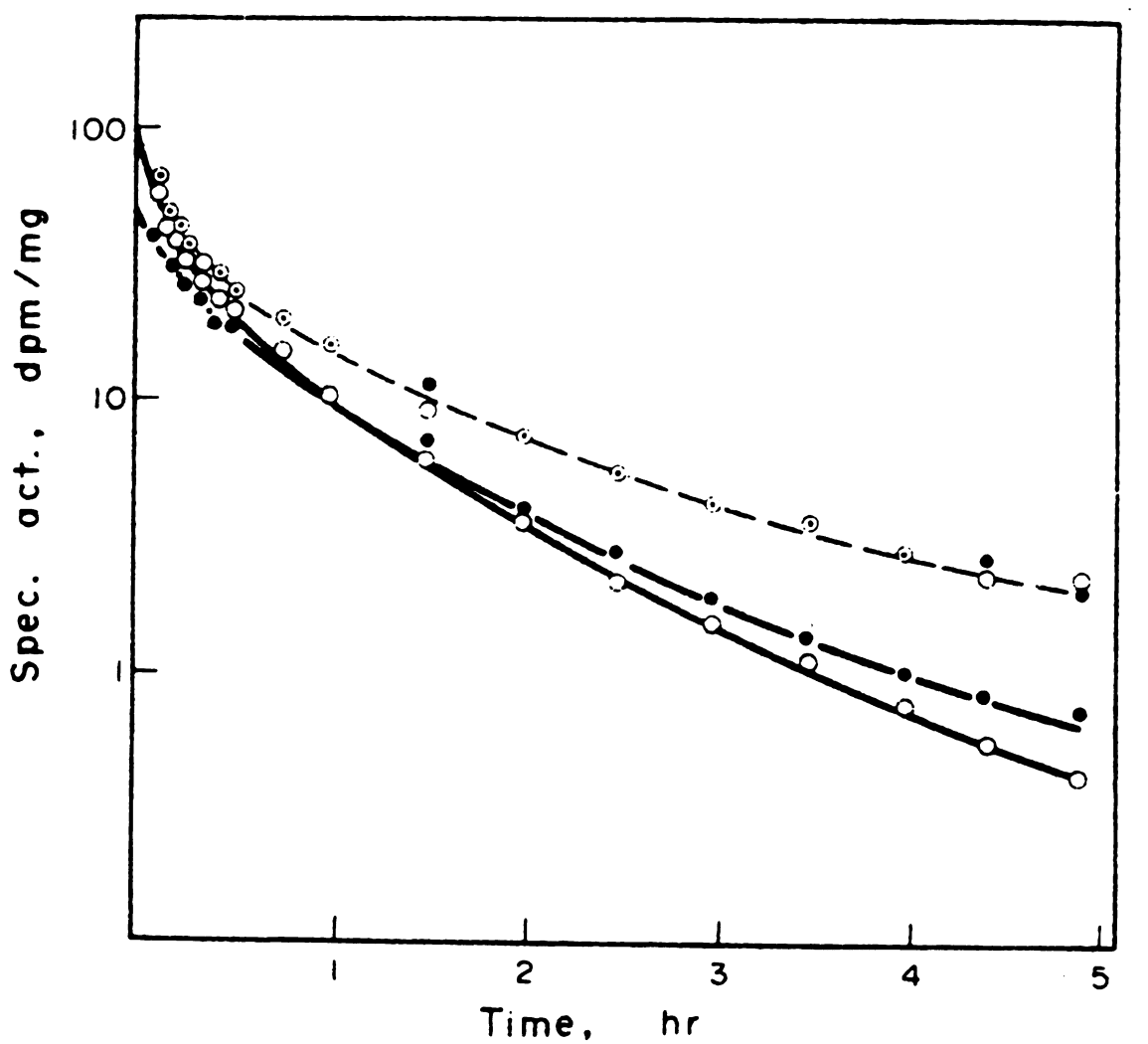


Fig. 2.

Quantitatively, the rate of glucose utilization in the monkey appears to be higher than 0.3 mg/min per kg for kelp bass (Bever, Chenoweth and Dunn, 1977); 0.65 mg/min per kg for fasted horses (Evans, 1971); 0.8 mg/min per kg fasted humans (Searle et al., 1974). The values for the monkey closely resemble the 3.7 mg/min per kg for the dog (Belo et al., 1976) and the 4.0 mg/min per kg for the rabbit (Katz et al., 1974b). Values as high as 10.8 mg/min per kg for rats (Katz et al., 1974) and 16.0 mg/min per kg for chickens (Brady et al., 1977) suggest that the monkey is intermediate relative to rates of glucose turnover. The reasons for interspecies differences in turnover rates are not entirely clear: however, it is likely that differences in metabolic rate, diet composition, insulin and glucagon sensitivity, metabolic compartmentation, and the ability of obligate glucose utilizing tissues, such as the brain, to adapt to altered physiological conditions, are partially responsible.

The formulae used to determine the rate of glucose utilization depend on the steady state assumption for validity. During the (2-^{3}H) glucose experiments, the plasma glucose levels of the monkeys declined significantly (Figure 1). This finding implies that the steady state assumption possibility was not valid during the (2-^{3}H) experiments. Consequently, these data were also calculated according to methods described by Shipley and Clark (1972) for non-steady state analysis of flux rate. Results obtained in these experiments were identical regardless of whether or not

the steady-state assumption was made; hence, the results from the non-steady-state analysis are not reported. The reason for the significant changes in plasma glucose during the (2-^{3}H) glucose experiments was not explored, but the majority of the (2-^{3}H) experiments were performed prior to the (6-^{3}H) experiments. Possibily the blood glucose levels changed as part of a stress response to catheterization and sampling procedures, both of which were initially unfamiliar to the monkeys.

The percentage of ^{14}C recycled to glucose in the monkey is about the same as that reported for dogs (Belo et al., 1976) but less than the 40% reported for chickens (Brady et al., 1977). Bergman et al. (1974) have suggested that the small amount of recycling that occurs in sheep (5%) may be due to the lack of glucokinase in ruminant liver.

When (2-^{3}H) glucose is injected into animals, the rate of glucose utilization appears to be higher than when (6-^{3}H) glucose is injected. Since (2-^{3}H) glucose is detritiated largely during the phosphoglucoisomerase reaction, and since (6-^{3}H) glucose is detritiated at the level of pyruvate, the difference between these estimates of the rates of glucose utilization may be considered as an approximation of the amount of substrate cycling which occurs between glucose-6-phosphate and fructose-6-phosphate. The calculation of substrate cycling in this reaction sequence is initially complicated by the isotope discrimination that phosphoglucoisomerase has against accepting (2-^{3}H)

glucose-6-phosphate as substrate for the reaction (Rose, 1970).

The values for substrate cycling obtained in the present study (Table 1) are somewhat higher than that reported for the dog (13%) by Issekutz (1977) and lower than that reported by Brady et al. (1977) for the chicken (25%). The validity of this comparison must be considered cautiously because the amount of substrate cycling calculated in the monkey and chicken experiments was based on average ($2-^3H$) and ($6-^3H$) values obtained on different days, in the case of monkeys, and in different animals, in the case of chickens. Issekutz (1977) injected ($2-^3H$) and ($6-^3H$) into the same dogs simultaneously. Until suitable methodologies and materials are available to account for in vivo isotope discrimination effects, the value given for glucose <-> fructose-6-phosphate percentage cycling (Table 1) should be considered only as a first approximation.

There is an apparent inconsistency in the rates of glucose utilization and transit time (Table 1). The rate of disappearance of ($2-^3H$) was greater than the rate of disappearance of ($6-^3H$) glucose; yet the transit time for ($2-^3H$) glucose was greater than the transit time for ($6-^3H$) glucose. One might have logically expected the tracer that disappeared at the fastest rate to remain in the body for the shortest length of time. This paradox may result from the way in which isotope discrimination initially affects the distribution of points on the transit time curve.

As Table 2 reveals, there was only a 4% probability of the (2-^{3}H) label irreversibly leaving pool 1, whereas there was a 23% probability of the (6-^{3}H) label leaving pool 1. Conversely, there was a 68% probability that the (2-^{3}H) label would leave pool 3 while there was only a 55% probability that the (6-^{3}H) label would leave pool 3. If one assumes that all species of glucose have an equal chance of being taken up by the cell, then it follows that (2-^{3}H) glucose suffers discrimination intracellularly. In vitro studies with purified enzyme preparations have established that phosphoglucoisomerase discriminates against (2-^{3}H) glucose (Rose, 1970). If one presumes that there is no discrimination against the (6-^{3}H) glucose, then the discrimination ratio K_{2H}/K_{6H}, can be calculated from isotope discrimination tables constructed by Lietzke and Collins (1959). The discrimination ratio calculated from pool 1 rate constants for the (2-^{3}H) and (6-^{3}H) experiments was 0.46. The numerical proximity of this value with that reported from in vitro studies heuristically suggests that isotope discrimination occurs in vivo to approximately the same extent as it does in vitro. An initial period of isotopic discrimination could substantially increase the overall mean transit time of a labeled compound.

If one further hypothesizes that the discrimination against (2-^{3}H) glucose is auto-eradicating because it results in an increase in the intracellular specific activity, then the first pool rate constant will be affected

Table 2. Probability of isotopic removal, turnover time (TT), and mean transit time (tm) expressed per pool.*

	Pool 1			Pool 2			Pool 3		
	Prob.	TT min	tm min	Prob.	TT min	tm min	Prob.	TT min	tm min
(2-^{3}H)	0.04	8.33	0.33	0.27	26.11	7.16	0.68	93.45	64.10
(U-^{14}C)	0.10	5.08	0.50	0.35	36.90	13.07	0.55	153.84	84.36
(6-^{3}H)	0.23	3.80	0.89	0.28	34.84	9.85	0.55	80.64	44.31
(U-^{14}C)	0.16	3.22	0.50	0.35	33.00	11.51	0.50	169.49	83.96

*Probability of isotopic removal is calculated as the fraction of the total dose removed from the pool in question. Turnover time of the pool is the reciprocal of the rate constant for that pool. Mean transit time for the pool is the product of the probability of removal and the turnover time (Shipley and Clark, 1972).

more than those of the second and third pools. Thus, the rate constants of the second plus the third pool would more accurately represent glucose flux through glycolysis, and substrate cycling between glucose and fructose-6-phosphate. If one uses only the second and third pool rate constants to calculate the rate of utilization in the (2-^{3}H) experiments, it is slightly higher than the value obtained when all three rate constants are used (5.2 versus 5.0). If the second and third pool rate constants of the (6-^{3}H) glucose experiments are used to calculate the rate of glucose utilization, a much greater value is obtained than when the rate constants for all three (6-^{3}H) pools are considered (5.4 versus 4.2). During the first 20 minutes subsequent to the tracer injection, little of the (2-^{3}H) was irretrievably lost. But this was clearly not the case when (6-^{3}H) glucose was injected. When the second and third pool rate constants of the (2-^{3}H) and (6-^{3}H) experiments were used to calculate the amount of glucose <-> fructose-6-phosphate substrate cycling, the value was 16%. This compares favorably with the values calculated directly from the overall rates of utilization (Table 1) and suggests that the first pool in the (2-^{3}H) experiments causes one to slightly underestimate the overall rate of utilization but contributes significantly to the mean transit time.

PART 3

EFFECTS OF ETHANOL, FRUCTOSE, AND ETHANOL PLUS FRUCTOSE INFUSIONS ON PLASMA GLUCOSE CONCENTRATION AND GLUCOSE TURNOVER IN MONKEYS (*MACACA FASCICULARIS*) AS MEASURED BY (6-^{3}H) GLUCOSE

INTRODUCTION

When ethanol is used as an energy source, it can cause hypoglycemia (Cummins, 1961; Tolis, 1965) and lactic acidemia (Ott et al., 1976). Since ethanol does not increase plasma insulin concentrations in rats, humans, or pigs (Singh and Patel, 1976; McMonagle and Felig, 1975; Nikkila and Taskinen, 1975; Kuhl et al., 1976) ethanol induced hypoglycemia is not secondary to an insulin mediated, glucose uptake.

The oxidation of 1,3-butanediol (BD) to 3-hydroxybutanal is analogous to the oxidation of ethanol to acetaldehyde: BD is oxidized by the cytosolic NAD^+-dependent, alcohol dehydrogenase (Mehlman, Tobin and Mackerer, 1975). Since the oxidation of BD is analogous to ethanol it may similarly induce hypoglycemia.

Fructose infusions have been reported by some investigators to increase the peripheral clearance rate and splanchnic uptake of ethanol in humans (Stuhlfauth and Neumaier, 1951; Tygstrup, Winkler and Lundquist, 1965). But, experimental results of others showed that fructose had little or no effect on ethanol clearance (Levy, Elo and Hanenson, 1977). Whether or not fructose affects ethanol clearance, fructose may prevent ethanol-induced hypoglycemia

if it, unlike lactate, pyruvate and alanine, can be rapidly converted to glucose during simultaneous ethanol metabolism. The objectives of the present investigations were to examine the effects of ethanol, BD, fructose, and ethanol plus fructose infusions on plasma glucose concentrations, glucose turnover rates and glucose clearance rates in Cynomolgus monkeys.

MATERIALS AND METHODS

Glucose turnover studies were performed on six adult, female Cynomolgus monkeys fed a commercially-prepared, biscuit-type diet (Purina Monkey Chow 25, Ralston Purina Co., St. Louis, MO). Allowing at least 10 days between infusions, each monkey was infused twice with each treatment and tracer solution. The monkeys were fasted 64 hours prior to the experimentation and the studies were conducted simultaneously on two monkeys while they sat in primate restraining chairs to which they were accustomed. Two 20-gauge around-needle catheters (Becton, Dickinson and Co., Rutherford, NJ) were inserted into antecubital or saphenous veins without surgery or anesthesia.

The tracer and treatment solutions were infused into one catheter and blood samples were withdrawn from the other. After catheterization, 3.0 uCi of (6-^{3}H) glucose

in saline was continuously infused for seven hours at a rate of 0.3 uCi/min x kg^{-1}. A Harvard Syringe Pump (Harvard Apparatus Co., Inc., Millis, MA) delivered 0.83 ml/min of tracer solution and a steady state specific activity of glucose (^{3}H dpm/mg glucose) appeared in the plasma within 2 hours of starting the tracer infusions.

In control experiments, monkeys were infused with tracer only for 7 hours. When treatment solutions were administered, monkeys were infused first with tracer for 3 hours and then with tracer and treatment solutions for 4 hours; thus, there was an initial control period associated with each treatment as well as a series of 7-hour control experiments. To minimize osmotic effects of treatment solutions, they were infused as 10% w/v solutions of ethanol in saline; fructose in distilled water; or 10% w/v of each, ethanol plus fructose in distilled water. BD was infused as a 20% w/v solution in distilled water. Ethanol and BD infusion rates were 110 umoles/min x kg^{-1}, while those for fructose were 30 umoles/min x kg^{-1}.

One ml blood samples were withdrawn every 15 minutes from the second through the third hour and every half-hour thereafter for glucose analysis. Blood samples were collected in chilled, heparinized tubes containing sodium fluoride. Samples were centrifuged within 30 minutes and the plasma was removed and frozen pending further analysis. Additional 1 ml blood samples were drawn every hour from the second through the seventh for pyruvate, lactate, ethanol

and beta-hydroxybutyrate determinations. Immediately after collecting a sample for these assays, it was mixed with 4 ml of cold perchloric acid (3% w/v) and kept on ice until the end of the experiment. The perchloric acid-treated blood samples were then centrifuged and the supernatant fluid was stored at $-40^{\circ}C$ until lactate (Hohorst, 1965), ethanol (Mattenheimer, 1970) and beta-hydroxybutyrate (Williamson and Mellanby, 1965) analyses were done.

Plasma samples were deproteinized with barium hydroxide and zinc sulphate (Somogyi, 1945). Aliquots of the supernatant fluid were used for the determination of plasma glucose (Glucostat, Worthington Biochemical, Freehold, NJ). Other aliquots of the samples were passed through 1 x 5 cm columns containing equal parts by weight of Dowex 1-x8, Cl^{-} and Dowex 50W-x8, H^{+} (Bio-Rad Laboratories, Rockville Centre, NY). The glucose was eluted from the columns by rinsing them with three, 5 ml portions of water. Glucose eluates were collected in scintillation vials, evaporated to dryness in a $37^{\circ}C$ water bath under an airstream, and redissolved in 1 ml of water prior to the addtiion of scintillation cocktail (3a70, Research Products International Corp., Elk Grove Village, IL). Radioactivity was quantified with a liquid scintillation spectrophotometer.

Rates of glucose turnover and clearance were calculated according to equations described by Shipley and Clark (1972). In the steady state, when neither the specific

acitivity of the plasma glucose pool nor the plasma glucose concentration changes, the rate of glucose production necessarily equals the rate of glucose disposal and is commonly called the glucose turnover rate. The equation used to calculate the glucose turnover rate is:

$$\text{Turnover Rate} = \frac{\text{Rate of Tracer Infusion (uCi/min)}}{\text{Plasma Specific Activity (uCi/mg glucose)}}$$

Once a steady state has been attained, the glucose turnover rate can only be affected by a change in glucose production. A decrease in glucose production increases the plasma specific activity and decreases the plasma specific activity; thereby causing an increase in the glucose turnover rate.

Changes in the glucose disposal rate will not affect the plasma specific activity. To determine whether there were changes in the glucose disposal rate, the clearance rate was calculated as follows:

$$\text{Clearance Rate} = \frac{\text{Tracer Infusion Rate (uCi/min)}}{\text{Plasma Tracer Concentration (uCi/ml)}}$$

Hence an increase in glucose disposal rate decreases plasma tracer concentration and increases clearance rate. Similarly, a decreased rate of glucose utilization increases the plasma tracer concentration thereby decreasing the clearance rate.

Changes in the rate of glucose turnover and clearance as well as plasma glucose concentrations were tested for

statisitical significance ($p<0.05$) by establishing three non-orthogonal contrasts that were labelled as segment I (2-3 hours), II (3.5-4.5 hours), and III (5.0-7.0 hours) in Figures 3 and 4. Each segment within an experiment was tested against another by using the Bonferroni t-test (Neter and Wasserman, 1974).

RESULTS

During the 18 months duration of these experiments, neither body weights of individual monkeys nor their average as a group (3.8 kg with a range from 2.7 to 5.3 kg) changed significantly.

In animals infused with tracer alone, the plasma glucose concentration declined significantly with time from a mean of 69 mg/dl during segment I to 54 mg/dl during segment III (Figure 3, panel A). This was caused by an increase in the clearance rate of glucose from 8.8 to 10.4 ml/min during the control experiments and not by a decrease in glucose production because the plasma specific activity and the glucose turnover rate (5.1 mg/min x kg^{-1}) were constant during the experiments.

Effects of ethanol on plasma glucose concentration and on (6-^{3}H) glucose turnover rate are shown in Figure 3, panel B. Plasma glucose concentration and turnover rate averaged 63 mg/dl and 5.1 mg/min x kg^{-1} prior to infusion of ethanol.

Fig. 3. Plasma glucose concentration and glucose turnover rate in monkeys infused with 0.3 uCi ($6-^{3}H$) glucose/min x kg^{-1} for seven hours. Each point represents values for six monkeys with two observations per monkey. Panel A, control experiments; panel B, ethanol infusions (110 umoles/min x kg^{-1}) initiated at the third hour (see arrow); panel C, 1,3-butanediol infusions (110 umoles/min x kg^{-1} initiated at the third hour (see arrow). Changes in plasma glucose concentration and rates of glucose turnover were tested for statistical significance ($p<0.05$) by establishing three non-orthogonal contrasts labeled as segments I (2-3 hours), II (3.5-4.5 hours) and III (5.0-7.0 hours). Each segment within an experiment was tested against another by using the Bonferroni t-test (Neter and Wasserman, 1974). Segments with similar superscript letters are not different ($p>0.05$). Standard errors for glucose concentration and glucose turnover in each panel were: (A) 3.1 mg/dl and 0.02 mg/min x kg^{-1}; (B) 3.7 mg/dl and 0.04 mg/min x kg^{-1}; and (C) 6.0 mg/dl and 0.2 mg/min x kg^{-1}.

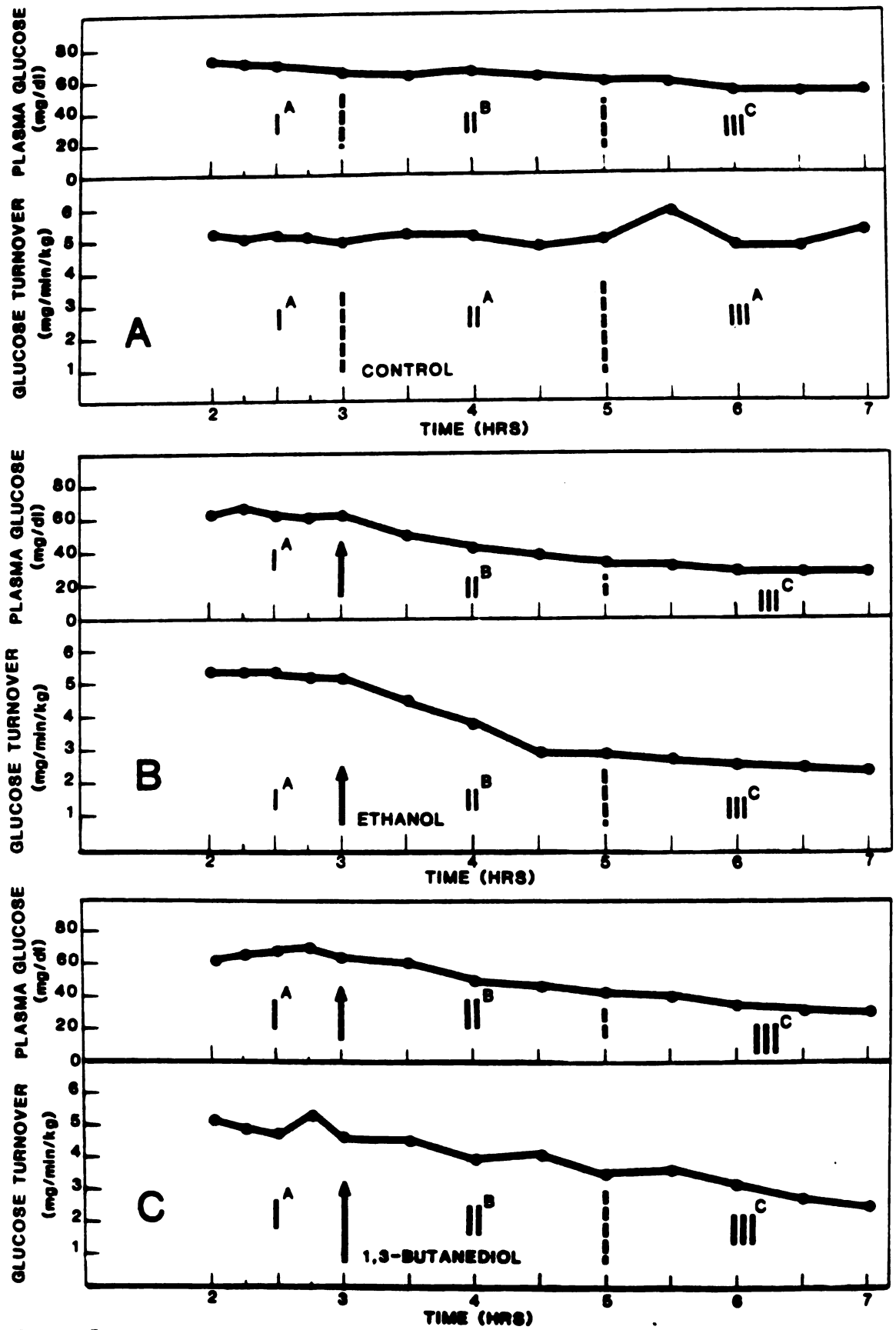

PLASMA GLUCOSE (mg/dl)
GLUCOSE TURNOVER (mg/min/kg)
A
CONTROL
B
ETHANOL
C
1,3-BUTANEDIOL
TIME (HRS)

Fig. 3.

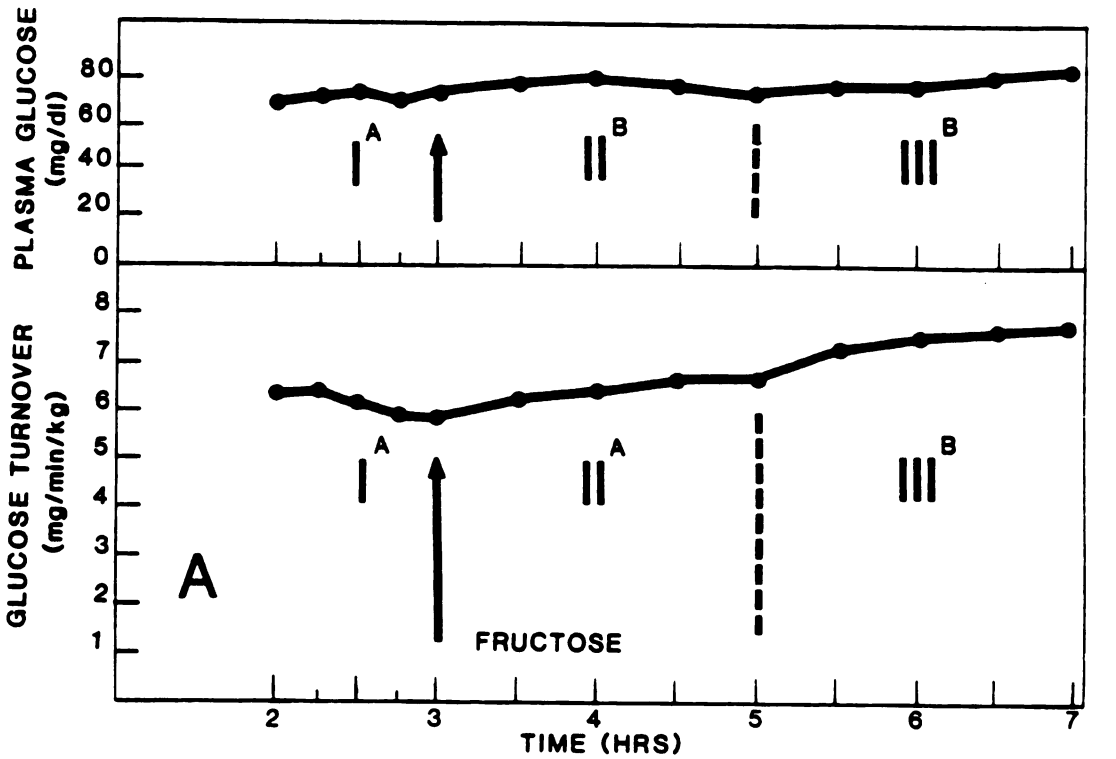
After two to four hours of ethanol infusion (segment III) the plasma glucose concentration and turnover rate had decreased to 30 mg/dl and 2.6 mg/min x kg^{-1}, respectively.

When BD was infused (Figure 3, panel C) plasma glucose levels declined from 67 mg/dl (segment I) to 56 mg/dl (segment II). Average plasma glucose levels during segment III (38 mg/dl) were less than averages in the other two segments. The rate of glucose turnover declined from 5.0 in segment I to 4.2 and 3.1 mg/min x kg^{-1} in segments II and III, respectively. Thus, responses to BD paralleled those observed when ethanol was infused.

Effects of fructose on plasma glucose concentration and on rates of (6-^{3}H) glucose turnover are shown in Figure 4, panel A. The concentrations of glucose in plasma increased from 69 mg/dl prior to fructose infusion to 76 mg/dl after fructose infusion. The glucose turnover rate increased from 6.1 (segment I) to 7.4 mg/min x kg^{-1} (segment III).

Plasma glucose concentrations and turnover values obtained after simultaneous infusions of ethanol and fructose are shown in Figure 4, panel B. Concentrations of glucose in plasma paralleled those observed when fructose was infused alone: they increased from 62 (segment I) to 72 mg/dl (segment III). The glucose turnover rate temporarily declined to 5.4 mg/min x kg^{-1} during segment II but returned to pretreatment, steady-state values during segment III. Thus, the hypoglycemic action of ethanol was eliminated by concommitant fructose infusion.

Fig. 4. Plasma glucose concentration and glucose turnover rate in monkeys infused with 0.3 uCi ($6-^{3}H$) glucose/min x kg^{-1} for seven hours. Each point represents values for six monkeys, with two observations per monkey. Panel A, fructose infusions (30 umoles/min x kg^{-1}) initiated at the third hour (see arrow); panel B, ethanol and fructose infusions (110 umoles and 30 umoles/min x kg^{-1}, respectively) initiated at the third hour (see arrow). Changes in plasma glucose concentration and rates of glucose turnover were tested for statistical significance ($p<0.05$) by establishing three non-orthogonal contrasts labeled as segments I (2-3 hours), II (3.5-4.5 hours) and III (5.0-7.0 hours). Each segment within an experiment was tested against another by using the Bonferroni t-test (Neter and Wasserman, 1974). Segments with similar superscript letters are not different ($p>0.05$). Standard errors for glucose concentration and glucose turnover in each panel were: (A) 2.6 mg/dl and 0.2 mg/min x kg^{-1}; and (B) 4.1 mg/dl and 0.4 mg/min x kg^{-1}.


PLASMA GLUCOSE (mg/dl)
80
60
40
20
0
GLUCOSE TURNOVER (mg/min/kg)
8
7
6
5
4
3
2
1
I A
II B
III B
I A
II A
III B
A
FRUCTOSE
2
3
4
5
6
7
TIME (HRS)

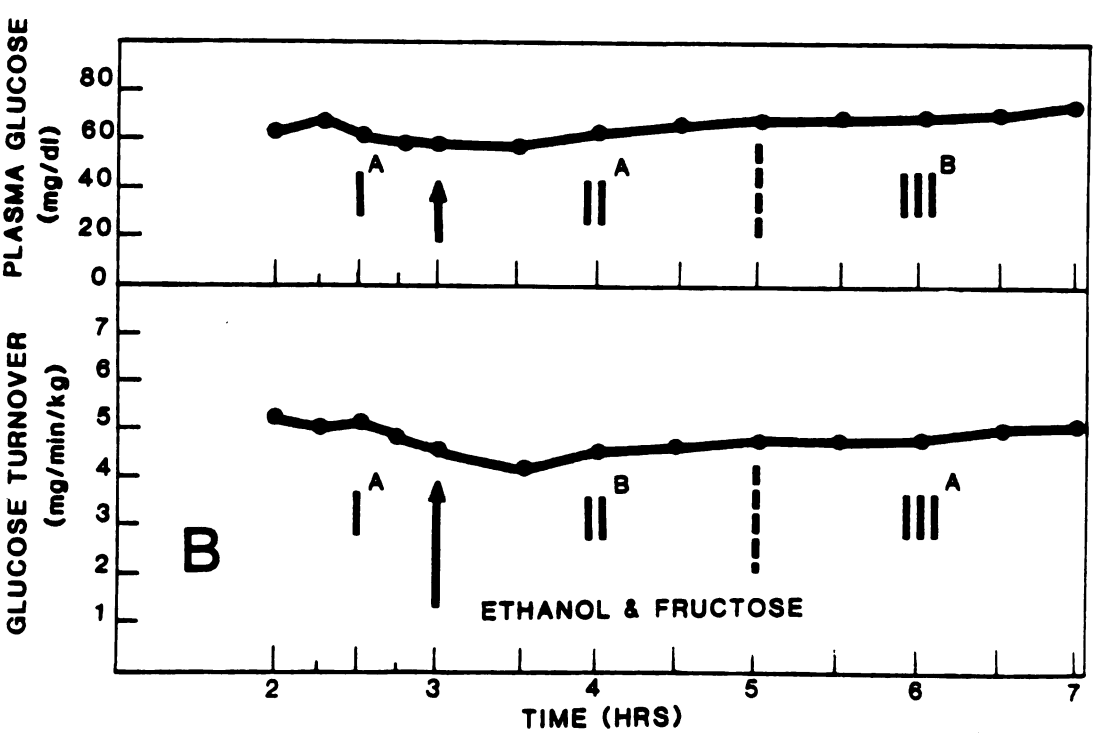

PLASMA GLUCOSE (mg/dl)
80
60
40
20
0
GLUCOSE TURNOVER (mg/min/kg)
7
6
5
4
3
2
1
I A
II A
III B
I A
II B
III A
B
ETHANOL & FRUCTOSE
2
3
4
5
6
7
TIME (HRS)

Fig. 4.

Figure 5 shows glucose clearance rates for each treatment as a percentage of clearance rates for the control experiments. The control clearance values were 8.8 ml/min for segments I and II and 10.4 ml/min in segment III. Similarly, the glucose clearance rate increased in segment III when the animals were treated with ethanol, fructose or BD. When ethanol and fructose were infused simultaneously, the clearance rate did not increase during segment III; thus, clearance as a percentage of control for the third segment of the ethanol plus fructose treatment does not represent an actual decline in metabolic clearance rate, but a failure of the clearance rate to increase as it did in the third segment of the other experiments. The decline in plasma glucose and increase in glucose clearance during the control experiments is difficult to explain; however, the decline in plasma glucose levels simply as a function of time of tracer infusion has appeared before in this group of animals (Armstrong, Romsos and Leveille, 1979).

Blood ethanol concentrations during the ethanol and ethanol plus fructose experiments rose to 9 mM during the 4-hour treatment infusion, but blood ethanol concentrations did not differ significantly between the two treatments at any time (Figure 6). The average rate of ethanol metabolism was approximated by subtracting the amount of ethanol estimated to be dispersed in total body water from the total amount of ethanol infused. Total body water has previously been reported to be 64% of total body weight for adult,

Fig. 5. Glucose clearance for each treatment expressed as a percentage of the control value. Six monkeys were used in each treatment, with two observations per monkey. Infusion periods were divided into three segments and each segment within a treatment was tested against another by the Bonferroni t-test (Neter and Wasserman, 1974). An asterisk indicates that the value is significantly lower ($P<0.05$) than the value in segment I for the corresponding treatment.

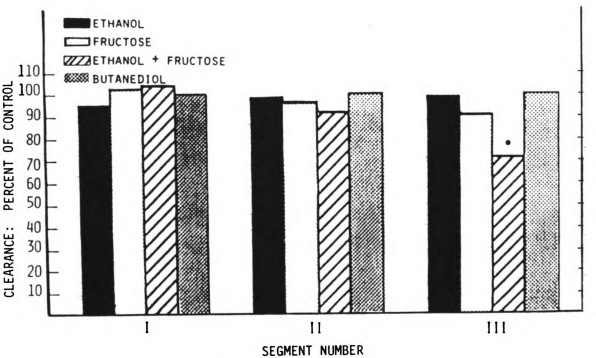

ETHANOL
FRUCTOSE
ETHANOL + FRUCTOSE
BUTANEDIOL
CLEARANCE: PERCENT OF CONTROL
110
100
90
80
70
60
50
40
30
20
10
I
II
III
SEGMENT NUMBER

Fig. 5

Fig. 6. Blood ethanol concentrations in monkeys infused with ethanol or ethanol plus fructose solutions starting at the end of the third hour of tracer infusion. Each point represents values from six monkeys, with two observations per monkey. The standard error was 0.6mM. An analysis of variance revealed no significant differences between the two treatments ($p>0.05$).

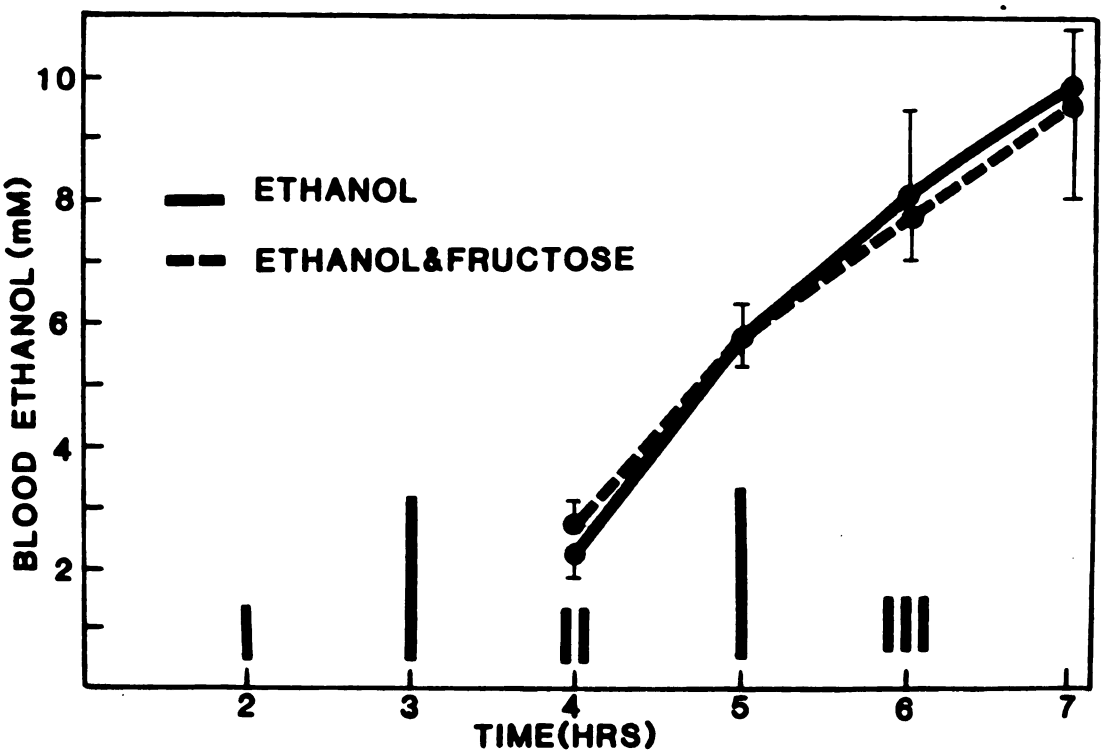

BLOOD ETHANOL (mM)
10
8
6
4
2
ETHANOL
ETHANOL&FRUCTOSE
I
II
III
2
3
4
5
6
7
TIME(HRS)

Fig. 6.

female Cynomolgus monkeys (Azar and Shaw, 1975). Thus:

$$\text{Rate}_{(ETOH)} = \frac{\text{Amount infused - (Concentration at 7 Hrs X 0.64 X Body Wt)}}{\text{240 Min X Body Wt}}$$

When ethanol was infused alone, the rate of ethanol metabolism was slightly, but not significantly ($p>0.05$), less than when ethanol plus fructose were infused (69 versus 74 umoles/min x kg^{-1}, respectively).

Blood lactate concentrations increased in monkeys infused with ethanol, fructose, or ethanol plus fructose, but there were no significant changes in blood pyruvate concentrations (Table 3). In the ethanol and the ethanol plus fructose experiments, the blood lactate/pyruvate ratio increased (Table 3).

Blood samples from animals infused with BD had greater concentrations of 3-hydroxybutyrate during segments II and III, but blood lactate concentrations did not change (Figure 7). Elevations in circulating levels of beta-hydroxybutyrate are consistent with conversion of BD to beta-hydroxybutyrate.

DISCUSSION

In agreement with observations in humans (Cummins, 1961; Tolis, 1965) ethanol infusions decreased the plasma glucose concentrations and glucose turnover rates in monkeys. The rates of peripheral glucose clearance were not significantly increased by the ethanol infusions when they

Table 3. Blood lactate, pyruvate, and lactate/pyruvate ratios in monkeys.

Treatment	Metabolite	Segment I	Segment II	Segment III	S.E.*
Ethanol	Lactate†	2.00‡a	2.62b	3.15b	0.51
	Pyruvate	0.16a	0.15a	0.14a	0.05
	Lactate/pyruvate	13a	22b	25b	4.85
Fructose	Lactate	3.15a	3.62a	4.34b	1.03
	Pyruvate	0.16a	0.18a	0.23a	0.08
	Lactate/pyruvate	20a	21a	24a	5.90
Ethanol + Fructose	Lactate	2.58a	3.89b	4.59b	0.63
	Pyruvate	0.18a	0.17a	0.15a	0.04
	Lactate/pyruvate	19a	32b	39b	0.49

*Standard error.

† Lactate and pyruvate concentrations in umoles/ml

‡ Values on the same line with similar superscript letters are not significantly different ($p>0.05$). Statistical tests and design are fully described in the text. Each value represents the mean of six animals.

Fig. 7. Blood beta-hydroxybutyrate (A) and lactate (B) concentrations in monkeys infused with 1,3-butanediol (110 umole/min x kg^{-1}) at the beginning of segment II. Each point represents values for six monkeys. Segments with similar superscript letters are not different ($P>0.05$). The standard error for beta-hydroxybutyrate values was 0.4 mM and that for lactate values was 0.3mM.

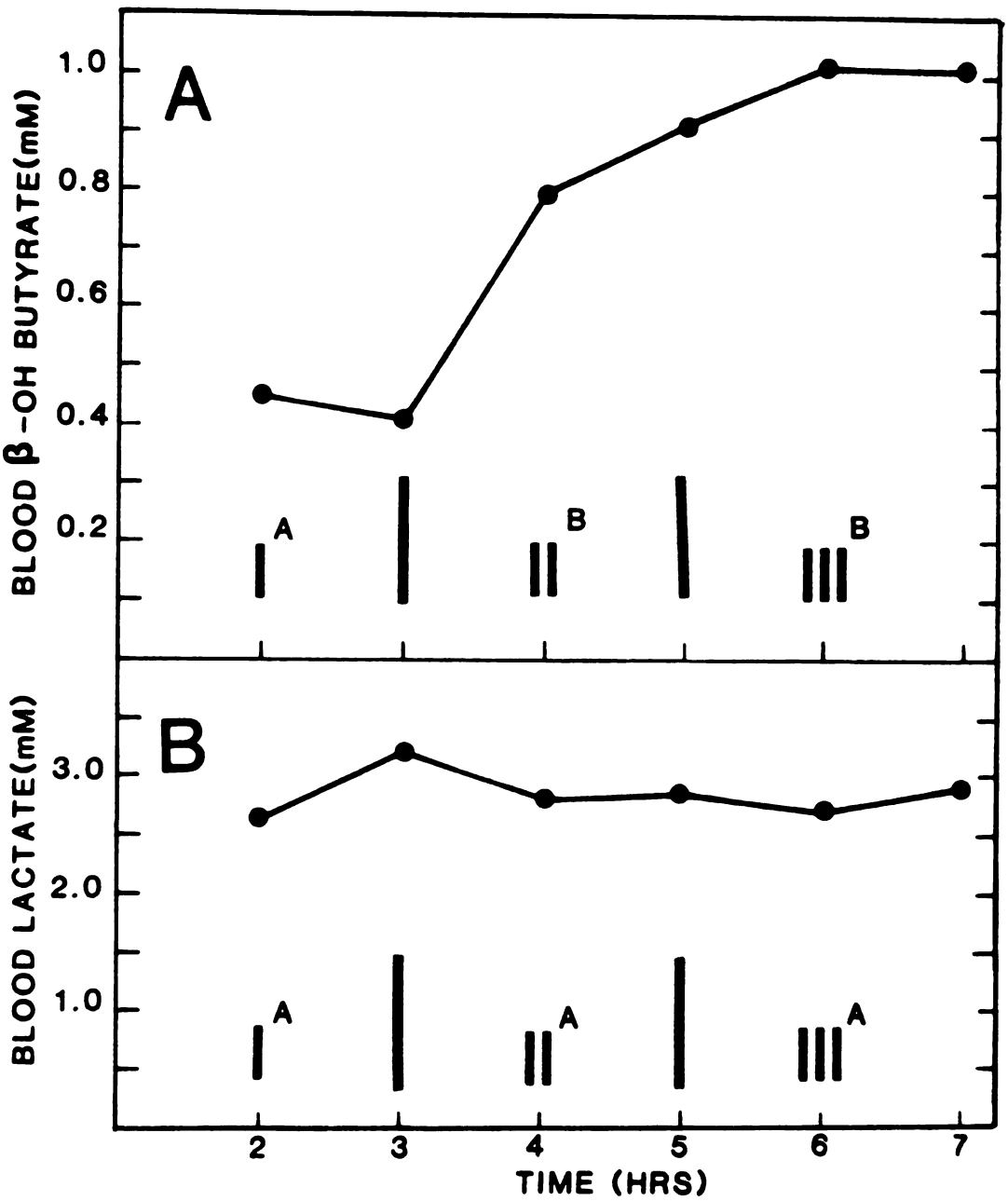

A
B
BLOOD β-OH BUTYRATE(mM)
1.0
0.8
0.6
0.4
0.2
BLOOD LACTATE(mM)
3.0
2.0
1.0
A
B
B
A
A
A
2
3
4
5
6
7
TIME (HRS)

Fig. 7.

were compared to the clearance rates observed during the corresponding segments of the control experiments.

While our results in monkeys that only glucose production was decreased by ethanol infusion, Lochner, Wulff and Madison (1967) found an immediate decrease in both glucose production (65%) and peripheral utilization (25%) when ethanol was infused into fasted dogs anesthetized with sodium thiopentol. It is possible that the decreased peripheral clearance in their experiments was due to anesthesia rather than the ethanol infusions because pentobarbital has been shown to decrease glucose uptake by the brain of anesthetized rats as much as 40% (Gjedde and Rasmussen, 1980). Other investigators (Dittmar and Hetenyi, 1978) examining the effects of ethanol infusions in unanesthetized, four-day fasted dogs, found that glucose production significantly decreased without a significant change in the metabolic clearance rate when ethanol was infused at a rate of 40 umoles/min x kg^{-1}. While their results are initially identical to those being reported here for monkeys, Dittmar and Hetenyi (1978) found that after 40 minutes of ethanol infusion the glucose production rate and plasma glucose concentration increased. The single dose priming injection they used prior to the start of the continuous infusion may have caused the initial decrease in glucose production; the relatively low rate of ethanol infusion thereafter (40 umoles/min x kg^{-1}) was probably insufficient to maintain the initial reduction in glucose

production. Others (Stifel et al., 1976) have shown that one umole of ethanol injected into rats as a single, intravenous dose, decreased activities of hepatic fructose-1,6-diphosphatase and aldolase within 10 to 15 minutes.

Since Zahlten et al. (1981) have recently observed that the intracellular lactate/pyruvate ratio in isolated, guinea pig hepatocytes was similar to the ratio observed in guinea pig plasma, the significant increase in the plasma lactate/pyruvate ratio seen in segments II and III when monkeys were infused with ethanol probably reflects an increased $NADH/NAD^+$ ratio in the hepatic cytosol. This change in $NADH/NAD^+$ would be expected to decrease glucose production by trapping major gluconeogenic precursors such as pyruvate and alanine, as lactate. In guinea pigs, ethanol increases the $NADH/NAD^+$ ratio in perfused livers (Jomain-Baum, et al., 1978) as well as isolated hepatocytes (Zahlten et al., 1981) and acutely decreases glucose production from lactate (Jomain-Baum et al., 1978). Considering that the intracellular compartmentation of glucose production in monkey liver (Hammond and Balinsky, 1978) is similar to that in guinea pigs, the ethanol-induced decrease in glucose production in our experiments with monkeys is consistent with observation in guinea pigs.

When BD was infused into the monkeys, the plasma glucose concentration and glucose turnover rates declined at about the same rate that they did when ethanol was infused.

Furthermore, the increased beta-hydroxybutyrate concentrations in the blood suggest that the oxidation of BD increases the hepatic $NADH/NAD^+$ ratio and inhibits glucose production similarly to ethanol. The BD infusions in monkeys did not decrease the rate of peripheral glucose clearance even though blood beta-hydroxybutyrate concentration increased. This agrees with results reported by Blasse et al., (1967) for dogs infused with sodium beta-hydroxybutyrate. They noted that beta-hydroxybutyrate infusions decreased the rate of hepatic gluconeogenesis, but did not affect the peripheral rate of glucose utilization.

When fructose was infused into the monkeys, the rate of glucose production increased while the clearance rate of glucose was unchanged. The increase in blood lactate during segment III is consistent with results reported for the effects of fructose in the perfused rat liver: a rapid increase in oxygen consumption and glucose production as well as a delayed increase in the production of lactate and pyruvate (Scholz et al., 1975). The same authors have shown that nearly 50% of the fructose added to the perfusion medium may be recovered as glucose while 46% of the fructose underwent fructolysis and was recovered as lactate and pyruvate. If one assumes for our experiments with monkeys, that the increase in glucose production during the infusion of fructose is due solely to the conversion of fructose to glucose, then the increase in glucose production following the infusion of fructose should approximate the percentage

of the fructose infused that was converted to glucose thus:

$$\% \text{ Fructose to Glucose} = \frac{(\text{Glucose Production})_{\text{fructose}} - (\text{Glucose Production})_{\text{control}}}{\text{Fructose Infusion Rate}}$$

When this calculation was performed with our data, we found that 26% of the infused fructose was converted to glucose. The unchanged metabolic clearance rate of glucose during fructose infusions is consistent with observations (Corvilain and Tagnon, 1961) showing that fructose causes little, if any, secretion of insulin.

When ethanol and fructose were simultaneously infused into monkeys, hypoglycemia did not occur even though the glucose production rate slightly decreased during segment II. The ability to remain euglycemic while glucose production rates fall can be explained by a simultaneous decrease in the clearance rate of glucose. The acetate produced from ethanol oxidation may have exerted a significant glucose sparing effect in skeletal muscle. When Jorfeldt and Juhlin-Dannfelt (1978) infused humans with ethanol they found that glucose uptake by the leg muscle was reduced from 120 umoles/min to 60 umoles/min. Simultaneously, acetate uptake by the leg was increased from 0 to 250 umoles/min and the arterial glucose concentration was 84 mg/dl prior to, and after, the ethanol infusion.

PART 4

ETHANOL ADMINISTRATION FAILS TO PRODUCE HYPOGLYCEMIA IN FASTED CHICKENS (*GALLUS DOMESTICUS*)

INTRODUCTION

Unprimed ethanol infusions into fasted animals have previously been shown to cause profound hypoglycemia by decreasing glucose production in several species; including, fasted dogs (Lochner et al., 1967), monkeys (Armstrong & Romsos, 1984), and humans (Searle et al., 1974). This effect of ethanol is likely linked to the increased reduction of pyridine nucleotides that results from hepatic metabolism of ethanol (Lochner et al., 1967; Krebs et al., 1969). Furthermore, inhibition of gluconeogenesis by ethanol in perfused livers of fasted rats, is more pronounced when a reduced substrate such as lactate is infused than when an oxidized substrate such as pyruvate is used (Krebs et al., 1969; Kaden et al., 1969).

Unlike in other species where ethanol inhibits glucose production, ethanol fails to inhibit glucose production from lactate in perfused livers or isolated hepatocytes of fasted chickens and actually increases glucose production from pyruvate (Brady et al., 1979; Dickson & Langslow, 1977; Sugano et al., 1982). Effects of ethanol on plasma glucose concentrations and in vivo glucose production in chickens have not been previously reported.

The present study was therefore conducted to evaluate the influence of ethanol on plasma glucose concentration and turnover in chickens. Since hepatic metabolism of ethanol may alter the cellular $NADH/NAD^+$ ratio, which in turn can influence the flux of gluconeogenic intermediates (Jomain-Baum et al., 1978; Krebs et al., 1969; Zahlten et al., 1982), we also determined the ability of chickens infused with ethanol to regulate their hepatic redox state.

MATERIALS AND METHODS

One-day-old, male, broiler chicks obtained from a commercial hatchery (Townline Hatchery, Zeeland, MI) were fed a high-carbohydrate stock diet (Master Mix Chick Starter; Central Soya, Fort Wayne, IN). Food and water were available ad libitum and room lights were on continuously. Experiments started when chickens weighed approximately 1500g (weight ranged from 1380 to 1620).

Experiment 1. This experiment was conducted to measure plasma glucose concentration and turnover in chickens infused with ethanol. Four chickens were fasted for 64 hours prior to the insertion of a 20-gauge, around-needle catheter (Becton, Dickinson & Co., Rutherford, NJ) into the external jugular vein, and another into the anterior jugular vein. A priming dose of 0.6 uCi (6-^{3}H)-glucose/kg body weight was injected via one catheter; immediately thereafter

(6-^{3}H)-glucose in saline was continuously infused (Harvard Syringe Pump, Harvard Apparatus Co., Inc., South Natick, MA) for six hours at a rate of 0.06 uCi (6-^{3}H)-glucose/min x kg^{-1} body weight. A steady state specific activity of glucose (^{3}H dpm/mg glucose) appeared in the plasma within two hours after starting tracer infusions.

During the last three hours of the six-hour infusion, chickens were infused with ethanol. Two chickens received unprimed ethanol infusions at a rate of 222 umoles ethanol/min x kg^{-1} body weight and the other two at a rate of 444 umoles ethanol/min x kg^{-1} body weight. This experiment was repeated with four other chickens; thus a total of four chickens were infused with each dose of ethanol.

One ml blood samples were withdrawn from the second catheter every 15 minutes from the second through the third hour and every half-hour thereafter. Blood samples were collected in chilled tubes containing heparin and sodium fluoride. Samples were centrifuged within 30 minutes of collection and the plasma was removed and frozen pending further analyses.

Additional one ml blood samples were taken every hour from the second through the sixth hour for blood lactate and ethanol determinations. Immediatedly after collecting a sample for these assays, it was mixed with four ml of cold perchloric acid (3% w/v) and kept on ice. Perchloric acid-treated blood samples were centrifuged at the conclusion

of the experiment and supernatant fluids were neutralized and refrigerated. Analyses for lactate (Hohorst, 1965) and ethanol (Mattenheimer, 1970) were done within 24 hours.

To determine the specific activity of plasma glucose (dpm/mg glucose), plasma samples were thawed and deproteinized with barium hydroxide and zinc sulphate (Somogyi, 1945). Aliquots of supernatant fluid were used to determine the concentration of plasma glucose (Glucostat, Worthington Biochemical, Freehold, NJ). Other aliquots of supernatant fluid were passed through 1 x 5 cm columns containing equal parts by weight of Dowex 1-X8, Cl^- and Dowex 50W-X8, H^+ (Bio-Rad Laboratories, Rockville Centre, NY) to separate radioactive anions and cations from radioactive glucose. Glucose was eluted from the columns by rinsing them with three, five ml portions of deionized water. The glucose eluates were collected in scintillation vials, evaporated to dryness in a 37°C water bath under an airstream, and redissolved in one ml of water prior to the addition of scintillation cocktail (3a70, Research Products International Corp., Elk Grove Village, IL). Radioactivity was quantified with a liquid scintillation spectrophotometer.

Rates of glucose turnover and clearance were calculated as shown below and on the following page (Shipley & Clark, 1972):

$$\text{Turnover Rate} = \frac{\text{Tracer infusion Rate (uCi/min)}}{\text{Plasma Specific Activity (uCi/mg Glucose)}}$$

$$\text{Clearance Rate} = \frac{\text{Tracer infusion Rate (uCi/min)}}{\text{Plasma Tracer Concentration (uCi/ml)}}$$

Values calculated from the preceding equations were tested for statistical significance by Dunnett's t-test (Winer, 1962).

Assuming that the volume of distribution for ethanol was 65% of body weight, the blood ethanol concentration after 180 minutes of ethanol infusion was used to approximate the rate of ethanol oxidation as follows:

$$\text{Rate} = \frac{\text{Amount infused - (Concentration at 6 Hrs X 0.65 X Body Wt)}}{\text{180 Min X Body Wt}}$$

Experiment 2. The purpose of this experiment was to obtain information on the hepatic redox state in chickens infused with ethanol. Sixteen chickens were fasted 64 hours prior to insertion of a 20-gauge, around-needle catheter into a jugular vein. Eight chickens were then infused with saline and the other eight were infused with 444 umoles ethanol/min x kg^{-1} for three hours.

Five minutes prior to discontinuing the infusion, a one ml blood sample was withdrawn from a wing vein for lactate and ethanol determinations as described for Experiment 1. At the end of each infusion period, chickens were killed by cervical dislocation. The liver was rapidly exposed and a portion of it seized with a pair of large-faced tongs precooled in liquid nitrogen. Samples (still held by tongs) were immediately plunged into liquid nitrogen. Less than

ten seconds elapsed from the time the infusions were discontinued until the samples were immersed.

After liver samples (1 to 2 g) were collected, each one was broken into small pieces, rapidly weighed and homogenized in eight ml perchloric acid (6% w/v) before thawing could occur. Homogenates were centrifuged and aliquots of supernatant fluid were neutralized with 2N KOH prior to the determination of metabolite concentrations.

Hepatic concentrations of lactate, pyruvate, glutamate, alpha-ketoglutarate and ammonium were determined using methods reported by Hohorst (1965), Czok and Lamprecht (1974), Bernt and Bergmeyer (1974), Bergmeyer and Bernt (1974) and Kun and Kearney (1974), respectively.

Hepatic alcohol dehydrogenase activity was measured in six additional chickens fasted 24 or 64 hours according to the method of Crow et al. (1977).

RESULTS

Ethanol infused into chickens at a rate of 222 umoles/min x kg^{-1} did not significantly affect plasma glucose concentration or the rate of glucose turnover (Figure 8). When ethanol was infused at a rate of 444 umoles/min x kg^{-1} for three hours, the plasma glucose did not change, but the rate of glucose turnover decreased by 15% (Figure 8). This necessarily means that the glucose

Fig. 8. Plasma glucose concentration and ($6-^{3}H$) glucose turnover rate in chickens infused with tracer for three hours alone and then simultaneously with ethanol (222 or 444 umoles/min x kg^{-1}) for an additional three hours. Each point represents the mean for four chickens. Standard errors for plasma glucose and turnover values in chickens infused with 222 umoles ethanol/min x kg^{-1} were 12.0 mg/dl and 0.6 mg/min x kg^{-1}, respectively. In chickens infused with 444 umoles ethanol/min x kg^{-1}, standard errors for plasma glucose and turnover values were 10.6 mg/dl and 0.4 mg/min x kg^{-1}, respectively. Asterisks indicate that a value was significantly ($p<0.05$) different from the respective second hour value, as determined by Dunnett's t-test (Winer, 1962).

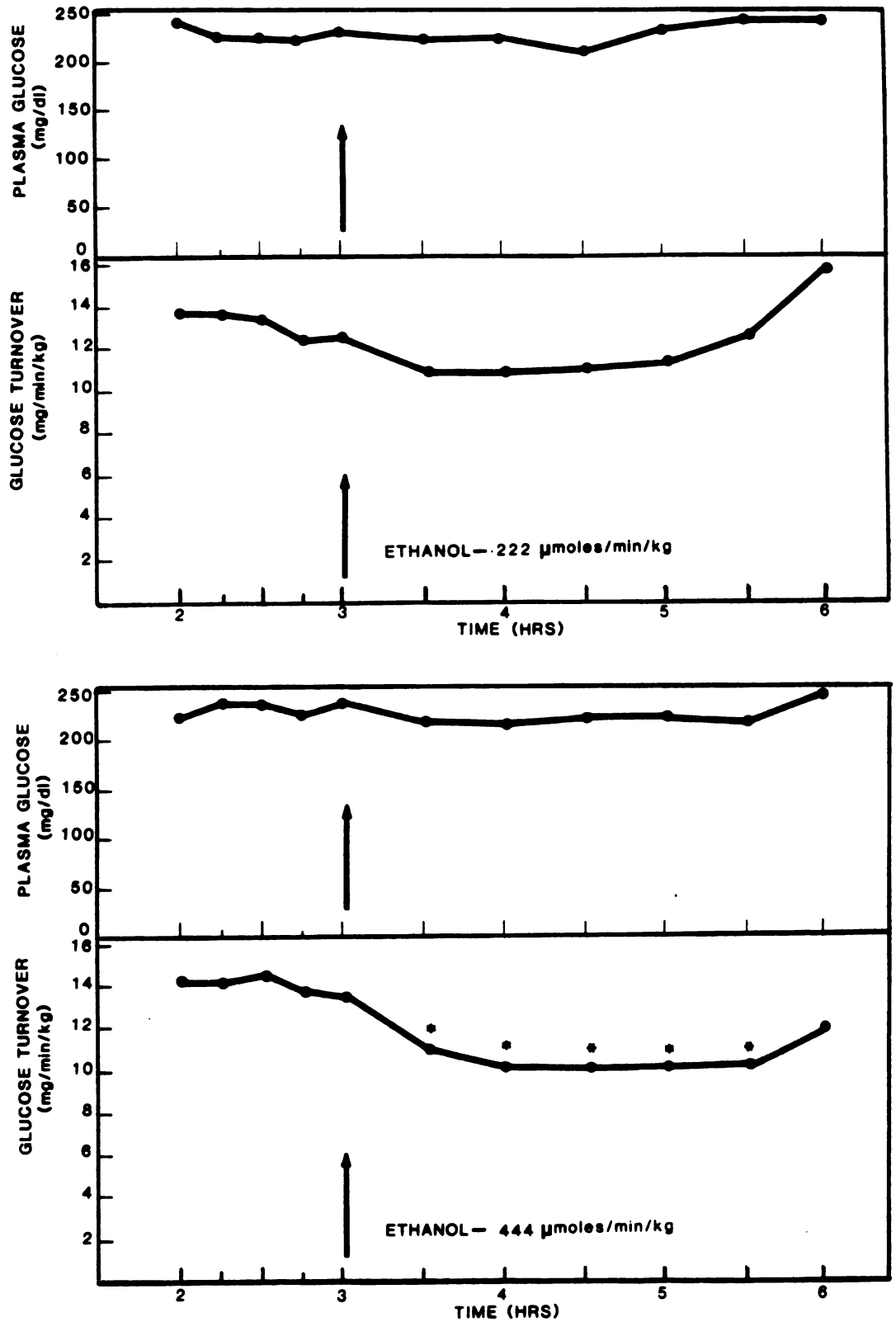

PLASMA GLUCOSE (mg/dl)
250
200
150
100
50
0
GLUCOSE TURNOVER (mg/min/kg)
16
14
12
10
8
6
4
2
ETHANOL— 222 μmoles/min/kg
2
3
4
5
6
TIME (HRS)
PLASMA GLUCOSE (mg/dl)
250
200
150
100
50
0
GLUCOSE TURNOVER (mg/min/kg)
16
14
12
10
8
6
4
2
ETHANOL— 444 μmoles/min/kg
2
3
4
5
6
TIME (HRS)

Fig. 8.

production rate and glucose clearance rate decreased in concert.

Blood lactate concentrations were not altered by ethanol infusions (Table 4). After 180 minutes of ethanol infusion, the concentration of ethanol in blood of chickens infused at the low dose increased from nondetectable levels to 21 mM; the increase in chickens infused with the high dose of ethanol was nearly threefold greater. Our approximation of the rate of ethanol oxidation in chickens infused with ethanol was 147 to 220 umoles/min x kg^{-1}, or about 50 to 65% of the ethanol infusion rate (Table 4). These values are consistent with in vitro measurements of hepatic alcohol dehydrogenase activity in fasted chickens. Hepatic alcohol dehydrogenase activity averaged 2.8 units of activity (umoles of NAD^+ converted to NADH/min x g^{-1} wet weight of liver) at 41°C in fasted chickens; or, approximately 170 units of actvity per liver.

To obtain information on the hepatic redox state of chickens we determined concentrations of several metabolites in freeze-clamped livers. The ratio of lactate to pyruvate was used as an indicator of the cytosolic $NADH/NAD^+$ ratio; whereas the ratio of glutamate to alpha-ketoglutarate and ammonium was used as a reflection of the mitochondrial $NADH/NAD^+$ ratio (Greenbaum et al., 1971). Hepatic lactate and pyruvate concentrations were slightly increased in chickens infused with ethanol for three hours, but lactate to pyruvate ratios were not changed (Table 5). Chickens

Table 4. Blood lactate and ethanol concentrations, and ethanol oxidation in chickens infused with ethanol.*

	Saline	Ethanol dose (umoles/min x kg^{-1})	
		222	444
	(n=8)	(n=4)	(n=8)
Lactate (mM)	2.28 ± 0.37[a]	2.37 ± 0.38[a]	2.63 ± 0.19[a]
Ethanol (mM)	-----	21 ± 1[a]	59 ± 3[b]
Ethanol oxidation (umoles/min x kg^{-1})	-----	147 ± 4[a]	220 ± 11[b]

*Values were obtained after 3 hours of infusion. Each value represents the mean ± SEM. Numbers in the same row with different superscript letters are significantly different ($p<0.05$).

See Materials and Methods for method of calculation.

Table 5. Concentration of metabolites in livers of chickens infused with saline or ethanol (444 umoles/min x kg^{-1}) for three hours.*

	Saline	Ethanol
	umoles/g liver	
Lactate	1110 ± 73[a]	1334 ± 55[b]
Pyruvate	102 ± 10[a]	128 ± 8[b]
Lactate/Pyruvate	11.5 ± 1.2[a]	10.6 ± 0.6[a]
Glutamate	2662 ± 123[a]	3145 ± 125[b]
Alpha-ketoglutarate	416 ± 40[a]	373 ± 46[a]
Ammonium	806 ± 67[a]	952 ± 97[a]
Glutamate/(Alpha-ketoglutarate)(Ammonium)	9.3 ± 1.2[a]	12.0 ± 2.0[a]

*Each value represents the mean ± SEM for 8 chickens. Different superscript letters on the same line indicate that the values were significantly different ($p<0.05$) as determined by Student's t-test.

infused with ethanol had 18% more hepatic glutamate and 10% less alpha-ketoglutarate concentrations than saline infused chickens, but this difference was not significant. As a result, the liver glutamate/(alpha-ketoglutarate)(ammonium) ratio was 29% higher in the ethanol infused chickens than observed in the control chickens, although the difference was not statistically significant ($p>0.05$).

DISCUSSION

Ethanol infused at the rate of 110 umoles/min x kg^{-1} into fasted monkeys markedly decreased plasma glucose concentrations and inhibited glucose production by 50% (Armstrong & Romsos, 1984). When chickens were infused with ethanol at the same rate during a pilot experiment, no effect on plasma glucose concentrations or glucose turnover were seen (results not reported). Likewise, ethanol infused at a rate of 222 umoles/min x kg^{-1} did not significantly affect plasma glucose concentration or rates of glucose turnover in chickens. When ethanol was infused at 444 umoles/min x kg^{-1}, the rate of glucose turnover decreased 15% ($p<0.05$) in chickens, but plasma glucose concentration did not change; therefore, glucose clearance rate and production rate had to have decreased simultaneously. This resistance to ethanol-induced hypoglycemia was apparently not related to an inability to metabolize ethanol since

our previous estimate of ethanol clearance in monkeys was 69 umoles/min x kg^{-1} (Armstrong & Romsos, 1984) and in keeping with the ethanol clearance rate of 147 umoles/min x kg^{-1} seen in chickens infused with ethanol at twice the rate. In addition, the hepatic alcohol dehydrogenase activity in chicken liver was similar to that reported by Crow et al. (1977) for the rat. Our ethanol infusion studies generally agree with results of in vitro experiments with chickens showing that ethanol fails to decrease hepatic glucose production (Brady et al., 1979; Sugano et al., 1982).

The measurement of metabolite ratios in experiment 2 revealed that chickens were able to maintain a nearly normal cytosolic $NADH/NAD^+$ ratio despite the infusion of large amounts of ethanol. The relatively stable lactate/pyruvate ratios seen in livers from ethanol treated chickens differs from the nearly two-fold increase seen in isolated guinea pig hepatocytes incubated with ethanol (Zahlten et al., 1982). Similiarly, ethanol more than doubles the lactate/pyruvate ratio in perfused rat liver (Williamson et al., 1969; Soboll et al., 1981).

Chickens, unlike guinea pigs and rats, were also able to maintain a nearly normal mitochondrial $NADH/NAD^+$ ratio despite the infusion of large amounts of ethanol. In chickens, ethanol slightly, but not significantly, increased the hepatic glutamate/(alpha-ketoglutarate)(ammonium) ratio while ethanol doubles the beta-hydroxybutyrate/acetoacetate ratios in isolated guinea pig hepatocytes (Zahlten et al.,

1982) and perfused rat liver (Williamson et al., 1969; Soboll et al., 1981).

It is unclear why chickens are so much more resistent to the hypoglycemic effects of ethanol than are other species, but their ability to maintain a relatively unchanged hepatic cellular redox potential may facilitate maintenance of gluconeogenesis in chickens.

PART 5

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

One of the continuing themes in this thesis has been the possible relevance of the monkey as a model for studies in human glucose production. Since the glucose turnover values reported for the monkeys in the first study are 3 to 4 times higher than glucose turnover rates cited for adult humans, the relevance of monkeys for glucose studies may seem uncertain. But it isn't. The apparent differences in glucose turnover rates result largely from expressing the data on the basis of body weight. Bier et al. (1977) have shown that the relationship between glucose production, plotted as a function of brain weight, is nearly linear. Considering our 3 kg monkeys to be approximately equivalent to a 3.5 kg infant with a 420 g brain, one would predict the rate of glucose turnover to be approximately 5.0 mg/min x kg^{-1}. This is very close to the values that are reported in this thesis for both the single dose and primed-continuous techniques. Thus monkeys may be good models for studies pertaining to human neonatal hypoglycemia.

The first study in this thesis used an unconventional approach to compare the stochastic and compartmental analyses for calculating the glucose turnover data. We assumed that the turnover rate obtained from the stochastic

analysis was the best estimate of the rate of glucose turnover because it isn't dependent on establishing the instantaneous specific activity of the glucose pool at zero time. The results obtained from the stochastic analysis were then substituted into a deterministic analysis and the radioactive glucose curves were "peeled" as many times as necessary to yield the same turonver rate as that obtained with the stochastic analysis. We found the (6-^{3}H) glucose curves could be represented equally well by 2 or 3 compartment models. According to convention, the (6-^{3}H) tracer studies are best represented as two pool models. The (2-^{3}H) glucose curves absolutely required 3 pools to yield the same turnover rate as that obtained by stochastic analysis. When one realizes that the (2-^{3}H) glucose label had a higher turnover rate and longer transit time than the (6-^{3}H) glucose label, a logical inconsistency becomes apparent. How can (2-^{3}H) glucose be leaving the system at a faster rate than (6-^{3}H) glucose while simultaneously remaining in the system for a longer length of time?

The rhetorical question is answered by recognizing that phosphoglucoisomerase discriminates against glucose that is tritium labelled at the number 2 carbon. An isotope discrimination ratio was calculated from the equations and when (2-^{3}H) glucose calculations were adjusted by the discrimination ratio, the logical inconsistency dissolved.

This method for discovering and resolving problems caused by isotope discrimination may be of future use in any turnover study performed with tracers.

The second study further demonstrates the metabolic similarity between monkeys and humans. Monkeys, like humans, responded to intravenous ethanol infusions with: 1) an acute hypoglycemia; 2) decreased rates of glucose production and unchanged metabolic clearance rates for glucose; 3) a significantly increased concentration of blood lactate; and 4) a significantly increased blood lactate/pyruvate ratio. These results suggested that the hepatic NADH/NAD+ ratio increased during ethanol infusions and gluconeogenesis from lactate was impaired as a consequence.

Intravenous fructose infusions significantly increased: 1) plasma glucose concentration; 2) the rate of glucose production; and 3) blood lactate concentration. The metabolic clearance rates of glucose and the blood lactate/pyruvate ratios were unchanged. This series of experiments suggested that fructose was converted to glucose as well as lactate and it was estimated that at least 26% of the fructose infused was converted to glucose.

When solutions containing ethanol and fructose were infused, plasma glucose concentration increased, the rate of glucose production initially decreased and then returned to the rate observed during the control period, and the blood lactate as well as the lactate/pyruvate ratio were significantly increased. Furthermore, the metabolic

clearance rates for glucose were significantly less than the values calculated for the series of control experiments.

The significant increase in the blood lactate/pyruvate ratio suggested that glucose production from lactate was decreased due to an increased hepatic $NADH/NAD^+$ ratio. However, glucose production from fructose was only slightly impaired and we estimated that at least 50% of the fructose infused was converted to glucose.

Based on results reported by others (Jorfeldt and Juhlin-Dannfelt, 1978), it seems that the decrease in the metabolic clearance rate of glucose might be accounted for by the glucose-sparing effect of acetate, the product of ethanol metabolism.

The results obtained from experiments where 1,3-butanediol solutions were infused suggested that butanediol solutions affected glucose homeostasis to the same extent as ethanol infusions: 1) a decrease in plasma glucose concentrations; and 2) a decrease in the rates of glucose production with no change in the metabolic clearance rate of glucose. Thus, relative to glucose metabolism, butanediol seems to have little advantage over ethanol as a parenteral energy source. However, our experiments revealed that monkeys infused with butanediol had significantly increased blood beta-hydroxybutyrate concentrations while blood lactic acid concentrations were no different than those seen during the control periods. This contrasts with the two-fold increase in blood lactic acid concentrations

seen when monkeys were infused with ethanol. Perhaps butanediol infusions may be advantageous in patients whose therapy is complicated by lactic acidemia.

In addition to lactic acidemia, ethanol oxidation is known to promote the development of fatty livers (Lieber et al., 1975; Abrams and Cooper, 1975). It is not currently known whether butanediol oxidation produces similar effects. Should future research demonstrate that butanediol oxidation does not promote the development of fatty livers, then butanediol would be preferable to ethanol as a parenteral energy source in this respect as well.

Since monkeys are able to convert oxalacetate to phosphoenolpyruvate in both the cytosol and the mitochondria, we could not ascertain whether ethanol primarily affected glucose production in the cytosol, the mitochondria, or both. In order to somewhat simplify the ethanol paradigm, chickens were chosen for further study because they represent a species where oxalacetate is converted to phosphoenolpyruvate exclusively in the mitochondria.

Results from the chicken experiments revealed that ethanol decreased glucose production only when it was infused at very high doses. Furthermore, the decreased glucose production was not accompanied by hypoglycemia because the metabolic clearance rate of glucose fell in concert with the glucose production.

The measurement of metabolite ratios in freeze-clamped chicken livers showed that chickens were able to maintain nearly normal cytosolic and mitochondrial redox ratios. This ability to maintain a relatively unchanged hepatic, cellualar redox potential may facilitate the maintenance of gluconeogenesis in chickens.

It was also shown that the chickens' extreme resistance to alcohol hypoglycemia was probably not related to an inability to metabolize ethanol since hepatic alcohol dehydrogenase activity was similar to that reported for rats. Furthermore, our calculated estimates of ethanol clearance suggested that chickens cleared ethanol at twice the rate of monkeys when the chickens were infused with twice the ethanolic dose.

Ethanol infusions in monkeys caused a 50% decrease in the rate of glucose production and plasma glucose concentration. Since PEPCK distribution in monkey hepatocytes is 50% cytosolic and 50% mitochondrial, we wondered whether ethanol might be exerting a primary hypoglycemic effect by interfering with phosphoenolpyruvate production in either the cytosol, or the mitochondria. In order to simplify the ethanol paradigm, we conducted ethanol studies on chickens because PEPCK is exclusively mitochondrial in this species. Our findings revealed that chickens were extremely resistant to ethanol-induced hypoglycemia, and even large doses of ethanol decreased glucose production by only 15%. Thus, one is tempted to

conclude that the resistance to ethanol-induced hypoglycemia is a consequence of mitochondrial PEPCK. Extending this conclusion to the monkey experiments, one might further conclude that the cytosolic production of phosphoenolpyruvate was sensitive to relatively low doses of ethanol, but the mitochondrial production of phosphoenolpyruvate continued unimpaired. Whether or not this conclusion is specious conjecture, depends on the validity of a number of assumptions--two of them being that: 1) ethanol exerts the hypoglycemic effect at the level of phosphoenolpyruvate formation, and 2) relative to ethanol hypoglycemia, the only important difference between chickens and monkeys lies in their distribution of PEPCK.

If it is true that ethanol infusions decrease glucose production by primarily inhibiting the cytosolic formation of phosphoenolpyruvate, then one would reasonably expect ethanol to produce a profound hypoglycemia in rats since their PEPCK activity is entirely cytosolic. But this is not the case: in fact, glucose production in rats is barely affected by ethanol (Jomain-Baum et al., 1978; Krebs, 1968). However, this may occur because the rat, unlike chickens, guinea pigs, humans, and presumably monkeys, has very high levels of beta-hydroxybutyrate dehydrogenase activity that enable it to use acetoacetate as a metabolic sink for the reducing equivalents produced during ethanol oxidation.

The question of whether or not ethanol specifically affects the cytosolic production of phosphoenolpyruvate in

species with low beta-hydroxybutyrate dehydrogenase activity could be answered by perfusing guinea pig livers with solutions containing either quinolinate--a known inhibitor of cytosolic PEPCK (Soling and Kleineke, 1976)--or quinolinate plus ethanol. If ethanol fails to decrease glucose production in the presence of quinolinate, then one might conclude with greater certainty that ethanol hypoglycemia initially results from a decrease in the cytosolic production of phosphoenolpyruvate.

Ethanol has been widely used to increase the caloric density of parenteral solutions and promote positive nitrogen balance in patients fed intravenously. However, ethanol is also known to precipitate metabolic complications resulting in lactic acidemia, hyperuricemia and fatty liver whether it is administered alone, or in conjunction with fructose or glucose. Relative to parenteral nutrition, our experiments tangentially suggested that 1,3-butanediol may prove to be a desirable alternative to ethanol.

PART 6

APPENDIX

APPENDIX

THE DERIVATION OF EQUATIONS USED TO CALCULATE TURNOVER RATES FOR DETERMINISTIC AND STOCHASTIC MODELS

Early methods for calculating turnover studies were established by Zilversmit et al. (1943a,b) when they suggested that the distribution space of a metabolite could be represented by a single, well-mixed compartment. If this assumption is valid, the ratio of the rate of disappearance of tracer to that of tracee should equal the ratio of the mass of tracer present to that of tracee. Thus,

$$\frac{dM^*}{dt} = - R_T \frac{M^*}{M} \qquad 1$$

where M^* = the mass of tracer in the system at any time, t,

M = the mass of tracee and is assumed to be constant,

R_T = the turnover rate of tracee at steady state.

Integrating equation 1 as follows, produces equation 2.

$$\int_0^a \frac{dM^*}{M^*} = - \frac{R_T}{M} \int_0^t dt$$

$$\ln M_a^* - \ln M_o^* = - \frac{R_T}{M} \cdot t$$

$$\ln M_a^* = - \frac{R_T}{M} \cdot t + \ln M_o^*$$

$$\ln M_a^* - \ln M = -\frac{R_T}{M} \cdot t + \ln M_o^* - \ln M$$

$$\ln \frac{M_a^*}{M} = -\frac{R_T}{M} \cdot t + \ln \frac{M_o^*}{M} \qquad 2$$

When the natural logarithm of M^*/M, the specific activity of the substance in the compartment of which blood plasma forms a part, is plotted against time, the slope of the resulting straight line has the value, $-R_T/M$. If M can be calculated, then the solution of the equation for R_T is straightforward. Since the real physiological system only approximates the one compartment model, the logarithms of specific activities plotted against time only become linear some time after injection of tracer. The authors extrapolated the terminal, linear portion of the curve back to t = 0 to obtain the specific activity at zero time had there been instantaneous and complete mixing. Knowledge of the value of specific activity at zero time (SA_0) is essential for calculating the concentration of tracer in the idealized system.

$$C_0^* = SA_0 \cdot C_0 \qquad 3$$

where C_0^* = concentration of tracer at t = 0,

C_0 = concentration of tracee at t = 0,

SA_0 = the extrapolated specific activity at t = 0.

Once the C_0^* has been calculated, the dilution principle may be used to calculate V, the volume of distribution.

$$V = \frac{M_0^*}{C_0^*} \qquad 4$$

where M_o^* = the dose of tracer injected

C_o^* = the concentration of tracer at t = 0.

Now that the volume of distribution is known, one can calculate M, the mass of tracee in the pool, as follows;

$$V = \frac{M}{C}$$

$$M = V \cdot C \qquad 5$$

Finally, once the mass, M, is obtained, it is possible to calculate R_T, the turnover rate, from the equation for the slope of the semi-logarithmic plot.

$$\text{Slope} = -\frac{R_T}{M}$$

$$R_T = -\text{Slope} \cdot M \qquad 6$$

An alternative way of calculating R_T is both interesting and useful. If one starts with equation 2,

$$\ln \frac{M^*}{M} = -\frac{R_T}{M} \cdot t + \ln \frac{M_o^*}{M}$$

and expresses it exponentially,

$$e^{\ln \frac{M^*}{M}} = e^{-\frac{R_T}{M} \cdot t} \cdot e^{\ln \frac{M_o^*}{M}}$$

$$\frac{M^*}{M} = e^{-\frac{R_T}{M} \cdot t} \cdot \frac{M_o^*}{M}$$

$$M^* = M_o^* \cdot e^{-\frac{R_T}{M} \cdot t} \qquad 7$$

equation 7 is obtained. If this equation is integrated from 0 to ∞, then the relationship between turnover rate and the area under the specific activity curve becomes apparent

in equation 8.

$$M_t^* = M_o^* \cdot e^{-\frac{R_T}{M} \cdot t}$$

$$\int_0^\infty M_t^* = \text{Area}$$

$$= M_o^* \int_0^\infty e^{-\frac{R_T}{M} \cdot t}$$

$$= M_o^* \cdot -\frac{M}{R_T} \int_0^\infty e^{-\frac{R_T}{M} \cdot t} \cdot -\frac{R_T}{M} \cdot dt$$

$$= M_o^* \cdot -\frac{M}{R_T} \left[e^{-\frac{R_T}{M} \cdot \infty} - e^{-\frac{R_T}{M} \cdot 0} \right]$$

$$= M_o^* \cdot -\frac{M}{R_T} \left[\frac{1}{e^\infty} - e^0 \right]$$

$$= M_o^* \cdot -\frac{M}{R_T} [0 - 1]$$

$$= M_o^* \cdot \frac{M}{R_T}$$

$$R_T = \frac{M_o^* \cdot M}{\text{Area}} \qquad 8$$

In practice, the area may be obtained by graphical analysis and the R_T calculated arithmetically; however, M must still be calculated from an extrapolated value.

Stetten, Ingle and Morley (1957) as well as Steele (1959) popularized the continuous infusion of tracer in order to calculate turnover rates. Reasoning as Zilversmit et al. (1943a, b) had done earlier, Stetten et al. (1957) recognized that the turnover rate of tracee to that of

tracer should equal the ratio of the mass of the tracee to that of the tracer.

$$\frac{R_T}{R_T^*} = \frac{M}{M^*} = \frac{1}{\text{Spec. Act.}}$$

$$R_T = \frac{R_T^*}{\text{Spec. Act.}} \qquad 9$$

R_T^* = the constant rate at which tracer is being infused.

Spec. Act. = the constant specific activity due to the steady states in which both tracer and tracee are held.

Both the single dose injection and the continuous infusion method should provide the same results; however, when investigators compared the two methods they found that the turnover rate of glucose in dogs was consistently higher (7%) in the experiments where the dose was administered as a single injection (Hetenyi and Norwich, 1974). An evaluation of the experimental curves revealed that this discrepancy exists when the terminal monoexponential portion of the curve is used in the calculations. When the turnover rate is calculated on the basis of the entire area under the curve there is no discrepancy between the single dose injection and the constant infusion results. Since the area is in the denominator (equation 8), using the smaller, monoexponential area yields higher turnover rates than those calculated from the equation when the total area is used.

When the tracer is injected as a single dose, the turnover rate is subject to error because the former model and method for calculating turnover rates depends on an extrapolation of the terminal, monoexponential portion of the curve back to the ordinate. The use of the y-intercept value in equation 3 is necessary to reduce the number of unknowns in equations 6, 7,and 8. One way to circumvent the problems associated with the preceding equations has evolved with the use of models lending themselves to stochastic analysis.

Stochastic, from the Greek word "stochastikos," means skillful in aiming. As the word is used in turnover studies, it means "random," in the sense that it involves a variate at each moment in time. The previously described compartmental models are deterministic because there is no allowance for scatter or randomness. Furthermore, deterministic models are explicit in reference to the interior structure of the model. That is, the number of pools, their interactions (caternary, mamillary, or mixed), and their internal kinetics are precisely described. One must question whether enough is really known about biological control systems to justifiably use a deterministic approach. Perhaps, in simple, isolated systems functioning within a carefully controlled environment, the use of a deterministic approach is valid; however, when whole animal studies are performed in vivo a stochastic approach can be used to advantage. The investigator need not make as many

assumptions nor does he have to specifically define the numerical parameters of the system. One reason biological systems are resistant to description by deterministic models is that the responses to a given input change from moment to moment. The systems are nonstationary. According to Bassingthwaighte and Ackerman (1967), apparent nonlinearities are usually due to uncontrolled and unrecognized inputs or cyclic fluctuations in subsystems. These unrecognized inputs may be continuous or quantized variables as well as pulse trains of varying frequency. A stochastic analysis incorporates changes such as these into the results without necessarily recognizing that they have occurred.

The basis of the stochastic approach is closely related to the application of the Fick Principle. In order to apply the Fick Principle, two assumptions must be made: 1) a tracer atom and the natural atom existing side-by-side at a given instant have an identical chance of appearing at another location in the system after a given time interval, and 2) individual tracer atoms or counterpart tracee atoms comprising a group will not all arrive at some other location simultaneously. However, there is a mean time for transit of the group as a whole. The latter assumption is the point at which the stochastic analysis mathematically departs from the deterministic models. Recall that the calculation for the volume of distribution in deterministic models was given in equation 4 as follows:

$$V = \frac{M^*}{C_o^*} \qquad 10$$

Solving this equation and substituting the volume obtained into equation 5 permits the calculation of the mass of tracee:

$$M = V(ml) \cdot C(mg/ml) \qquad 11$$

Finally, turnover rate is calculated from the slope of the semi-logarithmic plot where slope is equal to $- R_T/M$ (equation 6). This approach differs considerably from that used in the stochastic analysis.

The stochastic analysis presumes that the tracer is included in a discrete volume of fluids, for example, the plasma pool. The tracer, which is transversing the point of exit (the cell membrane) is present in a continuously changing concentration which is sampled in relation to elapsed time. If the mass of glucose which left the system and entered the cell was known in relation to some discrete time, T, then a mass flow or rate of utilization could readily be determined.

$$F_{mass} = \frac{Mass}{Time} \qquad 12$$

The first step is to measure M, the mass. If the mass were static, it could be measured by the principle of tracer dilution:

$$Mass = \frac{Dose}{Spec.\ Act.} \qquad 13$$

However, because of continual mixing and washout, the specific activity is not constant over the measured time period.

Therefore, the mean specific activity must be used in the calculation. Hence,

$$\text{Mass} = \frac{\text{Dose}}{\text{Spec. Act.}_{(\text{mean})}} \qquad 14$$

The mean specific activity is determined by measuring the area under the curve during the time interval and dividing by the elapsed time, T (Figure 9).

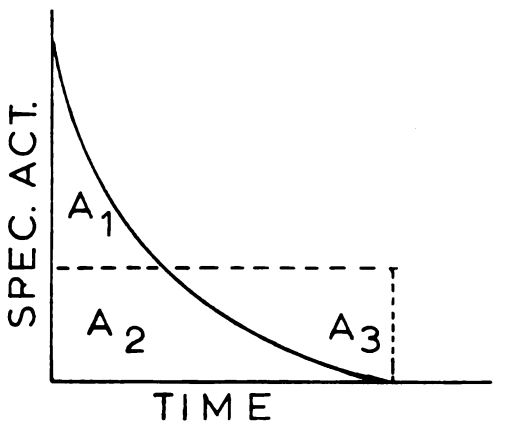


Figure 9 . Glucose turnover curve with dotted line showing normalized area under the curve.

When the area under the curve in Figure 9 is normalized we find that,

$$\text{Area}_1 + \text{Area}_2 = \text{Area}_2 + \text{Area}_3$$

and

$$\text{Spec. Act.}_{(\text{mean})} = \frac{\text{Area}_2 + \text{Area}_3}{\text{Time}} \qquad 15$$

Note that the y-intercept in Figure 9 is the mean specific activity. The relationships shown in equations 14 and 15 can be used to derive the Stewart-Hamilton equation as follows (equations 16-19):

$$\text{Flow} = \frac{\text{Mass}}{\text{Time}} \qquad 16$$

$$\text{Flow} = \frac{\text{Dose/Spec. Act.}_{(\text{mean})}}{\text{Time}} \qquad 17$$

$$= \frac{\text{Dose}/(\text{Area}/\text{Time})}{\text{Time}} \qquad 18$$

$$= \frac{\text{Dose}}{\text{Area}} \qquad 19$$

The ratio of dose to area under the time-concentration curve is the classical, working form of the Stewart-Hamilton equation; hence, the derivation of this equation by calculus is in order.

When time span, $\triangle t$, are considered to be so short that the instantaneous specific activities (SA_i) may be assumed to be constant during such times, then the amount of tracer lost during $\triangle t$

$$= \text{Flow} \cdot SA_i \cdot \triangle t \qquad 20$$

At $t = \infty$, all of the dose will be lost and the dose will be the sum of the series of the foregoing products. Thus:

$$\text{Dose} = \lim \sum_{t=0}^{\infty} \text{Flow} \cdot SA_i \cdot \triangle t \qquad 21$$

$$= \int_0^{\infty} \text{Flow} \cdot SA_i(t)\, dt \qquad 22$$

If flow is assumed to be constant, it can be removed from the integral. So,

$$\text{Dose} = \text{Flow} \int_0^{\infty} SA_i(t)\, dt \qquad 23$$

Rearranging the terms in the preceding equation yields the working form of the Stewart-Hamilton equation once again.

$$\text{Flow} = \frac{\text{Dose}}{\int_0^{\infty} SA_i(t)\,dt} \qquad 24$$

$$\text{Flow} = \frac{\text{Dose}}{\text{Area}} \qquad 25$$

Although individual molecules of glucose have different times of transit from one place or state in the system to another, a mean time may be determined for the entire dose. Assuming that tracer and tracee have an equal probability of exiting from the system, then the mean transit time is equally applicable to tracer and tracee: furthermore, the mean transit time is also related to the turnover time for the unlabeled material comprising the system. In keeping with the previous conditions set forth for stochastic analysis, the development of equations for mean transit time will be done in a system with an unspecified internal configuration.

Once again, assume a time interval, $\triangle t$, so short that the specific activity may be considered considered constant within that interval. Fm is the mass flow which was calculated in equation 25 and the amount of tracer lost during $\triangle t$ has been previously described in equation 20. It follows that the total amount of tracer lost is,

$$\text{Tracer lost}_{(\text{total})} = Fm \sum SA_i \cdot \triangle t \qquad 26$$

The average length of time, $\bar{t}$, that a molecule remains in the system is calculated from equations 20 and 26 as follows,

$$\bar{t} = \frac{Fm\,[t_1(SA_1 \cdot \triangle t) + t_2(SA_2 \cdot \triangle t) + \ldots + t_n(SA_n \cdot \triangle t)]}{Fm\,[SA_1 \cdot \triangle t + SA_2 \cdot \triangle t + \ldots + SA_n \cdot \triangle t]} \qquad 27$$

The Fm values cancel each other and the equation may be rewritten,

$$\bar{t} = \frac{\sum_{i=0}^{n} t_i \cdot SA_i(t) \cdot \Delta t}{\sum_{i=0}^{n} SA_i(t) \cdot \Delta t} \qquad 28$$

Finally, integration of the preceding equation yields,

$$\bar{t} = \frac{\int_{0}^{\infty} t \cdot SA(t)\, dt}{\int_{0}^{\infty} SA(t)\, dt} \qquad 29$$

Once the mass flow and the transit time are obtained it is possible to calculate the total amount of tracee in the body mass by multiplying mass flow by transit time. The derivations leading to this relationship are somewhat simplified if one first derives the equation for transit time based on the total quantity, q_t, of tracer remaining in the system at any particular time. If one views the entire system as a collection of particles which remain within the system for varying lengths of time, then the statistical concepts of class (time) and frequency (percent of particles remaining in the system) may be used to calculate the average time of residence for the collection of particles. The sum of $q_1t_1 + q_2t_2 + \ldots + q_nt_n$ is the grand total of times for each group of particles. When the grand total of times is divided by the total number of particles, the quotient is the mean transit time (equation 30).

$$\bar{t} = \frac{\sum_{i=0}^{\infty} q_i t_i}{\sum q_i} \qquad 30$$

If q_i is the amount of tracer remaining at t_i, the $\sum q_i$ is equal to the dose. Furthermore each of the separate multiples in the numerator, being a product of quantity (ordinate) and time (abscissa), represents a portion of area under the curve q(t). Thus the numerator is the total area under this curve and may be replaced by the integral, while the denominator may be replaced by dose.

$$\bar{t} = \frac{\int_0^{\infty} q(t)\, dt}{\text{Dose}} \qquad 31$$

Recognizing this form of the transit time equation will be useful during the following derivation of the equation for body mass of tracee.

A convenient starting point for deriving the equation used in determining the total body mass of tracee is a previously derived equation, equation 24.

$$\text{Flow} = \frac{\text{Dose}}{\int_0^{\infty} SA_i(t)\, dt}$$

Let q_i and Q_i respectively equal the mass of tracer and tracee in the system at t_i. It follows then, that

$$SA_i = q_i/Q_i$$

therefore,

$$\text{Flow} = \frac{\text{Dose}}{\int_0^\infty [q_i(t)/Q_i]\, dt}$$

$$\text{Flow} = \frac{Q_i}{\int_0^\infty [q_i(t)/\text{Dose}]\, dt}$$

$$Q_i = \text{Flow} \cdot \int_0^\infty q_i(t)/\text{Dose}\, dt$$

$$Q_i = \text{Flow} \cdot \frac{\int_0^\infty q_i(t)\, dt}{\text{Dose}} \qquad 32$$

Notice that the multiplier is the equation previously derived for mean transit time (equation 31). Hence,

$$Q_i = \text{Flow} \cdot \bar{t} \qquad 33$$

The usefulness and validity of the equation described for the stochastic analysis have been discussed and confirmed by Katz et al. (1974a,b) and Brady et al. (1977).

PART 7

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