DYNAMICS OF GLUCOSE AND CARBON DIOXIDE METABOLISM IN CATTLE GIVEN MOLASSES BASED DIETS

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Using cattle on molasses based diets, the irreversible loss rate (or entry rate) of glucose and the percentage of blood bicarbonate arising from glucose oxidation were estimated by infusing ^{14}C glucose and measuring the specific radioactivity of plasma glucose and blood carbon dioxide when these had apparently come to a plateau. The irreversible loss rate of carbon dioxide and the percentage of plasma glucose arising from blood CO_2 were estimated in the same animals four days later by infusing $NaH^{14}CO_3$ and measuring the specific radioactivity of blood carbon dioxide and plasma glucose. In both experiments, ^{3}H glucose was included in the infusion mixture to assess the total entry rate of glucose on the separate days. A model of glucose and carbon dioxide interrelationships in the animal was developed from these data. Almost all the glucose passing through the glucose pool was synthesised via reactions involving CO_2 fixation. Considerable glucose-C entered pools of carbon with a slow turnover rate possilby involved in tissue synthesis.

Key words: Cattle, molasses, glucose-carbon dioxide metabolism, radioisotopes, modelling

In many intensive cattle production systems where the diets are based on maize, considerable quantities of starch apparently escape rumen fermentation to be digested and absorbed as glucose from the lower intestinal tract (Armstrong and Smithard 1979). The absorption of starch on concentrate diets in ruminants may have caused scientists not to recognise the full significance of gluconeogenesis in high producing ruminants. On sugar based diets little or no sugar may escape rumen fermentation and if this is the case the animal is totally dependent on gluconeogenesis for its glucose supply. In cattle on sugar cane diets, responses in weight gain to

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supplements containing large quantities of starch (that is rice polishings) were obtained and were assumed to be, at least in part, due to starch apparently escaping rumen fermentation (Elliott et al 1978) which increased glucose entry rates considerably (Ferreiro et al 1979). Economedes et al (1980) showed that in lambs on sugar/oaten chaff based diets, (that were suboptimal in protein to support maximum feed intake) that extra glucose given postruminally stimulated liveweight gain significantly. These results suggest that at a low level of amino acid absorption from the digestive tract (which is suboptimal for animal production) more amino acids are available to the animal than are required and that the provision of extra energy postruminally may be used efficiently and increase production substantially.

The ruminant appears to have a need for glucose, since it synthesises considerable quantities of glucose particularly at times of high productivity (see Leng, 1970). Judson and Leng (1968) showed that 2025% of the digestible energy of a ration passed through the glucose pool in mature sheep on roughage diets and that the amount of glucose synthesised increased linearly with digestible energy intake. Smith et al (1979) showed that there was a linear relationship in cattle on molasses based diets between the rate of growth and glucose entry rates indicating the important role that glucose may have in providing substrates for growth.

The amount of glucose stored in body tissues is not known although glucose oxidation has been studied (see for review Leng, 1970). To study the partitioning of glucose utilisation an approach has been developed using isotope dilution techniques. A mathematical approach similar to that reported by Nolan et al (1976a) was used to investigate the destination of glucose carbon within the animal. The system depends on examining glucose and carbon dioxide irreversible loss rates from blood and measuring the proportion of each substrate pool which originates from the other.

Materials and Methods

Hereford cattle weighing approximately 200 kg were used. These were held in an animal house and were given ad libitum molasses containing 3% urea as a basal diet supplemented with 2 kg of oaten chaff.

Chemical methods: Plasma glucose levels were measured by the glucose oxidase method of Huggett and Nixon (1957). The specific radioactivity of glucose was measured as the penta-acetate derivative (Jones 1965) and assayed for radioactivity in a Packard Tricarb Scintillation Spectrometer 3320 using the channels ratio method for dual isotopes (see Judson and Leng 1972). The specific radioactivity of blood carbon dioxide was assayed as described by Leng and Leonard (1965).

Radioisotopes: All Radioisotopes were obtained from the Radio chemical Centre, Amersham, England. Labelled glucose and carbon dioxide in infusion solution were assayed for radioactivity as described for samples.

Experimental procedures: : The animals were infused on day 1 with a mixture of [U-^{14}C] and [2-^{3}H] labelled glucose for a period of 12 h. Blood samples were taken for assay of radioactivity in plasma glucose and in blood CO_2. These samples were taken at hourly intervals over the last 6 h of the experiment; 2-3 days later the animals were infused with a mixture of [2-^{3}H] glucose and Na$H^{14}CO_3$. Blood samples were taken for the assay of the specific radioactivity of glucose and of carbon dioxide over the last 6-12 h period.

Terminology for use in tracer studies

Since studies with isotopic tracers are beginning to be published in this journal, it is probably imperative that definition of the terminology is made here. The following terminology has been used (taken from Nolan et al 1976b).

Model: a representation of an hypothesis in which a formal structure for a system is proposed. Like other hypotheses, it may be based on previous knowledge, experimental observations and a number of assumptions. It is subject to amendment and revision with respect to qualitative or quantitative properties.

Tracer: defined substance labelled (e.g. by use of isotopes) so that its movement and behaviour in the system can be studied. Basic assumptions are that the tracer participates in physical, chemical and biological reactions, indistinguishably from tracee, and that its introduction causes no perturbation of the characteristics of the system.

Tracer dilution: isotope dilution: the change in the ratio of tracer and tracee following injection or during infusion of a tracer into a compartment.

Tracer kinetics: the movement and behaviour of tracer in a system with time.

Kinetic analysis: the interpretation of tracer behaviour in a system in terms of mathematical or physical models.

Steady-state: a condition of a system characterized by constancy with respect to concentrations, quantities and rates of flow of tracee during the experimental period.

Pool: a term reserved for compartment(s) of tracee which compose a biologically distinguishable entity.

Pool size: tile total mass of tracee distributed through all compartments within the biological system.

Space: the apparent volume of distribution of the tracee in the pool (i.e. in the biological system).

Turnover: the process of loss and replacement of tracee in a given compartment (or pool).

Turnover rate: the fraction of tracee present in a given compartment that is renewed per unit time.

Turnover time: the time required for an amount of tracee equivalent to that in the compartment, to be transferred into and out of the compartment; it is the reciprocal of the turnover rate.

Half-time: the time required for half the amount of tracee in a compartment at any given time to be removed by the turnover process.

Total flux rate: the rate (mass/unit time) at which all tracee enters and leaves a compartment which is in steady state. This parameter may be divided into:

Irreversible loss rate/entry rate: a fractional flux-rate equal to that fraction of the flux of tracee (mass/unit time) which leaves the compartment and does not return to it during the experimental period.

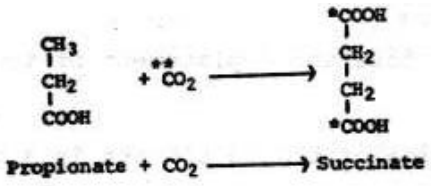
Recycling rate: a fractional flux-rate equal to that fraction of the flux of tracee (mass/unit time) which leaves the compartment and returns to it during the experimental period.

Theory

The basis of this study is that carbon dioxide is fixed into glucose precursors at two sites in the overall gluconeogenic pathways. These include the carboxylation of propionate to methylmalonate with the subsequent production of succinate and the conversion of this in the tricarboxylic acid cycle (TCA) to oxaloacetate. The second pathway is the carboxylation of pyruvate to give oxaloacetate directly. Oxaloacetate is then converted to glucose via phosphoenolpyruvate and the reversal of glycolysis (see Weekes 1979 for discussion).

It is assumed that dicarboxylic acid pools in the liver are in reversible equilibrium via reactions of the TCA cycle and therefore oxaloacetate synthesised from pyruvate is in equilibrium with malate, fumarate and succinate. The point here is that the radioactivity from $^{14}CO_2$ fixed into a compound that is converted to or in equilibrium with succinate will be equally distributed about the terminal carbon atoms because this is a symmetrical compound. The reactions are as follows, where the ^{14}C labelled atom in the compounds is indicated by an asterisk. Two asterisks indicating twice as much radioactivity as single asterisk.

(1)



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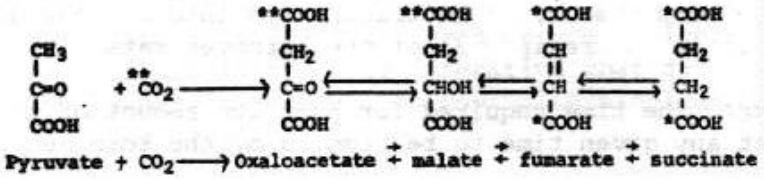

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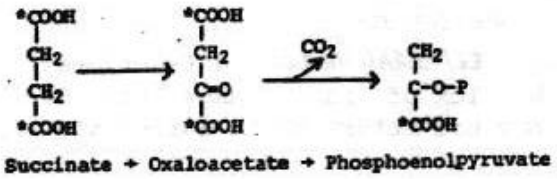

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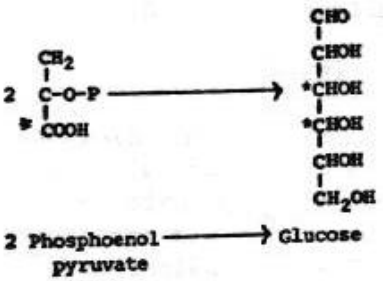
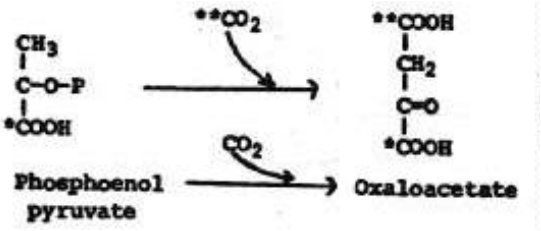


These equations only show the overall reactions and the integrated. pathways are shown in the Appendix.

Succinate is synthesised into glucose by first conversion to oxaloacetate, decarboxylation of the oxaloacetate to give rise to phosphoenolpyruvate and then by a reversal of the pathways of glycolysis. The net effect is that half the radioactivity fixed into the succinate molecule is lost as carbon dioxide at the decarboxylation of oxaloacetate to give phosphoenol-pyruvate.

Because two, 3 carbon compounds are required to synthesise glucose the net effect is that only half the radioactivity from carbon dioxide is fixed into glucose by these reactions. If all the glucose entering the blood pool was synthesised via carbon dioxide fixation reactions the ratio of specific radioactivities of carbon in glucose to blood carbon dioxide should be 0.167. Expressed in another way, 17% of the plasma glucose carbon arises from carbon dioxide carbon. A ratio less than .17 indicates (1) synthesis of glucose from compounds that do not undergo carbon dioxide fixation or (2) glucose absorption from the digestive tract. A ratio greater than .17 indicates that compounds that have undergone carbon dioxide fixation then leak from the metabolic pools (i.e. enter the blood pool) and then re-enter gluconeogenesis by a further carbon dioxide fixation reaction (i.e. lactic acid or alanine).

i.e.



A further consideration is that an increase in the ratio would occur if oxaloacetate synthesised from pyruvate did not completely equilibrate with succinate. However, the ratios obtained here are close to the theoretical and therefore this latter possibility seems to be unlikely.

Calculations

In studies in which a small quantity of isotopically labelled substrate is infused into blood the specific radioactivity of substrate will increase and eventually plateau (see Figs. 1 and 2). The irreversible loss rate (IL) of the substrate which under steady state conditions represents the entry rate of the substrate is calculated as follows

$$IL = \frac{I}{SR_M}$$

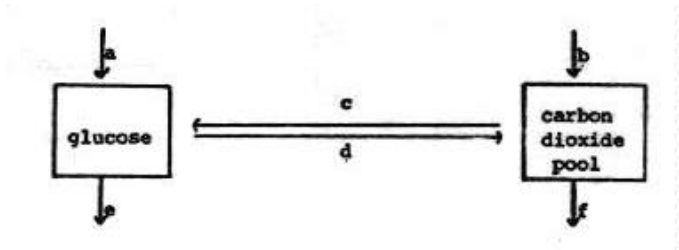
Where SR_M is the mean plateau specific radioactivity of the substrate (usually in mμc/mg C) and I (mμc/min) is the infusion rate.

If the substrate labelled by infusion is converted to another compound which also enters a blood pool then its specific radioactivity will increase to a plateau. The ratio of specific radioactivity of product to precursor represents the percentage of the product pool arising from the precursor pool.

In these studies ^{14}C-glucose was infused on one day and $^{14}CO_2$ at another time and the specific radioactivities of glucose and carbon dioxide were measured. From these experiments considerable information can be gained on glucose metabolism.

The following is an example of the method of calculating the flows of carbon in the model shown. It is taken from Depocas and DeFreitas (1970) and has been developed by Dr J.V. Nolan (see Nolan et al 1976a).

Consider the glucose-CO_2 model in cattle as follows:



Where a, b, c, d, e, and f represent the rates of flow of carbon in g of carbon per day (gC/d). The example given is for animal number 5 (see Table 1)

(1) The irreversible loss rate of glucose (ILA) = 141.6gc/d

(2) The irreversible loss rate of carbon dioxide (ILB) = 1175gC/d

(3) The proportion of the carbon in the glucose pool arising from carbon dixoide = 16.4%

(4) The proportion of the carbon in the carbon dioxide pool arising from glucose = 9.8%

Then the irreversible loss rates are as follows:

ILA = e + 9.8f = 141.6 gC/d
ILB = f + 16.4e = 1175 gC/d

Solving the simultaneous equations we have:

e = 27gC/d f = 1171gC/d

Similarly

ILA = e + d - 0.098f = 141.6 gC/d
ILB = f + c - .164d = 1175 gC/d

Substituting for e and f and then solving we have

c =118 gC/d d = 24 gC/d

Hence for both pools in equilibrium

a = e +d - c and therefore a = 120gC/d
b = f + c -d and therefore b = 1077gC/d.

In biochemical terms the rates of carbon transfer (in gC/d), i.e. a, b, c, d, e/, f, can be described as follows: c, is the glucose carbon arising from carbon dioxide fixation.

a, is the rate of entry of carbon into glucose that does not originate from blood CO_2. However, because for each $^{14}CO_2$ fixed into glucose five carbons arise from other substrate the value for(a - 5 c)is the amount of glucose that arises from reactions other than those involving carbon dioxide, plus glucose arising from glycogen or absorbed from the digestive tract, the value for 6 c is the net rate of gluconeogenesis from substrates involving carbon dioxide fixation.

d, is the net rate of glucose oxidation.

b, is the carbon dioxide from oxidation of substrates other than glucose.

f, is the carbon dioxide irreversibly lost from the blood carbon dioxide pool.

e, is the carbon of glucose entering pools with slow turnover, since in ruminants glycogen is labelled to only a small extent from plasma glucose (see Annison et al 1963) and in these studies the value for (a-5c) is very small, this appears to be glucose entering body tissue stores (i.e. growth).

Results

Pattern of specific radioactivity in blood bicarbonate and plasma glucose: The specific radioactivity in glucose and blood carbon dioxide during an infusion of (2-^{3}H) and (U-^{14}C) glucose is shown in Fig 1. The results for the infusion of (2-^{3}H) glucose and $NaH^{14}CO_3$ are shown in Fig 2.

Dynamic aspects of glucose and bicarbonate in cattle given a basal diet of molasses and oaten chaff: Kinetic data for the irreversible loss rate of glucose (as measured with ^{14}C glucose) and total glucose entry rate (as measured with ^{3}H glucose) are shown in Table 1 together with data for the irreversible loss rate of carbon dioxide from blood and the proportion of glucose arising from CO_2 and the proportion of the carbon dioxide arising from glucose oxidation. The flow rates of carbon through the glucose and carbon dioxide pools and between these pools are shown in Table 2.

Discusslon

In these studies the data used to develop the model of glucose carbon dioxide interrelationships were based on the results obtained when (U-^{14}C) glucose or $^{14}CO_2$ was infused intravenously into cattle. (2-^{3}H) Glucose was infused on both experimental days in order to check the constancy of glucose synthesis in the cattle between experimental days so as to check that the data collected on the two days were comparable. Although there were variations between days in this measurement the results for the three animals in the two experimental days were not significantly different.

The kinetic data obtained with (2-^{3}H) glucose approximate the total glucose entry rate (a + c) since the recycling of label from glucose-H is small (see Judson and Leng 1972). This is because in glycolysis the tritium label of (23- H) glucose is converted to water which enters a large body pool of water and is diluted to such an extent that any water that donates hydrogen to glucose in any way is negligibly labelled (see Judson and Leng 1972). This is supported by the fact that the difference between the total glucose entry rate (mean for both days) and glucose irreversible loss rate, was small.

The ratio of ^{14}C in carbon dioxide to that in glucose during an infusion of $NaH^{14}CO_3$ into blood indicates the percentage of the glucose arising from carbon dioxide fixation reactions. The main initial reactions in gluconeogenesis leading to carbon dioxide fixation are either at the propionate carboxylation or the carboxylation of pyruvate to oxaloacetate. This is discussed under the heading 'theory'. In these studies the ratio of ^{14}C in glucose to that in carbon dioxide was 0.16-0.24 which suggests that 20% of the glucose-C arose from blood carbon dioxide.

Figure 1:
Specific radioactivity of plasma glucose and blood carbon dioxide during a continuous intravenous infusion of a mixture of ^{14}C and ^{3}H glucose (0.242 µc/min in 0. 3 ml) into a steer. Tritium radioactivity in plasma glucose (!, nCi/mg glucose); ^{14}C radioactivity in plasma glucose (", nCi/mgC) and blood carbon dioxide (△ 4 nCi/mgC).

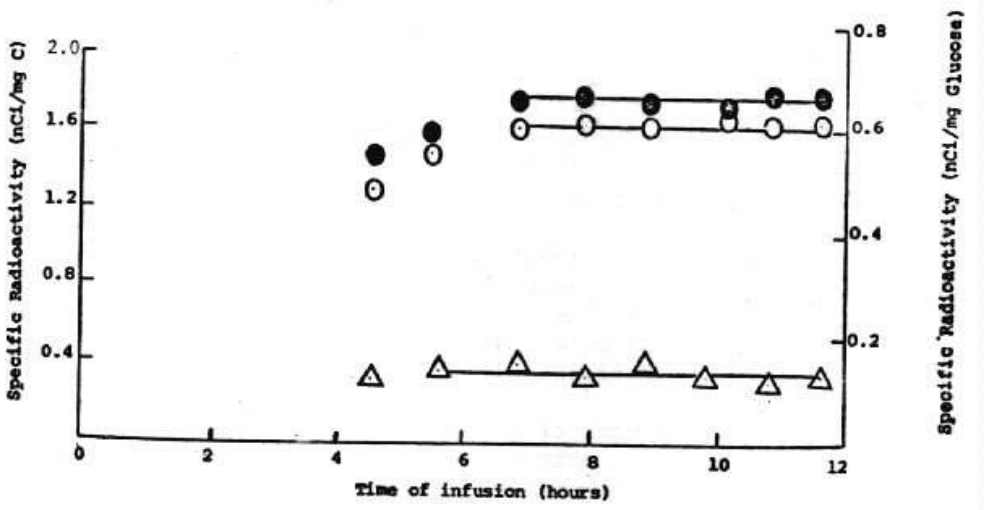


Figure 2 :
Specific radioactivity of blood bicarbonate and plasma glucose during a continuous intravenous infusion of a mixture of $NaH^{14}CO_3$ (0.351 µc/min) and 30 glucose (0.242 µc/min) into a steer. Tritium radioactivity in plasma glucose (!, nCi/mg glucose); ^{14}C radioactivity in plasma glucose (", nCi/mgC) and blood carbon dioxide (△ 4 nCi/mgC).

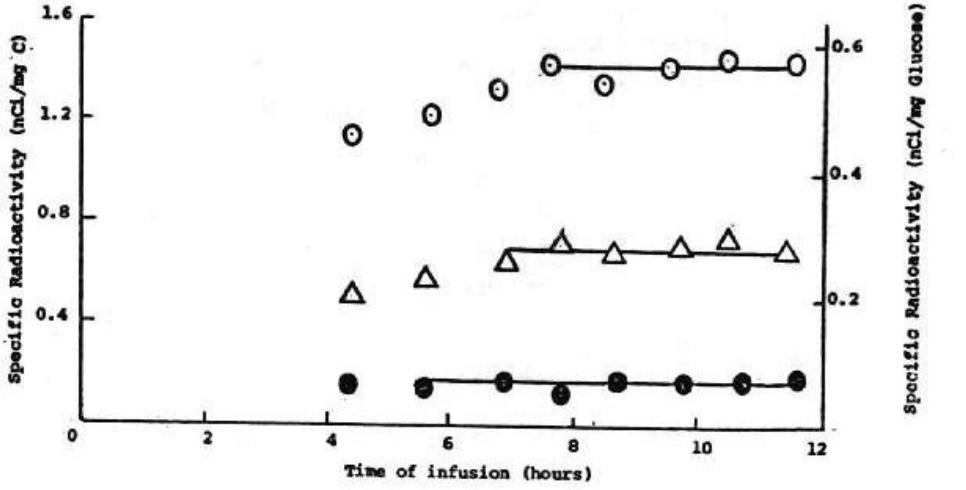
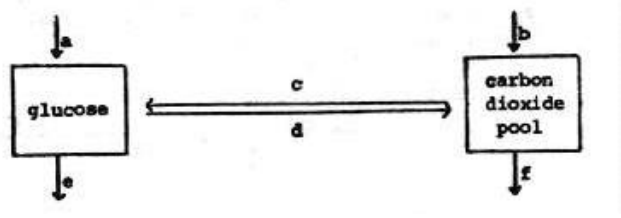


Table 1:
Dynamics of glucose and carbon dioxide metabolism in cattle given ad lib molasses and oaten chaff (2 kg/d)

Animal No	Plasma glucose conc. (mg/100 ml)		Glucose irreversible loss rate (g/d) [^{14}C]	Total glucose entry rate (g/d)	[^{3}H]	Carbon dioxide irreversible loss rate (gC/d)	Glucose from carbon dioxide (%)	Blood carbon dioxide from glucose (%)
	Day 1	Day 2	Day 1	Day 1	Day 2	Day 2	Day 2	Day 1
5	81	79	354	391	352	1,175	16.4	9.8
6	71	71	378	395	418	1,151	22.6	9.3
9	78	80 .	301	315	305	1,224	24.2	8.9

Table 2:
Values for carbon flux between and through the blood carbon dioxide and plasma glucose pools in cattle. The flows are given for each according to the labelling given in the figure.



Animal	Flows of carbon (gC/d)					
No	a	b	c	d	e	f
5	120	1077	24	118	27	1171
6	120	1067	35	109	46	1140
9	193	1140	30	111	13	1220

This indicates that about 83% of the glucose was synthesised via reactions in which the primary substrate is incorporated into the TCA cycle intermediates via CO_2 fixation reaction. A small leakage of substrates arising from the TCA cycle which reenters gluconeogenesis via a further CO_2 fixation reactions may explain the origin of the remainder of the carbon of glucose (see theory).

The results strongly indicate that virtually all the glucose synthesis in these animals arises from lactate or propionate with propionate probably being the major glucogenic precursor. Thus little or no glucose is absorbed from the digestive tract of cattle on these diets and during the course of these experiments, glycogen was not supplying significant amounts of blood glucose. It does not, however, exclude glucose synthesis from amino acids, but it indicates that the glucogenic amino acids are first converted to propionate or lactate before conversion-to glucose. This is conceivable for the amino acids which directly give rise to these intermediates, but seems unlikely for others, however, a possible explanation is that amino acids are deaminated at sites other than the liver (the main site of gluconeogenesis) and possibly transported to the liver to participate in gluconeogenesis.

Of major significance is that quite a large amount of carbon that entered the glucose pool did not reappear in blood carbon dioxide and therefore was not oxidised over the time course of these studies. If we consider that this carbon entered slowly turning over pools, which may be largely associated with tissue synthesis, then considerable amounts of glucose are deposited in tissue growth and therefore glucose provides a considerable proportion of the "building blocks" for tissue synthesis.

The results of the study clearly indicate that cattle on sugar based diets are dependent upon synthesis of glucose from short chain precursors absorbed from the gut. This means that ruminants are liable to suffer a shortage of glucose, particularly during periods of high productivity (see Kempton et al 1977) which are associated with high requirements for glucose, for instance, for milk lactose synthesis. In animals growing at high rates or in peak lactation in high yielding cows the glucose entry rate exceeds 22mg/kg $^{.75}$/min. In peak lactation in high yielding dairy cows up to 7 kg of glucose/day are synthesised (Weekes 1979). These high apparent requirements for glucose synthesis will increase the metabolisable energy requirements of lactating cows, since there is an energy cost associated with glucose synthesis. The use of a bypass starch source to provide the glucose (which is apparently required) for producing ruminants will be highly efficiently used and much more efficiently used than similar quantities of carbohydrate fermented to VFA in the rumen prior to absorption by the animal.

It appears highly desirable to initiate studies to find ways and means of causing dietary starch to escape fermentation particularly in lactating cows on basal diets of sugar and roughage. The mechanisms which allow rice polishings (high starch), to escape rumen fermentation on sugar cane based diets given to cattle (see Ferreiro et al 1979; Elliott et al 1978) are worthy of considerable study.

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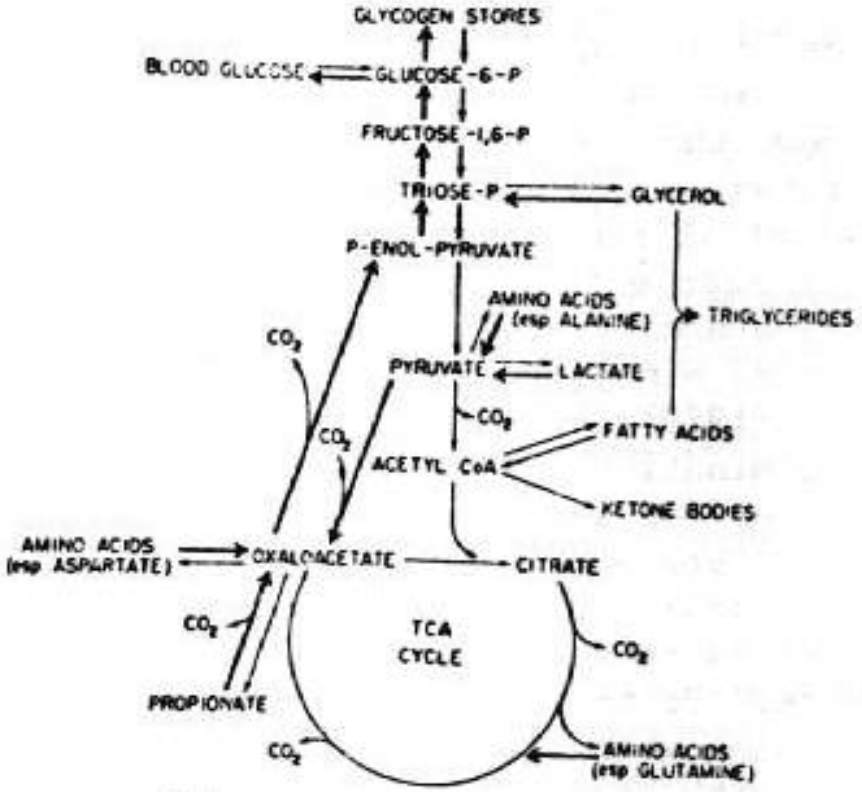
Appendix



Major metabolic pathways in ruminant liver (and kidney cortex). Heavy arrows indicate pathways for gluconeogenesis.