Metabolism in the whole animal

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Reviews of carbohydrate metabolism in ruminants have generally concentrated on gluconeogenesis. This emphasis has been prompted by the evidence that glucose is not normally absorbed from the gastrointestinal tract (this Symposium). Since the author has recently reviewed gluconeogenesis in ruminants (Lindsay, 1979*a*) it seems appropriate to discuss the extent to which carbohydrate and glucose utilization may be equated.

There are three logically distinct ways by which carbohydrate may be utilized by individual tissues. (1) There may be an absolute need for glucose which is not met by any other compound. (2) There may be a need for what I propose to call 'glycogenic' compounds. The use of glucose in this sense can be met, at least in part by other sugars such as fructose, or more commonly by compounds such as lactate, pyruvate and many amino acids. In this sense, these compounds spare glucose. (3) Glucose and other glycogenic compounds may be oxidized and thus serve as an energy source. Here, the compounds are all converted to acetyl CoA. A variety of compounds—acetate, ketones, long-chain fatty acids and leucine, as well as the glycogenic compounds (class 2 above)—may spare glucose in this sense. The distinction between these classes will be more obvious after considering metabolism in particular tissues.

Mammary gland. In lactating cattle, Bickerstaffe *et al.* (1974) and Annison *et al.* (1974) observed glucose uptake by the udder to range from 560–1509 mg/min, which accounted for from 50–90% of the glucose entry rate. The major function of glucose is in the synthesis of lactose, which is now known to determine the rate of formation of the aqueous phase of milk (Linzell & Peaker, 1971). In the final reaction of lactose synthesis, (glucose+UDP-galactose→lactose+UDP), free glucose is an essential component. However, glucose is not necessarily needed in forming the galactose portion. Because fructose:1,6 diphosphatase is present in the tissue, in principle C_3 compounds such as lactate could be converted to UDP-galactose. Although, however, the minimum requirement for glucose is thus only 50% of the lactose synthesized, in practice isotope studies (e.g. Bickerstaffe *et al.* 1974) suggest that at least 85% of lactose is derived from glucose. Even if all the lactose were derived from glucose, it would account only for 40–80% of glucose taken up. There is no certain evidence that the rest is taken up in response to a specific glucose requirement. Such a requirement could (but need not necessarily) include the known glucose oxidation through the pentose phosphate reactions as well as formation of glycerol for triacylglycerols. In the studies above, however, glucose uptake (—lactose output) is not correlated with milk output. There is known

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to be a significant uptake of lactate and non-essential amino acids (although uptake of the latter is frequently insufficient to meet the needs of protein requirements) (Linzell, 1974). It seems possible then that the sum of (glucose uptake-lactose output), lactate, pyruvate and the non-essential amino acids constitute a pool of 'glycogenic precursors' which meet a variety of needs. It is possible indeed that some is used as a non-specific energy source.

Testis. Linzell & Setchell (1969) have shown, using the isolated perfused ram or goat testis that 'normal function', in particular rete fluid formation requires a glucose concentration above about 1·5 mM in the arterial perfusing medium. The glucose uptake by this preparation (around 1–3 mg/min for the ram testis) was similar to that in vivo (Setchell & Waites, 1964). This utilization is maintained down to about 0·4 mM arterial glucose concentration (when fluid formation cannot be maintained). This suggests that the absolute glucose requirement is not a 'glycogenic' or oxidative requirement, but perhaps for some constituent such as inositol (Setchell *et al.* 1968) for which the total glucose requirement is quite small.

Pregnant uterus. Uptake of glucose by the pregnant uterus (Setchell *et al.* 1972; Christenson & Prior, 1978) is appreciably greater than estimates of fetal glucose uptake (e.g. Boyd *et al.* 1973). This reflects substantial metabolism of glucose by the placenta and uterus. Less than half the glucose is oxidized (Setchell *et al.* 1972) and a significant amount must be converted to lactate before being taken up by the fetal circulation (Burd *et al.* 1975; Char & Creasy, 1976). In unpublished studies on the pregnant uterus we have found, in addition to confirming that about 40% of glucose is oxidized, that variable amounts of lactate and pyruvate were released into the maternal circulation; similar findings are reported by Burd *et al.* (1975). By extending our studies using ^{14}C-lactate, we find that lactate can simultaneously be taken up and released by the pregnant uterus. It thus seems likely, at least to some degree, that glucose uptake reflects a 'glycogenic' requirement, some, although probably only a small part of which can be met by lactate (and perhaps some non-essential amino acids).

Brain. Glucose uptake by the sheep brain is about 37–45 μmol/100 g per min (Lindsay & Setchell, 1976). A very similar estimate (42 μmol/100 g per min) was obtained in one cow we studied. Assuming the rate of utilization of glucose is similar in most parts of the central nervous system, this corresponds to a total utilization of about 8–10 mg/min in sheep and 40–50 mg/min in cattle. Oxygen consumption by the sheep brain is about 235–270 μmol/100 g per min, that is, about six times the rate of glucose utilization that would be expected if glucose is almost completely oxidized; studies with ^{14}C-glucose are also consistent with this. We found only a small output of lactate and pyruvate by the brain and although this was not significantly different from zero, it may be real since it is very similar to the (statistically significant) estimate obtained by Jones *et al.* (1975). Although glucose serves mainly as an energy source, no other substrate has been found to 'spare' glucose oxidation. In part this may be due to the slow penetration by most small molecules across the blood-brain barrier. However, although ketones, for example, do slowly appear in cerebrospinal fluid, we were unable to obtain any

evidence that they can spare glucose oxidation (as has been shown in the human brain) (Owen *et al.* 1967). We have, however, recently obtained some indication that the branched-chain amino acids may be taken up to a small extent by the sheep brain, and this could spare glucose oxidation, although the extent to which this occurred was small—to about the same extent as the lactate output.

Cellular elements of blood. A small amount of glucose (less than 3% of glucose entry rate) can be metabolized by cellular elements in blood (Leng & Annison, 1962). For erythrocytes this must be an obligatory glucose requirement, since lactate cannot be metabolized. There is evidence, however, that the greater part of metabolism in blood is due to white cells and platelets (Leng & Annison, 1962; Anderson, 1969), which have some oxidative capacity. Thus lactate could to some extent spare glucose utilized.

Muscle. It is not possible to demonstrate unequivocally a requirement for glucose by muscle. There are, however, indications that there may be such a requirement. We have recently found that there is a significant uptake of glucose by sheep hind-limb muscle even in conditions such as pregnancy and lactation, where there is a large demand for glucose by reproductive tissues. There is also a significant utilization of glucose by the sheep heart even when blood ketones are elevated so that they become the main fuel of respiration (Lindsay & Setchell, 1976). Kempton, Smith *et al.* (1978) have shown that there is a close relation between glucose entry rate and growth in both lambs and cattle. This could simply reflect the relation between glucose entry and digestible energy intake (Judson & Leng, 1968; Lindsay, 1970) but Kempton, Hill *et al.* (1978) have shown that increasing glucose availability to young lambs, using a rumen bypass technique, results in increased growth even when protein availability is no longer limiting. Even if glucose does have an effect on growth, this would not necessarily show that the effect occurred in muscle; but a large part of the growth of young lambs does result in increased deposition of muscle; moreoever, the effect of glucose observed by Kempton *et al.* shows some specificity—it did not affect wool growth.

There is no indication as to whether the utilization of glucose is an absolute requirement, a need for a glycogenic compound or as a source of energy. The last possibility is unlikely since in most species the major sources of energy for muscle are fatty acids. In studies with ^{14}C-glucose, we have found that the fraction of CO_2 produced from glucose is variable, but generally about 0·05. It is certain, however, that glycogenic compounds must make some contribution. Although for example there is usually a net output of alanine by sheep muscle (Lindsay, Steel & Buttery, 1977) it has been shown by isotopic studies that there is a simultaneous uptake and output (Lindsay, Steel & Barker, 1977). A similar conclusion may be drawn from a study by Reilly & Chandresena (1977) in which they showed that the specific activity of arterial and jugular blood lactate differed following infusion of ^{14}C-lactate. Their results imply both uptake and release of lactate. The predominant tissue affected in this study was almost certainly muscle and in a study recently of lactate metabolism across the hind-limb muscles of sheep, we observed an uptake of lactate about one-half the size of the gross output. These input–output

relationships for lactate and alanine do not appear simply to be due to exchange reactions involving lactate dehydrogenase and alanine amino transferase since insufficient ^{14}C seems to be transferred from lactate to alanine or alanine to lactate. They may, however, relate to different muscle cells since they are heterogeneous with respect to fibre type or to different compartments within the same cells in the muscle studied. On occasion, however, a net uptake of lactate, or more rarely of alanine, can be demonstrated across the tissue as a whole. Clearly then some part of the carbohydrate utilized is accountable as lactate or alanine. Other amino acids such as glutamine or asparagine may also play a similar role, although there is as yet no direct evidence for this.

Adipose tissue. Although non-ruminant adipose tissue has a significant requirement for glucose, both as a source of reducing equivalents ($NADPH_2$) and as a carbon source, it has been generally accepted that glucose is less important than is acetate for ruminant adipose tissue both as a carbon source (Hanson & Ballard, 1967) and perhaps also for reducing equivalents (Bauman *et al.* 1970). However, in two recent studies, both in vivo in sheep (Prior, 1978) and in vitro with tissue from cattle (Whitehurst *et al.* 1978), evidence has been provided suggesting that lactate may be a more effective carbon source for fatty acid synthesis than is glucose. Whitehurst *et al.* observed a greater rate of synthesis of fatty acids from lactate than from acetate, although in terms of oxidation the reverse was true. These findings raise a number of difficulties. In particular, mitochondrial elongation of fatty acids seems unlikely to be the mechanism involved if acetate is more readily oxidized than lactate. On the other hand *de novo* cytosolic synthesis would seem to be limited as is glucose by the activity of ATP-citrate lyase. Further studies will clearly be needed to resolve these difficulties; for the present it is sufficient to recognize that lactate may be a more effective lipogenic substrate than is glucose.

We may now consider more fully the distinction made earlier, of the ways by which carbohydrate may be utilized. The absolute need for glucose is clearly demonstrated only in relation to the formation of lactose. It is perhaps striking that in several other tissues an apparent absolute need for glucose is associated with a continuous formation of fluid (in the testis, in the pregnant uterus and in the central nervous system) although if this is a significant function of glucose the absolute need is likely to be small since in all these cases the rate of fluid formation is relatively low. It is perhaps more likely that the particular significance of glucose is its ability to penetrate tissues more rapidly than other potential glycogenic compounds such as lactate.

The function inherent in the description 'glycogenic' compound is not clearly understood. It could be related to oxidation through the pentose phosphate series of reactions; or perhaps to the synthesis of ribose. However, in view of the generality of need (muscle and brain show very little pentose cycle activity) and the size of the flow it is more likely to be related to the reactions of the tricarboxylic acid cycle. Glucose and glycogenic compounds can function by supplying carbon for the cycle intermediates, in addition to the possible supply of acetyl CoA

through the oxidation of pyruvate, whereas energy sources such as acetate and ketones can supply only acetyl CoA. It is perhaps worth emphasizing that oxidation of glycogenic compounds can occur only through conversion to pyruvate and acetyl CoA, that is, the intermediates function only as catalysts. This makes it difficult to understand why there is a continual demand for the supply of intermediates. In gluconeogenesis this is due to the continual removal of intermediates in the synthesis of glucose. There must therefore be a similar depletion of intermediates in peripheral tissues. The only process that seems likely to be of sufficient magnitude to account for such a depletion is that of the synthesis of non-essential amino acids for protein synthesis. Such reactions would require a continual supply of amino groups. These would presumably be available from amino acids produced in protein degradation. There are some biochemical difficulties in accepting this notion. Nevertheless there is experimental support in that Shipley, *et al.* (1974) found that after injection of ^{14}C-glucose in rats a major fraction of ^{14}C found in rat tissues was in tissue protein. We can obtain some estimate of the amount of glucose used as an energy source in ruminants by estimating the amount of ^{14}C-glucose that appears as CO_2. In non-pregnant (Annison *et al.* 1967; Bergman, 1963) and in pregnant sheep (Bergman, 1963; Ford & Reilly, 1970) about one-third of the glucose appears as CO_2. Thus about two-thirds of the glucose circulating pass through pools with a long turnover time. In lactating cattle, one may calculate from the results of Bickerstaffe *et al.* (1974) and Brown & Lindsay (1966) that only about 18% of the glucose flow appeared as CO_2. However, about 50% could be accounted for as milk lactose. Thus the proportion of glucose passing through pools of long turnover time was in this case only about one-third. In recent experiments we have made with pregnant sheep we have recovered over 60% of glucose as CO_2. Thus, at least in some conditions (pregnancy and lactation), it seems that the amount of glucose passing through pools with a long turnover time can be reduced.

It may now be appropriate to re-examine some of the features of gluconeogenesis in ruminants. In one sense, the rate of glucose synthesis may give us a misleading indication of the amount of carbohydrate available to ruminants. Thus, (1) we know that the amount of propionate produced in the rumen is much more than the amount converted to glucose. The difference arises because substantial amounts are oxidized in the rumen wall (Annison *et al.* 1967). Propionate used in this way must be regarded as meeting a glycogenic function for the rumen wall, and thereby sparing carbohydrate that might otherwise be used. (2) Again we know (Armstrong & Smithard, 1979) that on certain diets a substantial amount of dietary α-glucose polymer escapes fermentation, passes to the duodenum but has substantially disappeared when the digesta reaches the ileum. There is no convincing evidence that a corresponding amount of glucose is absorbed, but even if this is not so, the carbohydrate is probably glycolysed in the intestinal wall and may spare glucose that would otherwise be used by the gut as well as contributing gluconeogenic substrate to the liver. (3) Amino acids catabolized in the wall of the gastrointestinal tract must also act as carbohydrate-sparing sources with

respect to the gut. Those absorbed from the gut, but not removed by the liver may equally spare glucose carbon in peripheral tissues. Thus although, as I have recently emphasized (Lindsay, 1979*b*), amino acids make a small, but limited, contribution to the synthesis of glucose carbon, glycogenic amino acids must be regarded as contributing 'available carbohydrate' to ruminants. Because of this partial 'blending' of glucose and gluconeogenic substrate, we cannot simply describe carbohydrate metabolism in ruminants as concerned with the synthesis of glucose from a number of exogenous precursors, and the outflow of glucose to various products. A significant part of the glucose synthesized is of endogenous origin. Thus when, as in pregnancy and lactation, there is an increased need for glucose as such, this may be met, not only by increasing the supply of exogenous precursors but also by reducing the recycling of endogenous glycogenic substrates.

REFERENCES

Anderson, D. M. (1969). *J. Comp. Path.* **79,** 525.

Annison, E. F., Bickerstaffe, R. & Linzell, J. L. (1974). *J. agric. Sci., Camb.* **82,** 87.

Annison, E. F., Brown, R. E., Leng, R. A., Lindsay, D. B. & West, C. E. (1967). *Biochem. J.* **104,** 135.

Armstrong, D. G. & Smithard, R. R. (1979). *Proc. Nutr. Soc.* (In the Press).

Bauman, D. E., Brown, R. E. & Davis, C. L. (1970). *Archs. Biochem. Biophys.* **140,** 237.

Bergman, E. N. (1963). *Am. J. Physiol.* **204,** 147.

Bickerstaffe, R., Annison, E. F. & Linzell, J. L. (1974). *J. agric. Sci., Camb.* **82,** 71.

Boyd, R. D., Morriss, F. H., Meschia, G., Makowski, E. L. & Battaglia, F. C. (1973). *Am. J. Physiol.* **225,** 897.

Brown, R. E. & Lindsay, D. B. (1966). *Fedn Proc. Fedn Am. Socs exp. Biol.* **25,** 343.

Burd, L. I., Jones, M. D., Simmons, M. A., Makowski, E. L., Meschia, G. & Battaglia, F. C. (1975). *Nature, Lond.* **254,** 710.

Char, V. C. & Creasy, R. K. (1976). *Pediat. Res.* **10,** 231.

Christenson, R. K. & Prior, R. L. (1978). *J. Anim. Sci.* **46,** 189.

Ford, E. J. H. & Reilly, P. E. B. (1970). *Res. Vet. Sci.* **11,** 575.

Hanson, R. W. & Ballard, F. J. (1967). *Biochem. J.* **105,** 529.

Jones, M. D., Burd, L. I., Makowski, E. L., Meschia, G. & Battaglia, F. C. (1975). *Am. J. Physiol.* **229,** 235.

Judson, G. J. & Leng, R. A. (1968). *Proc. Aust. Soc. Anim. Prod.* **7,** 354.

Kempton, T. G., Hill, M. K. & Leng, R. A. (1978). *Proc. Aust. Soc. Anim. Prod.* **12,** 143.

Kempton, T. G., Smith, G. H. & Leng, R. A. (1978). *Proc. Aust. Soc. Anim. Prod.* **12,** 139.

Leng, R. A. & Annison, E. F. (1962). *Aust. J. agric. Res.* **13,** 31.

Lindsay, D. B. (1970). In *Physiology of Digestion & Metabolism in the Ruminant,* p. 438 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Lindsay, D. B. (1979*a*). *Trans. Biochem. Soc.* **6,** 1152.

Lindsay, D. B. (1979*b*). In *ARC Seminar on Protein Metabolism in the Ruminant.* [P. J. Buttery and J. C. Tayler, editors]. Agricultural Research Council (In the Press).

Lindsay, D. B. & Setchell, B. P. (1976). *J. Physiol. Lond.* **259,** 801.

Lindsay, D. B., Steel, J. W. & Barker, P. J. (1977). *Proc. Nutr. Soc.* **36,** 80A.

Lindsay, D. B., Steel, J. W. & Buttery, P. J. (1977). *Proc. Nutr. Soc.* **36,** 33A.

Linzell, J. L. (1974). In *Lactation: a comprehensive treatise. Vol. 1. The Mammary Gland,* p. 143 [B. L. Larson & V. R. Smith, editors]. New York: Academic Press.

Linzell, J. L. & Peaker, M. (1971). *Physiol. Rev.* **51,** 564.

Linzell, J. L. & Setchell, B. P. (1969). *J. Physiol., Lond.* **201,** 129.

Owen, O. E., Morgan, A. P., Kemp. H. G., Sullivan, J. M., Herrera, M. J. & Cahill, G. F. (1967). *J. clin. Invest.* **46,** 1589.

Prior, R. L. (1978). *J. Nutr.* **108**, 926.
Reilly, P. E. B. & Chandresena, L. (1977). *Am. J. Physiol.* **223**, E138.
Setchell, B. P., Bassett, J. M., Hinks, N. T. & Graham, N. McC. (1972). *Q. Jl exp. Physiol.* **57**.
Setchell, B. P., Dawson, R. M. C. & White, R. W. (1968). *J. Reprod. Fert.* **17**, 219.
Setchell, B. P. & Waites, G. M. H. (1964). *J. Physiol. Lond.* **171**, 411.
Shipley, R. A., Gibbons, A. P. & Chudzik, E. B. (1974). *Can. J. Physiol. Pharmacol.* **52**, 797.
Whitehurst, G. B., Beitz, D. C., Pothoven, M. A., Ellison, W. R. & Crump, M. H. (1978). *J. Nutr.* **108**, 1806.

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