with the consequent induction of enzymes involved in fatty acid synthesis, and that any difference between the metabolism of fructose and glucose is comparatively unimportant as a factor determining the hyperlipidaemic effect of sucrose.

To conclude, I will return to the species with which I began. The few sucrose or fructose tolerance tests that have been conducted on human volunteers (Butterfield, Sargeant & Whichelow, 1964; Swann, Davidson & Albrink, 1966) have shown that neither of these sugars, given by mouth, produced as great a rise in blood reducing sugars as did glucose or 'liquid glucose' (partially hydrolysed starch); the increase in hexose sugars in the blood after fructose administration was less than half that found with glucose or 'liquid glucose', and approximately half of this increase was due to fructose. Furthermore, a significant rise in blood pyruvate was observed with both sucrose and fructose, which may be taken as an indication of augmented throughput of sugars in the Embden–Meyerhof pathway in the liver.

It appears, therefore, that in man, as in the rat, a substantial part of ingested sucrose does not reach the systemic blood as monosaccharides but is diverted by the liver, presumably for fat synthesis, and that the hyperlipidaemia encountered in human volunteers consuming sucrose-rich diets may also be explained on this basis.

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Glucose metabolism in the ruminant

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The metabolism of the tissues of the ruminant, in general, has been the subject of an excellent review by Ballard, Hanson & Kronfeld (1968). In this paper particular attention will be paid to the glucose metabolism of the lactating udder, since the

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glucose economy of the entire animal is dominated by the udder's requirements. Thus, for example, Annison & Linzell (1964) showed that the udder accounted for 60–85% of the total glucose utilization by lactating goats. Between 16–34% of the glucose taken up by the udder was oxidized and this represents 29–49% of the CO_2 produced by this organ. In contrast, the contribution of glucose to the CO_2 produced by the animal as a whole was only 7–14%. This suggests that, in the udder, glucose makes a major contribution to the energy supply—in contrast to other tissues of the ruminant where oxidation of acetate fills this role. On the other hand, other work does not seem to support this conclusion (see, for example Rook & Hopwood, 1970). What follows is an examination of some of the issues involved in this apparent contradiction.

It is well recognized that a supply of glucose is essential for the continued function of the lactating mammary gland. For example, the omission of glucose from the fluid perfusing an isolated udder leads to a rapid cessation of the secretion of milk (Hardwick, Linzell & Mepham, 1963). Similarly, hypoglycaemia, brought about in lactating goats and cows through the administration of insulin, results in a depression of milk yield (McClymont, 1951; Rook & Hopwood, 1970; Kronfeld, Mayer, Robertson & Raggi, 1963).

These effects upon the secretion of milk *per se* are, however, largely accounted for through the cessation of lactose production, since it is the osmotic pressure of this constituent which regulates the uptake of water for the formation of milk fluid; and, in fact, the synthesis of fat and protein tends to continue (Rook, Storry & Wheelock, 1965). Hence a shortage of energy need not be inferred.

The role of glucose in the production of lactose is not in doubt: plasma glucose is the major, if not the sole, precursor of milk lactose (Barry, 1964) and it has been estimated that 62% of the glucose utilized by cow mammary tissue in vitro is diverted to lactose production (Wood, Peeters, Verbeke, Lauryssens & Jacobson, 1965). Indeed, lactose production itself can account for one-half of the total circulating glucose in the goat (Annison & Linzell, 1964). It is, therefore, the role of the remainder of the glucose used by the gland which is uncertain.

A well recognized and major metabolic difference between the ruminant and non-ruminant is the failure of carbon from glucose to contribute to fatty acid synthesis within the tissues of a ruminant, including the mammary gland. For example, less than 0·5% of milk fatty acids were shown to arise from labelled glucose in the isolated, perfused, goat udder (Hardwick *et al.* 1963), and the rate of synthesis of fatty acids from glucose in slices of goat mammary gland was about one-tenth of that of acetate (McCarthy, 1970). Hanson & Ballard (1967) demonstrated that slices of adipose tissue and liver from sheep and cows behaved similarly.

This phenomenon is usually accounted for by the low activity of ATP citrate lyase (*EC* 4.1.3.8) and malate dehydrogenase (decarboxylating) (NADP) (*EC* 1.1.1.40) in ruminant tissues (Hardwick, 1966; Hanson & Ballard, 1967). These are enzymes of the 'citrate cleavage' pathway which in non-ruminants transports two-carbon units from the mitochondria where they are formed from glucose to the site of fatty acid synthesis in the cytoplasm (Fig. 1). Alternative precursors of

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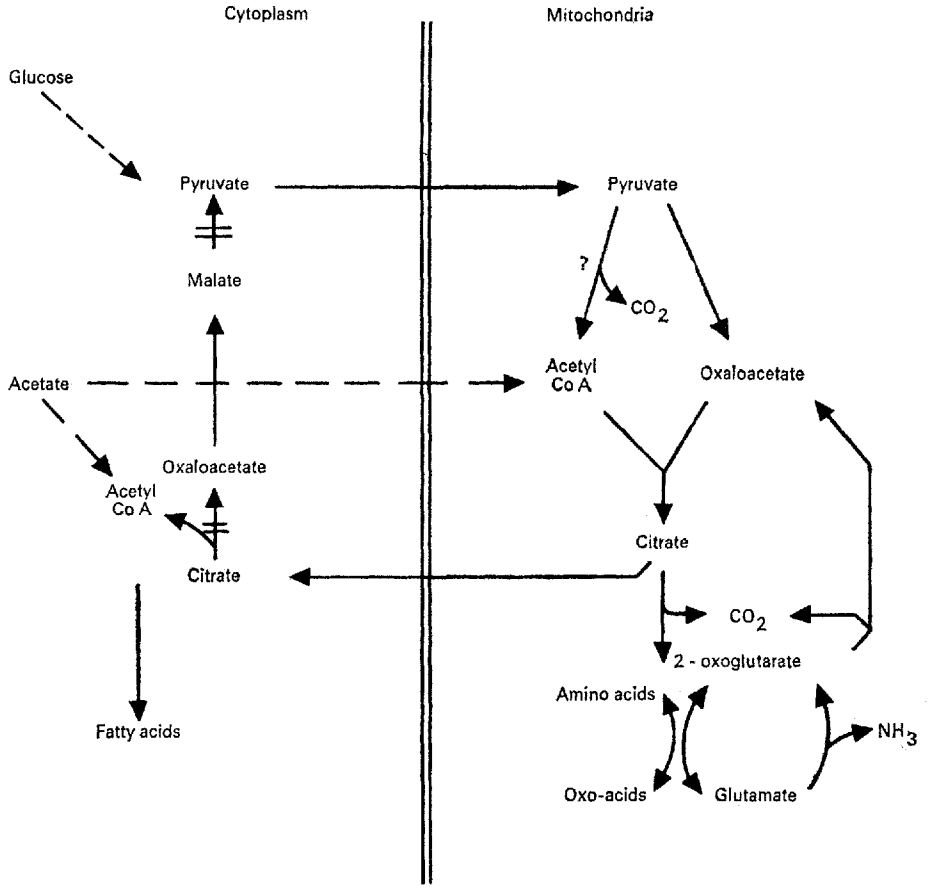

Fig. 1. Pathways of oxidation and lipogenesis for glucose and acetate. —— || ——, pathways not occurring in ruminant tissue.

fatty acids are available to the ruminant in the form of plasma β-hydroxybutyrate, and acetate which may be converted to acetyl-CoA by acetyl-CoA synthetase (*EC* 6.2.1.1) present in the cell cytoplasm (Hanson & Ballard, 1967).

The enzymes of the cleavage pathway are still present in the ruminant, albeit at low levels and, therefore, the genetic information for their synthesis is still available. The mechanism which leads to the repression of their synthesis has not so far been elucidated. A further control of this pathway may be exerted through the inhibition of ATP citrate lyase by acetyl-CoA (Chesworth & Smith, 1971). In certain circumstances therefore, both types of inhibition of the pathway could be removed, which may account for the observation that some glucose appeared to be used for lipogenesis after a long-term infusion of glucose into a fed sheep (Lindsay, 1970).

There is no doubt, however, that glucose plays an important role through the provision of reducing equivalents for fatty acid synthesis in the form of reduced NADP. One source of this co-enzyme – the oxidation of malate to pyruvate – is not available to the ruminant. Another source is NADP-specific isocitrate dehydrogenase

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(*EC* 1.1.1.42), but though this may be important for fatty acid synthesis from acetate when glucose is absent (Bauman, Brown & Davis, 1970) it seems certain that the major part of reducing equivalents arise in the ruminant mammary gland from the oxidation of glucose through the pentosephosphate pathway. It seems, moreover, that there is considerable interdependence between the rate of operation of the pentosephosphate pathway and the rate of fatty acid synthesis (Lindsay, 1970). This may account for the observation that the operation of the pentose pathway was more dependent upon the presence of acetate in sheep tissue than in rat tissue (Smith & Glascock, 1969). In the rat, unlike the sheep, lipogenesis may still occur from glucose in the absence of acetate.

In this sense therefore, glucose certainly provides energy (in the form of reducing equivalents) for fatty acid synthesis. Does it also provide energy in the form of ATP through the operation of the tricarboxylic acid cycle? Hardwick *et al.* (1963) concluded from figures produced by Black, Kleiber, Butterworth, Brubacher & Kaneko (1957) that there was extensive entry of glucose into the tricarboxylic acid cycle. A similar conclusion was reached by Smith & Glascock (1969). Certainly, the supposition that the primary block in glucose conversion to fatty acids in the ruminant lies in the absence of ATP citrate lyase allows the conclusion that glucose can contribute to acetyl-CoA in the mitochondria.

What proportion, therefore, of the CO_2 which arises from glucose represents oxidation in the tricarboxylic acid cycle? Methods that have been used to determine the proportion of glucose metabolized by the pentose pathway have been extensively reviewed by Katz & Wood (1960) and Landau & Katz (1965); however, when these methods are used, what is determined, in fact, is the proportion of triosephosphates formed from glucose which have arisen via the pentosephosphate pathway as alternative to the Embden–Meyerhof–Needham pathway (Fig. 2). Different authors use different definitions of the proportional occurrence of the pathways. For example, Wood *et al.* (1965) define the relative participation of the pathways as the proportion of glucose catabolized to triose by each of the two routes. Since one-half of the glucose so catabolized in the pentosephosphate pathway appears as CO_2, one would calculate from the figures produced by these authors (62% of glucose to lactose, 30% to pentosephosphate pathway and 8% to Embden–Meyerhof–Needham pathway) that 15% of the glucose had appeared as CO_2 by this route. But these calculations do not provide information as to the subsequent fate of the triosephosphates produced by either route: these may be used for synthesis, (for example of glycerol, serine or alanine) and are not necessarily oxidized.

A further possible fate of the triosephosphates, which has been deliberately excluded from the model systems used, is their recombination to reform hexosemonophosphate (Fig. 2). This seemed to be justified in most tissues, where recombination did not occur to any large extent (Katz & Wood, 1960). However, should this pathway occur it would have three consequences:

(1) it would result in an underestimation of participation of the pathway as defined above in methods based on the use of [1–^{14}C]- and [6–^{14}C]-glucose (e.g. Smith & Glascock, 1969). Methods based on randomization of [2–^{14}C]glucose (e.g. Wood

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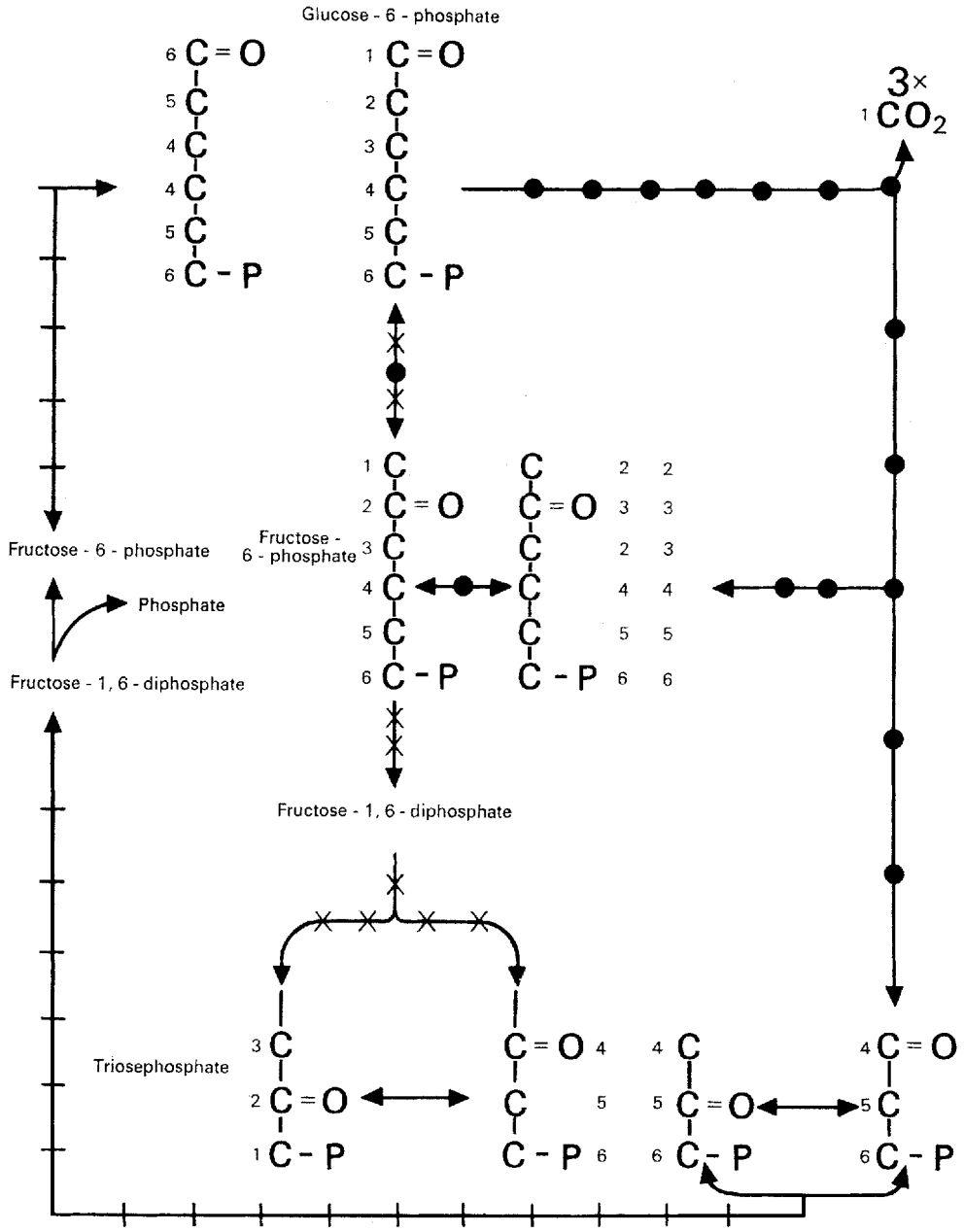

Fig. 2. Pathways of glucose metabolism. (For simplicity some possible randomizations of carbon atoms are omitted.) ——×——, Embden–Meyerhof–Needham pathway; ——●——, pentosephosphate pathway; —— | ——, recombination of trioses.

et al. 1965) would be unaffected; (2) it would lead to the possibility that more than one-half of the glucose calculated to be catabolized by the pathway would appear as CO_2 (even when this calculation itself is unaffected); (3) since carbon atom 6

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of glucose would be randomized with carbon atom 1, it would invalidate the conclusion (e.g. Smith & Glascock, 1969) that $^{14}CO_2$ from [6–^{14}C]glucose has arisen in the tricarboxylic acid cycle.

Is it justifiable to assume that recombination of triosephosphates does not occur in tissues of the ruminant, in particular the mammary gland? The absence of the pathway in the non-ruminant tissues studied, particularly adipose tissue, is almost certainly due to low activity of fructose-1, 6-diphosphatase. This enzyme, is however, a key enzyme of gluconeogenesis and may well be expected to be more active in ruminant tissues. The extent of gluconeogenesis (in the sense of the synthesis of glucose from non-glucose material – such as, for example, amino acids) in the mammary gland is uncertain (*vide infra*), but it is significant that fructose-1,6-diphosphatase is active in the mammary gland of the cow (Baird, 1969), and that author has suggested that its role may be specifically to allow recombination of the triosephosphates formed by the pentosephosphate pathway. Such a recombination does, in fact, occur in the udder as shown by the experiments of Wood *et al.* (1965), where C_1 of the galactose moiety of lactose produced from [6–^{14}C]glucose was one-fifth as active as C_6.

Calculations of the proportion of glucose catabolized to triose by the pentose-phosphate pathway are, therefore, of no use in deciding upon the origin of the CO_2 produced unless the extent of recombination is known. Furthermore, the apparent participation of the tricarboxylic acid cycle may have been overestimated.

The remaining question to receive consideration is whether all of the CO_2 arising from glucose, after the true extent of oxidation by the pentosephosphate pathway is subtracted, does, in fact, represent net oxidation of glucose.

Pyruvate formed from glucose may enter the tricarboxylic acid cycle by two routes; by oxidative decarboxylation of acetyl-CoA or by carboxylation to oxaloacetate (Fig. 1). Acetyl-CoA arising from exogenous acetate in the ruminant may divert pyruvate preferentially towards oxaloacetate rather than towards oxidation, since it inhibits the pyruvate dehydrogenase complex and activates pyruvate carboxylase (*EC* 6.4.1.1). If the rate of oxidation of acetyl-CoA in the cycle is high compared with the rate of entry of oxaloacetate, most of the labelled carbon which enters in this way will appear as CO_2, although there has, in fact, been no net oxidation since oxaloacetate is reformed in the cycle.

Hardwick (1966) showed that glucose contributed to both acetyl- and oxaloacetate-derived parts of the citrate molecule in perfused goat udder. We have, however, recently re-examined this problem using an intact goat (Chesworth & Smith, 1971). The specific activity of the oxaloacetate portion of milk citrate following intravenous infusion of [1–^{14}C]acetate was slightly less (by about 10%) than that of the acetyl portion, indicating that entry of unlabelled materials into the cycle other than through acetyl-CoA (from pyruvate or glucogenic amino acids) was small compared with the rate of oxidation of acetyl-CoA. After [U–^{14}C]glucose infusion, however, the specific activity of the oxaloacetate portion was eight to nine times that of the acetyl portion. Taken together these results may be interpreted as showing that the rate of entry of glucose carbon into the tricarboxylic acid cycle

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is low compared with acetyl-CoA, and that most of that which enters does so as oxaloacetate.

Since the entry of radioactivity from pyruvate to oxaloacetate probably represents net synthesis rather than exchange, the entry of glucose into the cycle and, therefore, the apparent oxidation accounted for by this route will correspond to the amount necessary to balance losses of intermediates from the tricarboxylic acid cycle (Heath, 1968). The extent of the utilization of tricarboxylic acid cycle intermediates for amino acid synthesis or, conversely, the extent of entry of intermediates from amino acid deamination is difficult to assess. Certainly there is transfer of radioactivity from tricarboxylic acid cycle intermediates to amino acids (Raafat, Verbeke & Peeters, 1963), but owing to the existence of the coupled transamination system (Fig. 1) an exchange of label between α-ketoglutarate and glutamate is possible, and this does not allow the estimation of net synthesis from these figures. More useful are the balance studies between uptake of amino acids and the plasma and their appearance in milk (Linzell & Mepham, 1968). The deficiency of non-essential amino acids observed in their experiments would, after allowance is made for synthesis from surplus arginine and orthinine, require about 4% of the milk protein to be synthesized from the tricarboxylic acid cycle. When the requirement for milk citrate synthesis is also taken into account one may conclude that there must be a small but positive entry of oxaloacetate into the cycle from pyruvate, which will carry in label from [^{14}C]glucose.

In summary, it is suggested that the extent of oxidation of glucose other than through the pentosephosphate pathway in the ruminant mammary gland may be less than the work examined, using radioisotopes, appears to indicate. How reasonable would it be to suggest that there is no other oxidation?

If one assumes that all of the milk lactose arises from glucose, that the amount of glucose oxidized by the pentosephosphate pathway is limited to the amount necessary to provide reducing equivalents for the fatty acid synthesis which occurs within the gland, and that the only other use of glucose is to 'top up' the tricarboxylic acid cycle as described above then, on the basis of an average milk composition (goat), one may estimate that 73% of the glucose is used for lactose, 23% oxidized in the pentosephosphate pathway and 4% used to 'top up' the cycle. The amount apparently oxidized will represent the sum of the last two values, or 27%. This amount is compatible with the amount of 25% for the extent of oxidation of glucose in the lactating goat udder (Annison & Linzell, 1964) and hence even the extreme conclusion, that no net oxidation of glucose occurs in the tricarboxylic acid cycle, is not impossible.

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Changes in the pattern of glucose metabolism in growth, pregnancy and lactation in ruminants

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The carbohydrate needs of pregnancy and lactation are of particular interest in ruminants because of their dependence on gluconeogenesis. The rate of gluconeogenesis (glucose entry rate) can therefore be determined by isotope dilution in fed or fasted ruminants. In sheep starved for 6 d the rate is about 120–140 mg/h per $kg^{0.75}$, which compares closely to values in fasted non-ruminants (Cowan, Vranic & Wrenshall, 1969; Owen, Felig, Morgan, Wahren & Cahill, 1969). The increased rate in fed ruminants may be related to either digestible energy (DE) or to protein intake (Judson & Leng, 1968), but over a wider range of rations and animals there is a much better relationship with DE than with protein intake (Lindsay, 1970). This is probably because the DE intake determines the rate of volatile fatty acid production and thus, also, the rate of synthesis of microbial protein, which probably accounts for most of the protein available to ruminants (Leng, 1970). It is assumed that the major precursors of glucose are propionate and glycogenic amino acids.

In pregnant sheep, Steel & Leng (1968) showed that the glucose entry rate is determined more by the food intake than by the stage of pregnancy. It is possible that on a fixed ration there is a slight increase in the glucose entry rate as pregnancy develops, but such increase is small compared with the effect of increased feed intake. When ewes were offered lucerne *ad lib.* they increased their intake markedly in late pregnancy; glucose entry rate was increased but not as much as might be

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