Glucose Utilization in Sheep

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In ruminants only small quantities of glucose are absorbed from the alimentary tract, most of the dietary energy being supplied by short-chain volatile fatty acids produced by the fermentation of ingested carbohydrates in the rumen. In addition, blood glucose concentrations in ruminants are considerably lower than those of non-ruminants, and ruminants are relatively insensitive to insulin. These major differences in carbohydrate metabolism have led to considerable speculation on the role of glucose in ruminant metabolism (Lindsay, 1959), but in the present studies measurements of glucose-utilization rates in sheep have demonstrated that glucose is quantitatively only slightly less important in this species than in non-ruminants.

Isotope-dilution techniques, which provide the only satisfactory methods for the measurement of turnover rates in the intact animal, have been used to determine glucose-utilization rates in rats (Feller, Strisower & Chaikoff, 1950; Baker, Shipley, Clark & Incefy, 1959), man (Baker, Shreeve, Shipley, Incefy & Miller, 1954) and dogs (Searle, Strisower & Chaikoff, 1954; Steele, Wall, de Bodo & Altszuler, 1956). Studies of ruminants, however, have been limited to those of Baxter, Kleiber & Black (1955) and Kronfeld, Tombropoulos & Kleiber (1959), who used single-injection procedures to measure glucose-turnover rates in cows. In the present investigation glucose-utilization rates in fed and starved sheep have been determined by isotope dilution with the constant-infusion method of Steele *et al.* (1956). The general procedure employed was similar to that described earlier for the measurement of acetate-utilization rates in sheep (Annison & Lindsay, 1961). Estimates of glucose-pool size and glucose 'space' (the volume of distribution of the glucose pool) were also obtained. In some experiments these parameters of glucose metabolism were measured by isotope dilution with a single-injection procedure, but utilization rates determined by this method were significantly higher than those obtained by constant infusion of ^{14}C -labelled glucose.

The effects of glucose load on the entry of endogenous glucose were examined by measuring utilization rates at high concentrations of plasma glucose. Entry of glucose appeared to be markedly inhibited under these conditions.

MATERIALS AND METHODS

Experimental animals. Twelve Merino ewes, age 2–3 years, were randomly divided into two groups of six animals, each of which was fed on one of the following diets: 400 g. of lucerne chaff (diet 1); 400 g. of lucerne chaff and 500 g. of maize (diet 2). The animals, which were housed indoors, were fed once daily and trained to eat their food within 2 hr. After transfer to new diets animals were allowed a period of 2–3 months to adjust to the new feeding régime before being used for experiments. All animals fed with diet 1 lost weight at an average rate of 0.2 kg./week during this time. This loss of weight continued at approximately the same rate for a further 3–4 months in half of the group, the remainder reaching equilibrium during this period. The second group (diet 2) gained weight at about 0.3 kg./week in the adjustment period, but subsequently showed variable weight changes which did not exceed 0.5 kg./week.

To accustom the animals to the conditions of the experiments they were restrained in the cage used for the infusion experiments for several hours each day over a period of 3–4 weeks. Experiments on fed animals were started 2 hr. after the end of the feeding period, and starved animals were not fed on the day of the experiment.

Experimental procedure. The general procedure described earlier (Annison & Lindsay, 1961) was used, with some modifications. Constant infusion (0.3 ml./min.) of labelled glucose was maintained with a continuous-injection apparatus (Palmer Instruments Ltd., London). Since utilization rates can only be measured under steady-state conditions, i.e. when the plasma glucose concentrations remain reasonably constant, care was taken to minimize disturbance of the animals before and during experiments. For this reason the polyethylene catheters were inserted into the jugular veins on the day before an experiment. Experiments were rejected if the plasma glucose concentration varied by more than $\pm 10\%$ of the mean value observed during the sampling period.

Radioactive glucose. Uniformly labelled D- ^{14}C glucose was obtained from The Radiochemical Centre, Amersham, Bucks. Aqueous solutions of labelled glucose were prepared (50 μC /mg./ml.) and stored at -10° until diluted in 0.1% glucose immediately before use. The radioactivity of each batch of labelled glucose was checked by wet oxidation of a sample to barium carbonate.

Chemical determinations

Determination of plasma glucose. The erythrocytes of mature sheep usually contain little or no glucose (Reid, 1953) and examination of the animals available to us in this laboratory indicated that the ratio plasma glucose concentration : erythrocyte glucose concentration was in most

cases at least 20:1. Erythrocyte glucose concentrations were calculated from the values obtained for whole blood and plasma, and from the haematocrit value. There is considerable uncertainty about correction factors for trapped plasma in haematocrit determinations in sheep (Turner & Hodgetts, 1959) and it is correspondingly difficult to decide whether or not glucose is present in the erythrocyte in low concentration or is absent. For this reason the experimental work was based on plasma glucose. This procedure also avoided possible variations in blood glucose concentration caused by the rapid release of erythrocytes from the spleen after even slight disturbance of the animal (Turner & Hodgetts, 1959). The specific glucose-oxidase procedure of Huggett & Nixon (1956) was used to estimate plasma glucose concentrations. Heparin was used as anticoagulant, and sodium fluoride (25 mg.) was added to each 10 ml. of blood to inhibit glycolysis.

Assay of labelled glucose in plasma. Two methods were used to determine the specific activity of plasma glucose. In the early part of the investigation, when only an end-window Geiger-Müller tube was available for radioactivity measurements, the periodate-oxidation procedure of Dunn, Friedmann, Maas, Reichard & Weinhouse (1957) was used. When more sensitive radioactive counting facilities became available (gas-flow counter) glucose was isolated from plasma as the osazone, oxidized to CO_2 by the method of Van Slyke & Folch (1940) and counted as barium carbonate. The two methods gave closely similar results when used to assay radioactive glucose of known specific activity contained in aqueous solution or in plasma. Although the periodate-oxidation procedure is more time-consuming and less specific than the osazone method, an important advantage of the technique is its greater sensitivity. This is in consequence of the much smaller amounts of added carrier glucose necessary in the periodate method, and from the inevitable dilution of glucose carbon with carbon from phenylhydrazine in the osazone derivative. The periodate-oxidation assay thus permits the use of lower concentrations of [^{14}C]glucose in the infusion experiments, which results in an important saving in costs when experiments with large animals are involved.

(i) Periodate oxidation of plasma glucose. The method of Dunn *et al.* (1957) was used, with some modifications. Carrier glucose (4 mg.) was added to plasma (5 ml.), which was diluted with water (5 ml.) before deproteinization with 0.3N-barium hydroxide (7.5 ml.) and 0.3N-zinc sulphate (7.5 ml.). Portions of the filtrate (10 ml.) were oxidized in an all-glass apparatus. Aeration of the acidified solution at room temperature to remove CO_2 before the oxidation of formate was found to be inadequate, but satisfactory results were obtained when filtrates were aerated at 90–95° for 15 min. Recoveries of CO_2 by this method were 95–100% of the theoretical yield. The periodate-oxidation method is not specific for glucose, a possible major source of error in some circumstances being the decarboxylation of aliphatic hydroxy and keto acids by mercuric oxide during the conversion of formic acid into CO_2 . To avoid errors from this source, samples of plasma obtained during each experiment were assayed with the omission of the periodate-oxidation stage, and the results used to correct those obtained after the complete oxidation procedure. Control experiments established that $^{14}\text{CO}_2$ was not obtained from labelled glucose if the periodate-oxidation step was omitted.

(ii) Isolation and oxidation of plasma glucose as osazone. Carrier glucose (20 mg.) was added to plasma (5 ml.), which was deproteinized by pouring into ethanol (20 ml.). After removal of precipitated protein, the filtrate was concentrated, by heating at 50–60° in a stream of air, to a volume of 4–5 ml. and the glucosazone prepared. The osazones were recrystallized twice from the system pyridine–water (1:4, v/v).

The osazones (2–3 mg.) were oxidized by the procedure of Van Slyke & Folch (1940), the liberated CO_2 being absorbed in 2 ml. of CO_2 -free 0.5N-sodium hydroxide. After the addition of 1 ml. of 5% ammonium chloride, 0.2 ml. of 10% barium chloride was added and the precipitated barium carbonate isolated and assayed for radioactivity.

Assay of specific activity of blood carbon dioxide. The method described by Annison & Lindsay (1961) was used.

Assay of radioactive barium carbonate. Radioactive barium carbonate was collected by filtration on Whatman no. 542 paper circles with an apparatus which eliminated the transfer of papers and precipitates to separate planchets. The paper circles were held on perforated-brass planchets by a stainless-steel Gooch-type funnel and the planchets then screwed directly on to filter stems. Weights of barium carbonate in the range 10–25 mg. were usually employed, but satisfactorily reproducible results were obtained down to weights of 5 mg. (2 mg./cm.²). The barium carbonate samples were assayed for radioactivity with an end-window Geiger-Müller tube or with a gas-flow counter fitted with a Micromil window (Nuclear-Chicago Corp., model D-47). Counts were corrected for self-absorption by the procedure of Hendler (1959), in which results are corrected to a standard weight of barium carbonate (15 mg.). Calibrated samples of sodium [^{14}C]carbonate and barium [^{14}C]carbonate obtained from Tracerlab Inc., U.S.A., were used as absolute standards.

Measurement of utilization rate. The term utilization, as used in this paper, has been defined earlier (Annison & Lindsay, 1961). In the studies with acetate (Annison & Lindsay, 1961) it was found unnecessary to use a priming dose of isotope (Steele *et al.* 1956) since the continuous infusion of labelled acetate gave rise to constant specific activities of plasma acetate after 30–60 min. With glucose, however, steadily increasing specific activities of plasma

Fig. 1. Plasma glucose specific activity in a typical experiment on a starved sheep. Priming dose, 15.4 μC ; infusion rate 0.17 $\mu\text{C}/\text{min}$. ●, Specific activity of plasma glucose; ▲, plasma glucose concentration.

Table 1. *Glucose-utilization rates in starved sheep measured by infusion and single-injection (S.I.) techniques*

Sheep no.	Date	Diet	Technique	Average concn. of plasma glucose (mg./100 ml.)	Glucose space (% of body wt.)	Glucose utilization (mg./min./kg.)
47	10. ix. 59	2	S.I.	85	20	2.2
47	24. ix. 59	2	S.I.	85	19	2.1
8816	12. iv. 60	2	S.I.	75	17	3.1
9397	12. x. 60	1	S.I.	70	16	2.5
10	13. x. 60	1	S.I.	71	18	2.8
58	13. x. 60	1	S.I.	72	16	3.1
47	22. ii. 60	1	Infusion	60	23	1.6
8816	24. ii. 60	2	Infusion	70	17	1.4
8816	2. iii. 60	2	Infusion	50	22	1.2
8816	10. iii. 60	2	Infusion	60	22	1.8
8816	16. ix. 60	1	Infusion	48	19	1.2
9397	25. x. 60	1	Infusion	73	21	1.5
10	18. viii. 60	2	Infusion	56	25	1.6
58	8. iii. 60	1	Infusion	41	24	1.1
58	18. x. 60	1	Infusion	75	22	1.1
51	11. xi. 59	*	Infusion	54	25	1.8
51	26. ii. 60	2	Infusion	73	21	1.7
51	4. iii. 60	2	Infusion	45	27	1.4
9	6. vii. 60	2	Infusion	57	30	1.8

* Diet: 400 g. of lucerne and 250 g. of maize/day.

glucose were obtained during the infusion of labelled glucose, as expected from the results of Steele *et al.* (1956) with dogs. When a priming dose was used, however, and specific activities of plasma glucose were plotted against time, in most experiments a straight-line plot was observed over the period 90–150 min. after the start of the infusion. This line was usually horizontal (Fig. 1), but occasionally showed a slight positive or negative slope. It was found that a priming dose of $15 \mu\text{C}$ of labelled glucose followed by the infusion of labelled isotope at $0.17 \mu\text{C}/\text{min.}$ was suitable.

In the single-injection experiments, blood samples were taken at 5 min. intervals after the injection of 50 or $100 \mu\text{C}$ of labelled glucose, and the specific activity of plasma glucose was plotted against time on a semi-logarithmic scale. In the calculation of utilization rate first-order reaction kinetics were assumed to apply (Dunn *et al.* 1957).

Glucose-utilization rates were calculated as mg./min./kg. body wt. (mg./min./kg.). A factor to be borne in mind when utilization rates measured in different species are compared on the basis of body weight is that in sheep a relatively larger proportion of body weight is represented by the alimentary tract than in some non-ruminants, e.g. dog.

RESULTS

Glucose utilization in starved sheep

The results obtained with the constant-infusion and single-injection techniques in starved (24 hr.) sheep are summarized in Table 1. Significantly higher results were given by the single-injection method, the mean value of six experiments being 2.9 ± 0.18 (s.e.) mg./min./kg. (range 2.1–3.1). Corresponding values for utilization rate by the continuous-infusion techniques showed a mean for

Table 2. *A comparison of results obtained for sheep in the present studies with those for starved dogs (Steele, Wall, de Bodo & Altszuler, 1956)*

Subject	No. of experiments	Mean value and range of body wt. (W) (kg.)	Mean value and range of utilization rate (g./hr./W ^{0.6})
Fed sheep	7	36.6 (31.4–44.0)	0.68 (0.51–0.91)
Starved sheep	13	33.5 (24.8–41.5)	0.37 (0.26–0.48)
Starved dogs	10	19.1 (13.8–24.1)	0.49 (0.42–0.67)

13 experiments of 1.5 ± 0.07 mg./min./kg. (range 1.1–1.8). It must be noted that average plasma glucose values were somewhat higher in the single-injection experiments than those observed in the continuous-infusion studies (Table 1), but since similar experimental techniques were used in both series of experiments these differences were presumably fortuitous. Some correlation between utilization rate and plasma glucose concentration was observed (Fig. 4) but a comparison of utilization rates measured in the same animals (sheep 47, 8816 and 58, Table 1) by both techniques suggests that the higher values obtained by single injection cannot be fully accounted for by differences in plasma glucose concentration. Lower values for glucose space were obtained by single injection (mean 18%, range 16–20%) than by constant infusion (mean 23%, range 17–30%).

When calculated in terms of metabolic body weight (Brody, 1945), utilization rates in starved sheep were about 75 % of those obtained from infusion experiments on starved dogs (Steele *et al.* 1956), as shown in Table 2.

There was no obvious correlation between diet and glucose turnover in starved sheep (Table 1) although the total number of experiments was too small for adequate comparison in view of the wide range of values obtained on each diet.

Glucose utilization in fed sheep

Plasma glucose concentrations were found to be sufficiently constant in the period 2–5 hr. after the end of feeding to allow glucose utilization to be measured by constant infusion. The specific activity of plasma glucose reached a plateau value after about the same time interval (90–100 min.) as with starved sheep (Fig. 2). Considerably higher entry rates were observed after feeding (Table 3) with a mean value of $2.6 \pm 0.2(7)$ mg./min./kg. (range 2.1–3.4) and plasma glucose values were significantly higher than in starved (24 hr.) animals on similar diets (Table 1). Measurements, with one exception, were made on animals fed with the high-starch diet (2), which would be expected to yield high glucose-entry rates.

Fig. 2. Plasma glucose specific activity in a typical experiment on a fed sheep. Priming dose, 15.0 μ c; infusion rate 0.17 μ c/min. ●, Specific activity of plasma glucose; ▲, plasma glucose concentration.

Effects of glucose load on utilization rate: The constant-infusion technique allowed the plasma glucose concentration to be raised by including carrier glucose with labelled glucose in the infusion solution. The results of a typical experiment are shown in Fig. 3, and the results of five experiments on starved sheep are summarized in Table 4. At enhanced concentrations of plasma glucose the measured glucose-utilization rates were only slightly higher than, or identical with, the entry rate of unlabelled glucose. In addition, the specific activity of plasma glucose in the period after the cessation of infusion of glucose was largely unchanged (Fig. 3). A wide range of values for glucose space was obtained during these experiments (Table 4).

Relationship of plasma glucose concentration to utilization rate. When the utilization rates obtained in all continuous-infusion experiments on fed and starved sheep were plotted against the corresponding plasma glucose values (Fig. 4) some dependence of utilization rate on glucose concentration was clearly evident. A line corresponding to the equation $y = 0.0273x + 0.04$ was found to be significant ($P < 0.01$). This linear relationship was more apparent when the results obtained with any single animal were considered, and six values obtained with one sheep (8816) are also shown in Fig. 4.

Glucose utilization on fasting. Measurements were made on two sheep in the immediate post-feeding period, and 24 and 48 hr. after feeding. A steady decline in plasma glucose value and utilization rate was observed in each case (Table 5).

Specific activity of blood carbon dioxide. Samples of venous blood were assayed at intervals during the infusion of labelled glucose and the results of a typical experiment are shown in Fig. 3. The specific activity of the CO_2 continued to rise throughout the infusion period.

A rough estimate of the contribution of glucose to overall oxidative metabolism was obtained by comparing the average specific activity of CO_2 with that of the specific activity of plasma glucose during the period of constant glucose specific

Table 3. Glucose-utilization rates measured after feeding

Sheep no.	Date	Diet	Concn. of plasma glucose	Glucose space	Utilization rate
			(mg./100 ml.)	(% of body wt.)	(mg./min./kg.)
8816	27. iv. 60	2	99	30	3.1
8816	29. iv. 60	2	77	29	2.4
51	16. iii. 60	2	79	30	3.4
9	5. vii. 60	2	99	19	2.2
9427	21. vi. 60	2	110	30	3.2
10	17. viii. 60	2	61	29	2.1
58	16. vi. 60	1	85	22	2.6

activity. In starved sheep the specific activity of blood CO_2 carbon was within the range 11–19% (five experiments) of that of plasma glucose carbon. Two experiments with fed animals gave values of 22 and 23%. In a further two experiments in which carrier glucose was infused with

Fig. 3. Effects of glucose load on endogenous entry of glucose. Priming dose, $15.4 \mu\text{C}$; infusion rate, $132 \text{ mg.}, 0.66 \mu\text{C/min.}$ Specific activity of infused glucose $5.0 \mu\text{mc/mg.}$; average specific activity of plasma glucose, $5.0 \mu\text{mc/mg.}$ ●, Specific activity of plasma glucose; ▲, plasma glucose concentration; ○, specific activity of blood CO_2 .

Fig. 4. Relationship between glucose-utilization rate and plasma glucose concentration, data obtained from all constant-infusion measurements on fed and starved sheep being used. ●, Starved sheep; ▲, fed sheep. Symbols with horizontal line (e.g. —●—) refer to sheep 8816.

Table 4. *Inhibition of entry of endogenous glucose at high concentrations of plasma glucose*

Sheep no.	Concn. of plasma glucose (mg./100 ml.)	Glucose space (% of body wt.)	Rate of infusion of unlabelled glucose (A) (mg./min.)	Total entry rate of glucose (B) (mg./min.)	Utilization rate (mg./min./kg.)	Entry rate of endogenous glucose (B - A) (mg./min.)
51	193	16	132	138	3.2	6
8816	154	20	132	132	4.0	0
9397	127	48	66	177	5.9	111
58	183	12	66	76	2.4	10
2589	254	10	132	158	5.6	26

Table 5. *Glucose-utilization rates and glucose spaces measured 4–5 hr. (A), 24 hr. (B) and 48 hr. (C) after feeding*

Sheep no.	Time interval	Body wt. (kg.)	Average concn. of plasma glucose (mg./100 ml.)	Glucose space (% of body wt.)	Utilization rate (mg./kg./min.)
9	A	44.0	99	19	2.2
	B	41.5	57	30	1.8
	C	40.7	45	26	1.2
10	A	38.3	61	29	2.1
	B	36.2	56	25	1.6
	C	38.5	54	22	1.2

labelled glucose at a rate of 132 mg./min., glucose made a greater contribution to overall oxidation, values of 33 and 36% being obtained.

DISCUSSION

Glucose-utilization rates, which measure the rate of entry into or removal from the glucose pool, are a quantitative estimate of the contribution of glucose to the overall metabolism of the animal. The magnitude of the rates determined in the present investigation, which were equivalent to an entry rate of 90 g./day for a starved sheep of 40 kg. body wt., indicate the central importance of glucose in sheep metabolism.

Only small amounts of glucose are absorbed from the alimentary tract of sheep (cf. Annison & Lewis, 1959) and the origin of the endogenous glucose entering the body pool is of considerable interest (Lindsay, 1959). Propionate is undoubtedly a major precursor, but the available estimates of propionate absorption from the alimentary tract of sheep (Annison, Hill & Lewis, 1957) suggest that the daily absorption rate of propionate is not more than 30–40 g., an amount which would account for only about half of the measured glucose-utilization rate. There is no unequivocal evidence that either acetate or butyrate is glucogenic, and it appears that gluconeogenesis from protein must make a major contribution to the glucose requirements of sheep (Lindsay, 1959). When glucose requirements are assessed, however, it must be noted that utilization rates measured by isotope dilution include entry to the body pool of glucose produced by 'internal' metabolic cycles, e.g.: muscle glycogen $\rightarrow$ lactate $\rightarrow$ liver glycogen $\rightarrow$ glucose $\rightarrow$ muscle glycogen.

The limitations of single-injection procedures in utilization studies were fully discussed by Steele *et al.* (1956), who concluded that straightforward mathematical treatment of the curve showing the decline in specific activity of plasma glucose after the injection of labelled glucose (cf. Dunn *et al.* 1957) seriously overestimated glucose-utilization rates. The results obtained in the present study with sheep (Table 1) support this view. Skinner, Clark, Baker & Shipley (1959) claimed to have overcome the intrinsic weaknesses of the single-injection method by using a mathematical approach based on the assumption that glucose in the intact animal is distributed throughout a three-compartment system. It seems doubtful, however, whether the parameters governing the entry and outflow of glucose from the multi-compartment system that comprises the intact animal can be defined well enough for adequate mathematical treatment of single-injection data. The difficulties inherent in this approach have been discussed by

Sheppard & Householder (1951) and by Hart (1955).

Raised circulating amounts of glucose appeared to inhibit or abolish entry of endogenous glucose, since for four of the five sheep examined calculated utilization rates were roughly equivalent to the rate of infusion of carrier glucose (Table 4). The widely variable apparent glucose spaces measured in these experiments, however, suggested that equilibrium between plasma glucose and the glucose of other body pools had not been fully established. Carrier glucose and [^{14}C]glucose, however, were infused as one solution, and during the period of constant plasma glucose concentration and specific activity (Fig. 3) infused glucose and plasma glucose were of almost identical specific activity. Even in the absence of complete mixing of body pools the simplest interpretation of these results is that hepatic entry of glucose was inhibited under glucose load. The rate of infusion of carrier glucose was not large enough to mask changes in endogenous glucose entry, being at most only three times as great as the usual endogenous entry rate. The unchanged specific activity of plasma glucose immediately after the cessation of infusion, which persisted until the plasma glucose concentration fell to the pre-infusion value (Fig. 3), was further evidence of inhibited glucose entry under glucose load, although recycling of labelled glucose and any production of labelled glucose from labelled liver glycogen in this period would tend to nullify the decline in specific activity due to glucose entry. Studies based on the application of glucose load during the decline in blood glucose specific activity in dogs after the injection of [^{14}C]glucose (Searle & Chaikoff, 1952; Reichard, Friedmann, Maas & Weinhouse, 1958) suggested that hepatic output of glucose was abolished in response to high blood glucose concentrations, but later it was convincingly shown that the plateau in blood glucose specific activity observed after the application of glucose load was not necessarily due to inhibition of endogenous glucose entry (cf. Steele, 1959). Steele & Marks (1958) produced evidence that hepatic output of glucose in dogs is not inhibited by the sudden application of glucose load. In the present experiments with sheep the procedure allowed time for the animal to adjust to higher circulating concentrations of glucose, but the conclusions must remain tentative until the apparent inhibition of hepatic glucose output can be confirmed by other experimental approaches. A possible explanation of this apparent species difference is that more effective mechanisms for the control of glucose production may exist in sheep, which depend almost entirely on endogenous glucose. The metabolic economy of ruminants appears to be delicately balanced with

respect to glucose availability, particularly in the pregnant or lactating animal (Annison & Lewis, 1959), and any mechanism which conserves glucose, or glucose precursors, is of obvious value.

The relatively small decline in glucose entry in starved sheep (Table 5) indicates the important contribution to gluconeogenesis of sources other than propionate in starvation, since concentrations of volatile fatty acids in the rumen of starved (48 hr.) sheep are low (Annison, 1954) and entry of propionate from endogenous sources is probably negligible. The absence of a correlation between glucose turnover and diet in starved animals maintained on rations differing widely in starch content (Table 1) emphasizes the important role of gluconeogenesis in sheep metabolism.

The continued rise in blood carbon dioxide specific activity during the infusion of labelled glucose was presumably largely due to the conversion of glucose into other metabolites, and their subsequent oxidation, since errors due to incomplete mixing of labelled carbon dioxide with body pool carbon dioxide are minimal after 2–3 hr. (Annison & Lindsay, 1961). This secondary oxidation of glucose implies that estimates of the direct contribution of glucose to oxidative metabolism are almost certainly too high, and should be regarded as maximum values. Moreover, the use of venous blood carbon dioxide in place of expired carbon dioxide when measuring the contribution of labelled substrates to oxidative metabolism may give erroneous results unless mixed venous blood is used, since different tissues may vary considerably in their oxidative capacities. Clearly, jugular blood obtained during the infusion of labelled glucose would be expected to contain carbon dioxide of higher specific activity than that of mixed venous blood, since sheep brain utilizes glucose, but not acetate (McClymont & Setchell, 1956). In the present studies values of 22–23% and 11–19% respectively were obtained for the contribution of glucose to total carbon dioxide production in fed and starved animals. These values are much higher than the value (10%) reported by Baxter *et al.* (1955) in experiments on a lactating dairy cow, but the entry of labelled glucose into milk constituents in this animal makes comparison difficult. In sheep under glucose load about a third of the expired carbon dioxide was derived from pathways involving glucose oxidation. Estimates of the amount of glucose oxidized were not available since total carbon dioxide output was not measured in these experiments.

Steele *et al.* (1956) reported a mean value for the glucose space of fasted dogs of 25% (range 19–29%) of body weight, after correction for the glucose content of erythrocytes. In dogs the glucose space was approximately the same as the

inulin space (20–25%), which is a rough measure of the volume of extracellular fluid. The apparent distribution of the glucose pool throughout the extracellular fluid led Steele *et al.* (1956) to suggest that glucose space is a fundamental parameter of glucose metabolism. The values obtained for starved sheep by constant infusion (mean value 23%, Table 1) were somewhat lower than those reported for dogs, but the relatively larger proportion of body weight represented by the alimentary tract of sheep must be borne in mind. Although a wide range of values for glucose space were obtained in the present experiments (Tables 1 and 3), the results obtained in different experiments on the same animal were in reasonably close agreement. Glucose spaces measured after feeding (Table 3) were significantly higher than those of starved animals (Table 1). Hix, Underbjerg & Hughes (1959) have recently reported values of about 30% of body weight for the extracellular fluid of sheep, based on thiocyanate distribution, but this estimate included alimentary-tract contents. It would appear therefore that the glucose space of sheep, as measured by the constant-infusion method, is approximately the same as the extracellular space.

When the values for glucose utilization obtained in all constant-infusion experiments in fed and starved sheep were plotted against plasma glucose concentrations a roughly linear relationship was obtained (Fig. 4). This was unexpected in view of the findings of Bing *et al.* (1953) on the relationship between glucose utilization by the human heart and blood glucose concentration. Only small amounts of glucose were extracted at blood glucose concentrations below about 80 mg./100 ml., and glucose utilization reached its maximum value at blood concentrations of 120 mg./ml. The present findings, which were based on the intact sheep, indicate the ability of sheep tissues to utilize glucose at low plasma concentrations, and suggest the absence under these conditions of mechanisms to 'spare' glucose by the preferential oxidation of other substrates. At the highest glucose concentrations obtained there was no evidence of utilization reaching a maximum, but insufficient results were obtained at high plasma glucose concentrations to decide this point.

The wide range of plasma glucose concentrations and glucose-entry rates observed in different animals on similar diets and maintained under similar conditions (Table 1) presumably reflects differences in endocrine balance. These findings emphasize the need for caution when interpreting results obtained on small numbers of animals.

The measurement of the two fundamental parameters of carbohydrate metabolism, glucose-utilization rate and glucose space, constitutes a

valuable tool in the investigation of certain metabolic disorders. This approach is being currently used in this laboratory in the study of ovine pregnancy toxæmia, a condition characterized by impaired carbohydrate metabolism.

SUMMARY

1. Measurement of glucose utilization in sheep by isotope dilution has indicated the central importance of glucose in sheep metabolism. Glucose-utilization rates in fed and starved (24 hr.) animals, measured by a constant-infusion method, were $2.7 \pm 0.2(7)$ and $1.5 \pm 0.09(13)$ mg./min./kg. body wt. respectively. When calculated in terms of metabolic body weight ($W^{0.6}$) utilization rates in starved sheep were about 75 % of those reported for starved dogs. Corresponding values for the volume of distribution of the body glucose pools, expressed as a percentage of body weight, were $27 \pm 1.7(7)$ and $23 \pm 1.2(13)$.

2. A comparison of single-injection and constant-infusion methods for the measurement of glucose-utilization rates indicated that significantly higher results were given by the former technique.

3. Evidence of some inhibition of endogenous glucose entry under glucose load was obtained when utilization rates were measured at high concentrations of circulating glucose achieved by constant infusion.

4. A roughly linear relationship was observed when glucose-utilization rates obtained in all constant-infusion experiments were plotted against corresponding plasma glucose concentrations.

5. Rough estimates of the contribution of glucose to overall oxidative metabolism were obtained by a comparison of the specific activities of plasma glucose and blood carbon dioxide. The results suggested that in fed and in starved animals 22–23 % and 11–19 % respectively of blood carbon dioxide was derived from glucose. Under glucose load this value rose to about 30 %.

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