glucosylase is confined to the vascular tissue (Hatch *et al.* 1963), whereas uridine diphosphatase is only present in the storage parenchyma.

SUMMARY

1. A phosphatase that attacks certain nucleoside 5'-diphosphates, but not the corresponding mono- and tri-phosphates, was purified 43-fold from sugar-cane storage tissue.

2. The relative activities for the 5'-diphosphates of uridine, of inosine, of guanosine, of deoxyguanosine, of cytidine, of thymidine and of adenosine were 1.0, 0.45, 0.38, 0.11, 0.08, 0.06 and 0.0 respectively. The relationship between activity and the structure of substrates is discussed.

3. Added Mg^{2+} ions increased activity by only 40% but the enzyme was completely inhibited by EDTA. Activity was depressed to some extent by fluoride, inorganic phosphate and Mn^{2+} ions.

4. The enzyme operated at more than 50% of its maximum rate between pH 5.8 and 8.9. The Michaelis constant for UDP was less than 0.1 mM.

5. Phosphatase preparations that attacked UDP much more rapidly than they did either UMP or UTP were obtained from a number of other plant tissues.

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Glucose Utilization in Pregnant Sheep

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There has been much speculation on the role of glucose in ruminant metabolism. This has, in part, arisen because the concentration of blood glucose is lower than in non-ruminants, and because the evidence from many experiments in ruminants is that little glucose is absorbed from the alimentary canal. For example, Allcroft & Strand (1933) and Bell & Jones (1945) found that no obvious hyperglycaemia developed in the sheep and cow after a test meal of glucose; Schambye (1951) and Annison, Hill & Lewis (1957), working with sheep, were unable to demonstrate significant portal-carotid differences in glucose concentration. Also, most of the dietary energy of the ruminant is absorbed in the form of short-chain volatile fatty acids that are produced by fermentation of carbohydrates in the rumen. It is therefore likely that metabolism in the ruminant is adapted towards acetate as the main

source of energy and the main substrate for lipogenesis. Lindsay (1959) reviewed the available evidence on carbohydrate metabolism in the ruminant and concluded that most of the glucose presented to the tissues must arise by gluconeogenesis from propionate and protein. During pregnancy, however, the foetal demand for fructose (Huggett, Warren & Warren, 1951) is believed to cause a relatively great increase in overall hexose requirements (Kronfeld, 1958).

It was therefore decided to measure turnover rates of glucose in pregnant sheep and compare these rates with those obtained by other investigators in non-pregnant sheep.

The constant-infusion isotope-dilution method has been used by Steele, Wall, de Bodo & Altszuler (1956) for measuring glucose-utilization rates in dogs and by Annison & White (1961) and Kronfeld

& Simesen (1961*b*) in sheep. Both the latter groups of investigators used non-pregnant non-lactating female sheep. The present investigation was designed to study utilization rates in ewes pregnant with twins and in ewes showing signs of pregnancy toxæmia. By using, in separate experiments, glucose labelled at C-1 and C-6 and measuring the specific radioactivities of expired CO_2 , it has been shown that glucose is degraded in sheep by a pathway that incorporates C-1 in preference to C-6 into CO_2 . A note on the results of these experiments has been published (Ford, 1963).

The rate of utilization of glucose by pregnant sheep was comparable with that obtained by Kronfeld & Simesen (1961*b*), but markedly lower than the value obtained by Annison & White (1961) with fed non-pregnant sheep. Two further experiments were therefore carried out on non-pregnant females, receiving the same diet and kept under the same conditions as the pregnant sheep. The technique was similar, except that uniformly labelled glucose ($[\text{U-}^{14}\text{C}]\text{glucose}$) was infused at a lower level of radioactivity.

MATERIALS AND METHODS

Animals. After adult Clun Forest ewes had been run with a vasectomized ram they were served by an entire ram on known dates. The ewes were fed on pasture supplemented with hay and a mixture of oats, flaked maize, linseed cake, molasses and beet pulp at a rate sufficient to produce a small but steady increase in body weight and to keep the concentration of plasma ketone bodies low. Towards the end of pregnancy ewes carrying twins were selected by X-ray examination and trained to mount a ramp and stand in the stocks where the perfusions were to be carried out. Ewes to be made ketotic were taken into the animal house and confined in pens without food but with ample water. They were starved for periods up to 96 hr. and were considered suitable for experiment when a high concentration of plasma ketone bodies was accompanied by dullness, impaired or absent eye-preservation reflex and signs of nervous derangement of the type found in idiopathic pregnancy toxæmia.

Radioactive glucose. High-radioactivity D- $[\text{U-}^{14}\text{C}]\text{glucose}$ (2 mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks. Glucose was eluted from the filter paper with a minimum amount of water and the solution stored at -20° until diluted with 0.9% sodium chloride containing 0.01% of glucose for use. The stability of the infused radioactive glucose was checked by preparing and counting recrystallized potassium gluconate. In half of the experiments the same pregnant animal was infused on one day with $[\text{U-}^{14}\text{C}]\text{glucose}$ and 24 hr. later with $[\text{6-}^{14}\text{C}]\text{glucose}$; in the other half of the experiments the order of infusion was reversed. The specific radioactivity of $^{14}\text{CO}_2$ in expired air before the beginning of the second infusion was generally about double the background value of the previous day. In determining the specific radioactivity of $^{14}\text{CO}_2$ on the second day, the radioactivity of CO_2 before infusion began was taken as the background value. In the same way the residual plasma radioactivity at the

commencement of infusion on the second day was subtracted from the total radioactivity of the plasma glucose before calculation of the specific radioactivities of plasma glucose. The radioactivity of the preinfusion plasma sample on the second day was 1–2% of that of plasma samples collected during the steady-state period. The two non-pregnant animals were each infused once with $[\text{U-}^{14}\text{C}]\text{glucose}$.

Experimental procedure. Ewes were brought into the experimental shed at 10 a.m. on the day on which they were to be perfused and, after injection of a local anaesthetic, polythene cannulae were inserted in a downward direction into both jugular veins. To avoid contamination of blood samples by the infusion fluid, the infusion cannula was inserted about 12 in. into the left jugular, and the cannula for withdrawal of blood samples about 6 in. into the right jugular vein.

Experiments were begun at 2 p.m. by the injection of a priming dose of $30\text{ }\mu\text{C}$ of labelled glucose in 3 ml. of 0.9% sodium chloride, followed by the continuous infusion of $70\text{ }\mu\text{C}$ of labelled glucose in 0.9% sodium chloride containing 0.01% of glucose over 150 min. at the rate of 1.1 ml./min. Infusion was by means of a velodyne-controlled pump as described by Annison & Lindsay (1961). The velodyne and pump were made in the workshop of this Institute. Approximately 50% less radioactivity was infused in the non-pregnant sheep with a D.C.L. micro-pump (The Distillers Co. Ltd.).

Blood samples (about 15 ml.) were collected with heparin and sodium fluoride at intervals of about 12 min. beginning 1 hr. after the beginning of the infusion so that seven samples in all were obtained. The blood was centrifuged at 0° within a few minutes of collection, and the plasma separated and stored at -20° until radioactivity was assayed. During the last 50–60 min. of the infusion period the animal's head was enclosed in a polythene box with entry and exit valves for air. Expired air passed through the exit valve via a flow-meter into a spirometer. The bulk of the air was exhausted from the spirometer to the atmosphere, but a constant flow of 500 ml./min. was dried by passing through a tower of magnesium perchlorate, then through a 250 ml. ionization chamber and finally through a katharometer (Cambridge Instrument Co.). The current from the ionization chamber was measured with a Dynacon vibrating-reed electrometer (Nuclear-Chicago Corp.) and recorded on a Honeywell-Brown chart recorder. The katharometer gave a tracing of the concentration of CO_2 on a Cambridge recorder calibrated from 0 to 5% of CO_2 . After the completion of each infusion, normal sheep were fed and ketotic animals were starved until the second infusion had been carried out.

Chemical methods and assay of radioactivity. For the reasons given by Annison & White (1961) all glucose determinations were made on plasma, which was deproteinized with 0.3*N*-barium hydroxide and 5% (w/v) zinc sulphate. Glucose in the filtrate was determined by the enzymic method of Huggett & Nixon (1957), and ketone bodies were determined by a modification (Ford & Boyd, 1960) of the method of Bakker & White (1957).

In preliminary trials, samples of sheep plasma with added radioactive glucose were deproteinized with barium hydroxide and zinc sulphate, carrier glucose was added and the solution was evaporated to dryness under reduced pressure. The residue was extracted with hot methanol, in-

soluble material was discarded, and potassium gluconate was prepared and recrystallized by the method of Blair & Segal (1960). Recoveries of 65% of added radioactivity were obtained by this method and the specific radioactivity of the potassium gluconate was 95–100% of that of the added glucose. The non-glucose radioactivity of methanolic extracts of plasma samples that had been collected during the period 80–150 min. after the beginning of infusion, i.e. during the period of constant plasma-glucose specific radioactivity, was about 2–5% of glucose radioactivity. By counting concentrated methanolic extracts of plasma filtrates and omitting the final preparation of gluconate, recoveries of 80% of added radioactivity were obtained, and the slight loss in specificity was regarded as acceptable in view of the increased recovery of radioactivity. As a result of these findings the following procedure was adopted. The methanolic extract of dried deproteinized plasma was evaporated to dryness at 37° in a stream of air and dissolved in 1.5 ml. of water to which had been added 0.1 ml. of a 10% (w/v) solution of Triton X-100 (Rohm and Haas Co.). Plastic scintillator beads (NE-102; Nuclear Enterprises Ltd.) (5 g.) were poured into the vial, and, after the mixture had been kept overnight at 0°, radioactivity was counted in a Panax refrigerated scintillation counter with an efficiency of 20%. Samples of the glucose solutions that had been used for each infusion were subjected to the same procedure and plasma specific radioactivities in each experiment were calculated by reference to the infusion fluid used in the particular case. Clearly the counting of methanolic extracts of protein-free plasma filtrates can give plasma-glucose specific radioactivities up to 5% greater than would the counting of a recrystallized specific derivative of glucose, and a corresponding 5% error can arise in the calculation of utilization rates. Specific radioactivities of CO₂ in expired air were calculated direct from the recordings of radioactivity and CO₂ content of air by using a calibration constant for the ionization chamber.

Utilization rate. During the constant infusion of radioactive glucose of negligible carrier content and when plasma-glucose specific radioactivity is constant, the rate of addition of glucose to plasma (measured in m-moles/min.) is given by the rate of infusion of ¹⁴C (μmc/min.) divided by the specific radioactivity of plasma glucose (μmc/m-mole). With a constant plasma-glucose concentration, the rate of addition of glucose to the plasma is equal to the rate of removal of glucose from the plasma, i.e. the transfer or utilization rate.

RESULTS

Particulars of the six sheep used are given in Table 1. Two of the pregnant sheep (J 27 and G 104) had high concentrations of plasma ketone bodies and showed definite signs of abnormality resembling those found in clinical cases of pregnancy toxæmia or ovine ketosis. The other two pregnant animals (L 5 and K 111) were clinically normal in every respect, although one animal (K 111) had a mean concentration of plasma ketone bodies at the upper end of the normal range. The concentrations on the day of the second infusion were higher than during the first infusion; in view of the well-known sensitivity of ketone-body concentration in pregnant sheep to very slight changes in diet this rise can be attributed to deprivation of food for the period of infusion during the first day. The plasma-glucose specific radioactivity was constant during the period beginning 80 min. after the commencement of infusion in three of the four animals used (Fig. 1). With K 111, however, the plasma-glucose

Fig. 1. Specific radioactivities of plasma glucose and of CO₂ in expired air during infusion of sheep J 27. ●, [1-¹⁴C]-Glucose was infused (priming dose, 30 μc; infusion rate, 460 μmc/min.); ○, [6-¹⁴C]glucose was infused (priming dose, 30 μc; infusion rate, 472 μmc/min.). Experimental details are given in the text.

Table 1. *Characteristics of sheep used in studies of glucose utilization*

Experimental details are given in the text. The values given for the concentrations of plasma glucose and plasma ketone bodies are means ± s.e.m. with the numbers of determinations in parentheses. The signs of pregnancy toxæmia shown by J 27 and G 104 are described in the text.

Sheep no.	Age (years)	Wt. (kg.)	No. of days pregnant	Concn. of plasma glucose (mg./100 ml.)	Concn. of plasma ketone bodies (mg./100 ml.)	Signs of pregnancy toxæmia
L 5	3	73	142	52.5 ± 1.5 (16)	5.0 ± 0.6 (16)	Absent
K 111	4	84	130	38.0 ± 0.7 (16)	10.7 ± 1.1 (16)	Absent
J 27	5	68	135	44.4 ± 1.5 (14)	29.8 ± 2.0 (11)	Present
G 104	7	67.5	140	25.7 ± 0.7 (16)	18.1 ± 1.5 (14)	Present
L 79	4	70	—	85.0 ± 2.7 (8)	5.0 ± 0.35 (6)	Absent
L 274	4	59.5	—	78.5 ± 2.4 (9)	6.6 ± 0.9 (6)	Absent

specific radioactivity did not reach a plateau until about 120 min. after the commencement of infusion (Fig. 2). Curves of the same shape were obtained during both infusions, suggesting that the delay in reaching constant plasma specific radioactivity was a characteristic of the individual animal. The delay could have arisen from a slow mixing of radioactive glucose or from a decreasing glucose-transfer rate in the period 0–120 min. on both days. Whatever the cause, equilibrium was reached at 120 min. and maintained from then until the end of the infusion. Although infusions of the same radioactivity/unit volume were used in all experiments on pregnant sheep, the overall infusion periods varied by several minutes. This variation in the infusion periods, together with the lower radioactivity of the infusion fluid given to the non-pregnant sheep, caused the differences in the rates of infusion of radioactivity shown in column 3 of Table 2.

Fig. 2. Specific radioactivities of plasma glucose and of CO_2 in expired air during infusion of sheep K 111. ●, $[1\text{-}^{14}\text{C}]$ -Glucose was infused (priming dose, $30\text{ }\mu\text{C}$; infusion rate, $470\text{ }\mu\text{mc/min.}$); ○, $[6\text{-}^{14}\text{C}]$ glucose was infused (priming dose, $30\text{ }\mu\text{C}$; infusion rate, $465\text{ }\mu\text{mc/min.}$). Experimental details are given in the text.

For all animals, utilization rates (Table 2: column 6) were calculated on the basis of plasma-glucose specific radioactivities prevailing at 140 min. The consistency in duplicate determinations on the same animal shows that daily variation in glucose utilization was slight. There are, however, differences between utilization rates of individual pregnant animals and these differences are maintained when the rates are adjusted for body weight (Table 2: column 7) or metabolic body size (Table 2: column 8) as suggested by Kleiber (1947).

When the utilization rates of the normal pregnant animals are compared with those of the ketotic pregnant animals, the former have a mean rate of 1.35 ± 0.18 (4) mg./min./kg. and the latter a mean rate of 1.22 ± 0.13 (4) mg./min./kg. This difference is not significant ($P > 0.5$). Possible differences between the results from the normal and ketotic pregnant sheep are not the only sources of variation in utilization rates of pregnant sheep. The variance is analysed in Table 3. None of the sources of variation is significant when compared with the error, but the greatest mean square arises from differences between sheep. When the sums of squares of all other sources of variation are combined as one error and the mean square of the difference between sheep is compared with this error (Table 3), F equals 11.3 and is significant ($P < 0.05$). This means that the differences in utilization rates of pregnant sheep are due to differences between individual animals irrespective of their metabolic states. Clearly, the utilization rates of normal and ketotic sheep can be combined to give a mean of 1.29 ± 0.11 (8) mg./min./kg. for pregnant sheep. This value is significantly lower than the mean value of 1.83 ± 0.10 (2) mg./min./kg. for non-pregnant sheep ($P < 0.05$).

Table 4 gives the specific radioactivity of expired CO_2 at 140 min. after the commencement of infusion, i.e. when CO_2 specific radioactivity and

Table 2. Results of experiments on glucose utilization in sheep

The methods of infusion and of measuring plasma-glucose specific radioactivity are given in the text. Utilization rates have been calculated in three different ways to permit comparison with results obtained by other investigators.

Condition of sheep	Sheep no.	Infusion details		Sp. radioactivity of plasma glucose at 140 min. ($\mu\text{mc/m-mole}$)	Utilization rate		
		Glucose label	Rate ($\mu\text{mc/min.}$)		(m-mole/min.)	(mg./min./kg.)	(g./hr./kg. ^{0.75})
Normal pregnant	L 5	$6\text{-}^{14}\text{C}$	495	680	0.73	1.80	0.31
		$1\text{-}^{14}\text{C}$	450	750	0.60	1.48	0.26
	K 111	$1\text{-}^{14}\text{C}$	470	910	0.53	1.14	0.21
		$6\text{-}^{14}\text{C}$	465	1000	0.47	1.00	0.19
Ketotic pregnant	J 27	$1\text{-}^{14}\text{C}$	460	850	0.54	1.43	0.25
		$6\text{-}^{14}\text{C}$	472	830	0.57	1.50	0.26
	G 104	$6\text{-}^{14}\text{C}$	430	1250	0.35	0.93	0.16
		$1\text{-}^{14}\text{C}$	455	1150	0.39	1.04	0.18
Non-pregnant	L 79	$\text{U-}^{14}\text{C}$	270	400	0.67	1.73	0.30
	L 274	$\text{U-}^{14}\text{C}$	205	320	0.64	1.94	0.32

plasma-glucose radioactivity had been steady for at least 20 min. and in most cases for longer. One factor that will affect the labelling of CO_2 during the infusion of any ^{14}C -containing substrate will be the degree of labelling of the substrate presented to the tissues, i.e. the plasma specific radioactivity at equilibrium. Although the same total amount of radioactivity was administered in each experiment on pregnant animals, the plasma-glucose specific radioactivities differed between successive experiments on the same animal as a result of slight changes in utilization rates on succeeding days, and also after differences in the rate of infusion of radioactivity as a result of variations in overall perfusion time.

For these reasons, in order to make a direct comparison of specific radioactivities of CO_2 in expired air during separate infusions carried out on the same animal it is necessary to adjust the observed CO_2 specific radioactivity to a standard plasma-glucose specific radioactivity. The arbitrary value of $1000 \mu\text{mc}/\text{m-mole}$ has been chosen and adjusted values are given in column 5 of Table 4. From these

values the C-1:C-6 ratios of column 6 have been calculated. The mean ratio of 1.89 ± 0.15 for ketotic sheep does not differ significantly from the mean ratio of 1.31 ± 0.07 for the normal pregnant sheep ($0.1 > P > 0.05$). The four ratios can therefore be grouped to give a mean value of 1.60 ± 0.18 (4) for pregnant sheep.

Table 5 gives the CO_2 output of each sheep at 140 min. in m-moles/min. The values are derived from the total production of expired air/min. as given by the flow-meter and the percentage content of CO_2 in expired air given by the katharometer. Some incompetence of the face-mask valves and leakage of air where the sheep's neck passes into the mask probably caused the CO_2 -output values to be underestimated.

Table 5 also gives information on the sources of CO_2 . If 100% of the CO_2 were produced by the oxidation of glucose under steady-state conditions, its specific radioactivity, on a molar basis, would be one-sixth of that of the glucose supplied to the tissues, i.e. of plasma-glucose specific radioactivity. Any lower percentage contribution would be

Table 3. *Analysis of variance for glucose-utilization rates of pregnant sheep*

The first part of the Table (a) shows that none of the individual sources of variation listed in the first column is significantly greater than the error. The second part of the Table (b) shows that differences between individual sheep are significant when compared with all other sources of variation. N.S., Not significant.

Source of variation	Sum of squares	Degrees of freedom	Mean square	<i>F</i>	
(a) Between normal and ketotic groups	0.0338	1	0.0338	1.44	N.S.
Between sheep	0.5892	3	0.1964	8.4	N.S.
Between infusions	0.0024	1	0.0024	0.41	N.S.
Between days of infusion	0.0094	1	0.0094	0.10	N.S.
Error	0.0234	1	0.0234		
Total	0.6586	7			
(b) Between sheep	0.5892	3	0.1964	11.3	$P < 0.05$
Error	0.0694	4	0.0175		
Total	0.6586	7			

Table 4. *Carbon dioxide specific radioactivities and carbon dioxide ratios of pregnant sheep infused with [^{14}C]glucose*

The C-1:C-6 ratio is defined in the text. The plasma-glucose specific radioactivity and the specific radioactivity of CO_2 in expired air were measured as described in the text. The CO_2 specific radioactivities of column 5 are adjusted to a plasma-glucose specific radioactivity of $1000 \mu\text{mc}/\text{m-mole}$ to permit calculation of the C-1:C-6 ratios of column 6.

Sheep no.	Glucose label	Sp. radioactivity of plasma glucose at 140 min. ($\mu\text{mc}/\text{m-mole}$)	Sp. radioactivity of CO_2 at 140 min. ($\mu\text{mc}/\text{m-mole}$)	Adjusted sp. radioactivity of CO_2 ($\mu\text{mc}/\text{m-mole}$)	C-1:C-6 ratio
L 5	$1\text{-}^{14}\text{C}$	750	3.5	4.7	1.38
	$6\text{-}^{14}\text{C}$	680	2.3	3.4	
K 111	$1\text{-}^{14}\text{C}$	910	2.8	3.1	1.24
	$6\text{-}^{14}\text{C}$	1000	2.5	2.5	
J 27	$1\text{-}^{14}\text{C}$	850	3.0	3.5	2.05
	$6\text{-}^{14}\text{C}$	830	1.4	1.7	
G 104	$1\text{-}^{14}\text{C}$	1150	5.2	4.5	1.74
	$6\text{-}^{14}\text{C}$	1250	3.5	2.6	

Table 5. *Production of carbon dioxide by sheep from variously labelled [^{14}C]glucose*

The methods of measurement and calculation are given in the text.

Sheep no.	Glucose label	Sp. radio-activity of plasma glucose at 140 min. ($\mu\text{mc}/\text{m-mole}$)	Sp. radio-activity of CO_2 at 140 min. ($\mu\text{mc}/\text{m-mole}$)	CO_2 from glucose (%)	CO_2 output at 140 min. (m-moles/min.)	CO_2 produced from glucose (m-mole/min.)	Glucose oxidized to CO_2 (%)
L 5	$1\text{-}^{14}\text{C}$	750	3.5	2.8	2.4	9.8	0.235
	$6\text{-}^{14}\text{C}$	680	2.3	2.0			
K 111	$1\text{-}^{14}\text{C}$	910	2.8	1.9	1.7	10.2	0.176
	$6\text{-}^{14}\text{C}$	1000	2.5	1.5			
J 27	$1\text{-}^{14}\text{C}$	850	3.0	2.1	1.55	11.1	0.180
	$6\text{-}^{14}\text{C}$	830	1.4	1.0			
G 104	$1\text{-}^{14}\text{C}$	1150	5.2	2.7	2.2	9.6	0.210
	$6\text{-}^{14}\text{C}$	1250	3.5	1.7			
L 79	$\text{U-}^{14}\text{C}$	400	1.4	2.1		6.8	0.143
L 274	$\text{U-}^{14}\text{C}$	320	2.0	3.7		4.8	0.180

expected to produce a proportionately lower CO_2 specific radioactivity.

It is known, however, from the C-1:C-6 ratios that these two carbon atoms contribute to CO_2 labelling at different rates, and column 5 of Table 5 gives for the pregnant sheep the percentage contribution of C-1 and C-6 of glucose calculated from the information in the preceding two columns. The mean of these two values gives a rough approximation of the contribution of the glucose molecule to CO_2 production. In the two non-pregnant animals infused with uniformly labelled glucose, the CO_2 labelling will arise from all six carbon atoms of the glucose molecule and the calculated percentage contribution should be a reasonable assessment of the contribution of glucose to CO_2 production.

Thus although there is variation from 1.55 to 3.7 % between the six animals, the ketotic pregnant sheep do not differ from the normal pregnant or from the non-pregnant sheep. As the rate of CO_2 production at 140 min. is known (column 6) the amount of the CO_2 derived from glucose has been calculated and is given in column 7.

The total amount of glucose utilized is already known (Table 2), and the percentage of glucose utilized that is oxidized to CO_2 has been calculated and is given in column 8 of Table 5.

DISCUSSION

Choice of methods. Annison & Lindsay (1961) have considered the relative merits of tolerance tests, of single-injection and of continuous-infusion isotope-dilution techniques for determining uptake rates of metabolites. They concluded that the last-named method was most suitable for measuring the uptake of metabolites by tissues at normal plasma concentrations. Kronfeld & Simesen (1961*a*) discussed the application of isotope-dilution methods to the study

of glucose utilization in pregnancy toxæmia and suggested that the use of the continuous-infusion method might be restricted because of the difficulty of establishing steady-state conditions in ketotic sheep. For this reason they employed the single-injection technique. In the present investigation the plasma-glucose concentration was constant throughout the experiments, and plasma-glucose specific radioactivity was steady during the period 80–150 min. after the commencement of infusion in five of the six animals and during the period 120–150 min. in the sixth animal. The latter was one of the two normal pregnant animals. It is therefore feasible to compare results in the ketotic ewes with those in the normal pregnant ewes and in the non-pregnant ewes.

Cases of pregnancy toxæmia that have occurred spontaneously present one or more of a number of symptoms to a greater or lesser degree, and, when brought to the Laboratory, their capacity to survive long enough to allow infusion on two consecutive days is not always certain. For this reason artificial induction of the syndrome was employed and it was possible to induce typical cases with a reasonable degree of regularity. Concentrations of plasma ketone bodies were high, and plasma-glucose concentrations, although low, were not necessarily lower than those in symptomless pregnant sheep. This variation in the mean plasma-glucose concentrations and the lack of negative correlation with ketone-body concentrations is well known to occur in cases that arise spontaneously. One important characteristic of naturally occurring cases is their irreversibility. After the completion of experiments, both J 27 and G 104 continued to refuse food, their symptoms became progressively more severe and the animals were both killed when death was imminent. It therefore seems reasonable to equate the status of the two ketotic sheep with that

of field cases, although Gallagher (1959) has shown that liver mitochondria from animals with pregnancy toxæmia were incapable of oxidizing fatty acids, whereas the liver mitochondria of starved asymptomatic pregnant ewes did not show this abnormality.

Utilization rates. Although other workers have determined utilization rates of glucose in sheep by isotopic techniques, differences in the conditions of the experiments and in the nutritional and reproductive status of the animals used make the results difficult to compare with the present ones. For example, Annison & White (1961) used the continuous-infusion method on non-pregnant females receiving a diet rich in starch and protein, but not fed for 24 hr. before the experiment. They obtained a utilization rate of 1.5 ± 0.07 (13) mg./min./kg. compared with the mean rate in the present study of 1.84 ± 0.10 (2) mg./min./kg. for non-pregnant animals that had been feeding up to 4 hr. before the commencement of infusion.

Those of their results that it is most reasonable to compare with the present ones were obtained 2–5 hr. after feeding and gave a mean value of 2.6 ± 0.2 (7) mg./min./kg. Annison & White (1961) suggested that the high-starch diet of their animals might have been responsible for the high rate. Presumably some of the carbohydrate could escape degradation in the rumen to be absorbed as hexose from the small intestine. Annison & White's (1961) animals had, after feeding, a high plasma-glucose concentration, which was reflected in higher glucose-utilization rates than were obtained in animals with lower plasma-glucose concentrations. A similar relationship emerges when plasma-glucose concentration and utilization are compared in the pregnant and non-pregnant sheep in the present study.

Kronfeld & Simesen (1961*b*), using the continuous-infusion method on two non-pregnant females, found a mean rate of 0.3 g./hr./kg.^{0.75}, compared with a mean of 0.31 g./hr./kg.^{0.75} in the present study. Kronfeld & Simesen (1961*a*) used the single-injection technique on pregnant ewes and obtained rates ranging from 1.4 to 3.6 mg./min./kg. These values are appreciably higher than the present ones, but Annison & White (1961), when comparing both methods in the same sheep, found that the single-injection method gave appreciably higher results than the continuous-infusion method. Nevertheless, it is reasonable to compare the results that Kronfeld & Simesen (1961*a*) obtained by using the single-injection method on ketotic and on normal pregnant sheep; the utilization rates in the former were about one-third to one-fifth of the latter. This is a large difference; the present studies with continuous infusion showed no significant difference at all. One possible explanation is that

results with the single-injection method are affected by changes in the rate of mixing of isotope throughout the glucose pool, a variable that does not affect results calculated during the steady state reached after a period of constant infusion. The absence of a significant difference between utilization rates of ketotic and normal pregnant sheep does not support the theory of Reid (1958), who suggested that the basic change in ketosis was an adrenal-steroid diabetes, and that hyperglycaemia did not develop because of the foetal utilization of hexose. If Reid's hypothesis were correct, normal pregnant sheep would be expected to utilize glucose at a significantly greater rate than ketotic sheep.

An objection might be raised to the comparison of the present results in pregnant sheep with those from the two non-pregnant sheep on the grounds that the true body weight of the sheep used in the two series of experiments differed. The pregnant sheep used in the present work were well-grown and well-fleshed but clearly part of their body weight was that of their two foetuses and foetal fluids. This portion of the body weight consumes, but does not produce, hexose; so that to compare endogenous production of glucose (assuming negligible alimentary contribution) it would be feasible to deduct 15 kg. from the body weight of the pregnant sheep, an assessment of the average weight of foetal fluids, membranes and two foetuses near to term. When this is done, the mean production rate for the fed pregnant sheep becomes 1.69 ± 0.24 (4) mg./min./kg. of maternal tissue, a value not significantly different from but still lower than that of fed non-pregnant sheep. The result of this type of adjustment of the entry rate is compatible with the suggestion that endogenous glucose production in the pregnant ewe does not increase with increasing foetal demand and that the maternal tissues are relatively undersupplied compared with the tissues of the non-pregnant ewe.

According to Huggett *et al.* (1951) foetal requirements are 12–22 g./day/foetus at term, which can be taken as about 40 g./day for twin foetuses at 135 days. The rate of 1.29 mg./min./kg. for pregnant sheep is equivalent to a glucose supply of 145 g./day in an animal of 78 kg. body wt. A foetal requirement of 40 g./day is therefore about 28 % of the available maternal supply.

These calculations contain many approximations but they do suggest that tissue glucose supplies are marginal, and if maternal body weight is to be increased or even only maintained the amount of glucose directly oxidized by both the pregnant and non-pregnant ewe seems likely to be low.

Production of carbon dioxide from glucose. Table 5 shows the percentage of glucose that is directly oxidized. This varies from 3.5 to 9.5 %, with no

clear-cut difference between the fed, ketotic or non-pregnant sheep. During infusion, the activity of the sheep is necessarily restricted and it is doubtful whether one is measuring the maximum rate at which glucose may enter the circulation or the full contribution that glucose may make to respiratory CO_2 during normal exercise.

Nevertheless, as all groups of sheep were infused under the same conditions it seems reasonable to compare groups and also to compare these results with those of other workers, e.g. Annison & White (1961), who carried out their experiments under similar conditions.

The contrast lies between these results and those of Annison & White (1961) in non-pregnant sheep on a high-starch diet. They found that the percentage of CO_2 derived from glucose was 11–19 in starved sheep, 22–23 in fed sheep, and 33–36 when plasma-glucose concentrations were raised by infusion to over 120 mg./100 ml. It appears from the results of Annison & White (1961) that the contribution of glucose to oxidative metabolism rises in proportion to the concentration of glucose offered to the tissues, and from the present results that the proportion of glucose directly oxidized is low when the supply is marginal.

From the percentage of glucose directly oxidized and the overall glucose-utilization rate, the amount of glucose available for synthetic purposes becomes 1.27 mg./min./kg. in the fed pregnant animals, and 1.76 mg./min./kg. in the fed non-pregnant animals. If by allowing 15 kg. for foetuses and foetal fluids a 63 kg. non-pregnant sheep is compared with a 78 kg. pregnant sheep, the daily supply of glucose for synthesis becomes 160 g. in the former and 143 g. in the latter. In the latter case, the foetal requirement is 40 g., leaving 93 g. for maternal synthetic processes.

Thus, when glucose utilization in pregnant sheep is compared with that in non-pregnant females on a similar diet, there are marked differences in the amount available, but not in the proportion that is oxidized.

The requirements of twin foetuses are a small proportion (about 28 %) of the maternal supply; but, as the total supply is only 70 % of that in non-pregnant sheep, the extra drain on supplies may well be critical.

C-1:C-6 ratios. Pentose-cycle activity in sheep tissues and homogenates has been demonstrated by Raggi, Hansson, Simensen, Kronfeld & Luick (1961) and the mean ratio of 1.60 in the whole animal is consistent with this finding. Tombropoulos & Kleiber (1961), using cattle, calculated the percentage of glucose oxidized by the pentose cycle from CO_2 ratios and from the degree of labelling of alanine derived from milk protein. Katz & Wood (1960), however, have underlined the possible errors

of such calculations in the absence of information on the percentage of glucose utilized by non-triose pathways and on the proportion of triose carbon oxidized to CO_2 . In the absence of such information in the present study the only conclusion to be drawn from the ratio is that pentose-cycle activity in the pregnant sheep has been confirmed.

SUMMARY

1. By using the constant-infusion isotope-dilution method, pregnant sheep have been shown to utilize glucose at the rate of 1.29 ± 0.11 (8) mg./min./kg. There was no significant difference between normal and ketotic sheep.

2. Under steady-state conditions the CO_2 ratio, i.e. the specific radioactivity of expired CO_2 during the infusion of [$1\text{-}^{14}\text{C}$]glucose divided by the specific radioactivity of expired CO_2 during the infusion of [$6\text{-}^{14}\text{C}$]glucose, was 1.60 ± 0.18 (4).

3. Under steady-state conditions the mean CO_2 production was 10.2 ± 0.35 (4) m-moles/min. The percentage of CO_2 derived from glucose ranged from 1.7 to 2.4 and the percentage of glucose utilized that was oxidized to CO_2 ranged from 5.5 to 9.5.

4. These results have been contrasted with those obtained from two non-pregnant sheep on a similar diet. The latter utilized glucose at the rate of 1.84 ± 0.1 (2) mg./min./kg. and produced CO_2 at the rate of 6.8 and 4.8 m-moles/min. The percentage of CO_2 derived from glucose was 2.1 and 3.7 and the percentage of glucose utilized that was oxidized to CO_2 was 3.5 and 4.7.

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Studies on the Reaction between the Blood-Group Antibody Anti-D and Erythrocytes

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Radioactive iodine (^{131}I) was first used as a label for the blood-group antibody anti-D by Boursnell, Coombs & Rizk (1953). These authors estimated the antibody content of a particular example of anti-D serum to be 54 $\mu\text{g./ml.}$ and also estimated the total number of antigen sites on Rh-positive cells (probable genotype CDe/CDe) to be 5500/red cell. Masouredis (1959) carried out similar experiments and estimated the number of antigenic sites to be between 2000 and 3000/red cell. In later observations, Masouredis (1960b) estimated that cells homozygous for the antigen D had either 10 300 or 7400 antigen sites/red cell, depending on the absence or presence respectively of the blood-group antigen C. Masouredis (1960a) also estimated the extent of the transfer of ^{131}I -labelled anti-D antibody from sensitized red cells to unsensitized cells. For one particular example of anti-D he found that approx. 15% was transferred in 1 hr. and thereafter no further transfer could be demonstrated. Evans, Mebust & Hickey (1960), using conventional techniques with antiglobulin sera, found that the extent and rate of the transfer of anti-D between red cells was consistent with the law of mass action and they confirmed their results by using ^{131}I -labelled anti-D.

Hughes-Jones, Gardner & Telford (1962) presented the results of investigations on the kinetics of the reaction between the human blood-group antibody anti-c and erythrocytes by using ^{131}I as a label for the antibody. An analysis of the law of mass action first suggested by Scatchard (1949) was used to demonstrate heterogeneity of the intrinsic binding constant between antigen and antibody and also to determine the number of antigen sites

on the red cell. Similar investigations on three examples of the human blood-group antibody anti-D are the subject of the present paper.

METHODS

Antibody-containing sera. Three sera were investigated: Av, Ke and Wa. In each case the antibody was an incomplete anti-D, each known to have the characteristics of a 7s γ -globulin. Serum Av had a titre of 1000 (indirect anti-globulin method) and was obtained 3 months after the injection of Rh-positive red cells that were given to re-stimulate antibody production. γ -Globulin was prepared by ether fractionation from sera Ke and Wa by Dr R. Kekwick of the Lister Institute; these γ -globulin preparations had titres of 1000 and 500 respectively.

Red cells. All the red cells used were of the phenotype CCDee; they were probably (P 0.9) of the genotype CDe/CDe and are referred to below as $R_r R_r$. The control cells were of the genotype cde/cde (referred to as rr cells).

Labelling of γ -globulin solutions with ^{131}I and determination of the specific activity. These were done by the methods described by Hughes-Jones *et al.* (1962). γ -Globulin was prepared from serum Av by precipitation with 1.8M-ammonium sulphate before labelling. The uptake of iodine by γ -globulin was usually between 1 and 2 atoms of iodine/molecule of protein.

Calculations. The rate constants for association and dissociation were calculated on the assumption that the reversible reaction of antigen and antibody could be represented by:

where $[\text{Ag}]$, $[\text{Ab}]$ and $[\text{AgAb}]$ represent antigen, antibody and complex concentrations respectively. The following assumptions were also made: (1) that the specific activity of the ^{131}I -labelled-antibody molecules was the same as that of ^{131}I -labelled γ -globulin; (2) that the molecular weight of the antibody was 160 000; (3) that anti-D was univalent;