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Acetate Utilization and the Turnover of Citric Acid-Cycle Components in Pregnant Sheep

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Acetate is an important metabolic substrate in the ruminant. It is the major product of ruminal fermentation and is readily absorbed by the ruminal epithelium and transferred to the blood stream. A number of workers (Jarrett, Potter & Filsell, 1952; Pugh & Scarisbrick, 1952; Reid, 1958) have studied the rate of fall of plasma acetate concentration after intravenous administration, but it is difficult to assess the significance of utilization rates obtained when plasma concentration is high and is falling rapidly. Annison & Lindsay (1961) studied acetate utilization in non-pregnant sheep by a constant-infusion isotope-dilution technique similar to the one used by Steele, Wall, de Bodo & Altszuler (1956) for the measurement of glucose-utilization rates in dogs. Annison & Lindsay obtained rates varying from 0·7 to 5·5 mg./min./kg. in animals that had plasma acetate concentrations of 0·25–1·09 m-moles/l. Essig, Norton & Johnson (1961), using a similar technique, obtained rates of 9·2 and 12·0 mg./min./kg. in two castrated male sheep 3–4 hr. after feeding.

Clearly there is a considerable range of acetate-ultilization rates in non-pregnant sheep. Increased utilization rates might be expected in pregnant animals if rapidly growing foetal tissues utilize acetate at a greater rate than maternal tissues. It was decided to measure acetate-utilization rates in early and late pregnancy in normal sheep and in late pregnancy in under-fed sheep by the continuous-infusion isotope-dilution method. In the under-fed animals, the amount of carbohydrate synthesized from propionate is believed to be low, and overall acetate-utilization rate might well be increased as a result of the mobilization of depot fat to satisfy energy requirements. In most animal species the administration of [1-^{14}C]acetate gives rise to a higher rate of labelling of expired carbon dioxide than does the administration of [2-^{14}C]acetate. Weinman, Strisower & Chaikoff (1957) suggested that the carbon dioxide ratio, i.e. the ratio of the specific activities of expired carbon dioxide when [1-^{14}C]- and [2-^{14}C]-acetate respectively are given, is an indication of the turnover of citric acid-cycle components. These carbon dioxide ratios have been determined in non-pregnant sheep and in well-fed and under-fed pregnant sheep.

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MATERIALS AND METHODS

Experimental animals. Clun Forest ewes were used throughout. When the presence of a normal oestrus cycle had been confirmed by the use of a vasectomized ram, they were run with a normal ram until two oestrus cycles had been missed. Two series of experiments were performed (1958–1959) and (1959–1960). Pregnancy was confirmed radiographically about 4 weeks before expected parturition. Animals were maintained in paddocks until required for experiments, and were allowed to graze freely. In the last 2 months of pregnancy, they were also given concentrates (a mixture of rolled oats, linseed cake, flaked maize and bean meal) sufficient to permit a small but steady increase in weight throughout the period. Under-fed animals (1958–1959) received no concentrates for 6 days before experiment, but grazed freely on what little grass was available on an almost bare pasture. Partially starved

animals (1959–60) were maintained indoors for the 6-day experimental period, and received only 150 g. of chopped hay/day during this time.

The effect of these dietary changes on the animals' weight is shown in Table 1.

Technique of experiment. In each experiment an animal was brought from pasture into the experimental room in the evening and fed if necessary. The following morning polythene catheters (gauge 53a; Portland Plastics Ltd.) were inserted into each jugular vein, that in the left jugular being inserted 10–12 in. and that in the right about 6 in. Infusion of [^{14}C]acetate was made through the long catheter to ensure that sampled blood was not contaminated by infused [^{14}C]acetate. [^{14}C]Acetate (30 μc) in 3 ml. of 0·01 N-NaOH (with negligible carrier acetate) was given as a single injection, followed by 70 μc in 180 ml. of 0·4 mN-NaOH over the next 2 hr. Constant infusion over this period was by means of a Velodyne as described by Annison & Lindsay (1961). Blood samples were taken at approximately equal intervals throughout the last hour of infusion. Samples of expired air were taken at intervals throughout the infusion, by using a rubber face mask (in the 1958–1959 series) as described by Annison & Lindsay (1961). In later experiments a different arrangement was used. Expired air was passed, after being dried with magnesium perchlorate, through a 250 ml. ionization chamber (Nuclear–Chicago Dynacon) for measurement of radioactivity in CO_2 and then through a CO_2-meter for measurement of CO_2 concentration. The CO_2-meter, constructed in this Institute, consisted essentially of a bottle containing 5 mM-sodium hydrogen carbonate solution plus phenol red. A beam of light passed through an Ilford 603 green filter, through the bottle, and then into a photocell (VA26T), the current produced being recorded on a galvanometer. Changes in the CO_2 content of the air bubbled through the bottle produced changes in the pH of the solution and in the intensity of the indicator colour. These colour changes affected the transmission of light through the bottle, thus varying the output of the photocell. The entire apparatus was calibrated by passing a series of gas mixtures of known CO_2 content through the solution of bicarbonate. In half the experiments [2-^{14}C]acetate was given first, followed by [1-^{14}C]acetate 24 hr. later. In the other half the order was reversed. The radioactivity of CO_2 in expired air, before the beginning of the second infusion, was generally about double the background value on the preceding day. In determining the specific activity of CO_2 on the second day, the activity of expired CO_2, before infusion began, was taken as the background value.

The concentration of blood glucose was determined by the method of Huggett & Nixon (1957), and that of blood ketone bodies by a slight modification of the method of Bakker & White (1957), as described by Ford & Boyd (1960).

The specific activity of plasma acetate was measured by the methods of Annison & Lindsay (1961).

The specific activity of the CO_2 of the expired air was initially measured as described by Annison & Lindsay (1961) but, in later experiments, directly as described above. However, the calibration constant of the ionization chamber was known only when 100% CO_2 was the filling gas. The CO_2 of the expired air was therefore absorbed in alkali on three occasions during the experiment, and its specific activity determined, either as solid barium carbonate, as described by Annison & Lindsay (1961), or by using the ionization chamber as a closed chamber with 100% CO_2 as filling gas, as described by Neville (1948). Apparent specific activities, as measured directly, were then converted into true values, by using an empirical correction constant. The required correction was in the range 3–5%.

RESULTS

Concentrations of glucose and ketone bodies. The concentrations of blood or plasma glucose and of ketone bodies, at the times of the various infusions, are shown in Table 1. Blood, for these analyses, was taken at the beginning and end of each infusion. The reduced plane of diet in late pregnancy produced a rise in the concentration of blood ketone bodies and a fall in that of blood glucose. In other animals, values are within normal limits, except for sheep nos. 10 and 11, where the blood glucose concentration was low although the blood ketone body concentration was within the normal range.

Acetate-utilization rates. From the rate of infusion of [^{14}C]acetate and the specific activity of plasma acetate during steady-state conditions, it is possible to compute the turnover rate of acetate (or utilization rate). This is given in m-moles/min. by dividing the rate of infusion of [^{14}C]acetate (μmc/min.) by the mean specific activity of plasma acetate (μmc/m-mole) calculated from values obtained during the period when the latter had reached a constant level. Such rates are given in Table 2, as are the average values of plasma acetate concentration, obtained when the specific activity of the plasma acetate was constant. In group A animals, acetate turnover was $2{\cdot}44 \pm 0{\cdot}17$ (6), in group B animals $2{\cdot}08 \pm 0{\cdot}27$ (8) and in group C animals $2{\cdot}37 \pm 0{\cdot}23$ (8) mg./kg./min. These values do not differ significantly from each other.

The group on a low plane of nutrition in late pregnancy show a substantially lower mean acetate-turnover value of $1{\cdot}00 \pm 0{\cdot}07$ (6) mg./min./kg. ($P < 0{\cdot}001$). These values do not directly show the amount of acetate absorbed from the gastrointestinal tract, since the turnover of endogenous acetate is unknown.

Production of carbon dioxide from acetate. In expressing specific activities of carbon dioxide, it is necessary to consider the relation between expired $^{14}CO_2$ and the time of infusion. With a constant infusion of [^{14}C]acetate, the specific activity of carbon dioxide has generally reached a constant value after about 3 hr. By using an appropriate ratio of priming dose to infusion rate, as was done in these experiments, constancy of the specific activity of expired carbon dioxide is generally reached within 2 hr. and this value was used in most of the experiments described. However, in two cases the specific activity did not reach a

constant value in this time and, here, the mean of values obtained in the last 30 min. was used. This approach is valid because, although the ratio:

$$\frac{\text{Sp. activity of } CO_2 \text{ from } [1\text{-}^{14}C]\text{acetate}}{\text{Sp. activity of } CO_2 \text{ from } [2\text{-}^{14}C]\text{acetate}}$$

is appreciably higher at 30 min. after infusion than at later times, the ratios at 90 min. and at 120 min. are not appreciably different. The situation is the same whether or not the individual specific activity–time curves have reached a constant value.

The final values for the specific activity of carbon dioxide for all experiments, obtained either as a plateau value or as 90–120 min. average values, are shown in Table 3.

In each experiment, the proportion of the expired carbon dioxide that was derived from the carbon atom of acetate that was labelled can be calculated. It is given by dividing the final specific activity of carbon dioxide by the mean specific activity of the labelled carbon atom of plasma acetate. By adding, for each sheep, the proportions derived from C-1 and C-2, the proportion of the expired carbon dioxide derived from the carbon of acetate is found. These values are shown as percentages in Table 4. As Table 4 shows, there is a significant fall in the percentage of carbon dioxide derived from acetate in animals on a low plane of nutrition in late pregnancy. In some cases, where the output of carbon dioxide is known, it is also possible to estimate the percentage of acetate utilized which is converted into carbon dioxide. The carbon dioxide output is about the same in all four groups. Thus, as shown in column 5 of Table 4, the amount of acetate oxidized to carbon dioxide is lowest in group D. This fall in acetate oxidation by group D sheep suggests an increase in the rate of oxidation of other substrates. Glucose cannot be one of these, as Ford (1963*a*) has shown that carbon dioxide production from glucose by starved pregnant sheep is similar to that of normal pregnant sheep.

Carbon dioxide ratios. The final values for the specific activity of carbon dioxide (column 3 of

Table 1. *Particulars of animals used in acetate-utilization experiments*

The values given for the concentrations of blood or plasma glucose and for that of plasma ketone bodies are means ± S.E.M. with the numbers of determinations in parentheses. Experimental details are given in the text.

Condition of sheep	Sheep no.	Reproductive state	Nutrition	Concn. of blood glucose (mg./100 ml.)	Concn. of blood ketone bodies (mg./100 ml.)	Wt. change in 7 days before experiment (kg.)
Group A (non-pregnant)	1	Non-pregnant	Good pasture + concentrates	38·0 ± 1·2 (4)	3·3 ± 0·2 (4)	+3
	2	Non-pregnant	Good pasture + concentrates	30·0 ± 1·1 (4)	2·8 ± 0·5 (4)	−1
	3	Lactating for 26 days	Good pasture + concentrates	38·5 ± 1·2 (4)	2·7 ± 0·2 (4)	Not weighed
Group B (in early pregnancy, i.e. < 107 days)	4	Pregnant (twins) for 86 days	Good pasture + concentrates	*55·0 ± 6·5 (4)	3·9 ± 0·1 (2)	+2
	5	Pregnant (twins) for 97 days	Good pasture + concentrates	*56·0 ± 4·4 (4)	3·9 ± 0·1 (2)	0
	6	Pregnant (single) for 106 days	Good pasture + concentrates	*58·0 ± 2·0 (2)	2·8 ± 0·9 (4)	+3
	7	Pregnant (single) for 104 days	Good pasture + concentrates	*65·0 ± 4·0 (2)	2·6 ± 0·2 (4)	+2
Group C (in late pregnancy, i.e. > 126 days; well-fed)	8	Pregnant (single) for 133 days	Good pasture + concentrates	*57·0 ± 2·2 (4)	6·7 ± 1·2 (4)	+1
	9	Pregnant (single) for 128 days	Good pasture + concentrates	*65·5 ± 4·1 (4)	4·6 ± 0·5 (4)	+1
	10	Pregnant (single) for 134 days	Good pasture + concentrates	27·0 ± 0·7 (4)	3·2 ± 0·6 (4)	+7
	11	Pregnant (single) for 140 days	Good pasture + concentrates	*40·0 ± 1·0 (4)	3·3 ± 0·4 (4)	Not weighed
Group D (in late pregnancy; under-fed)	12	Pregnant (twins) for 131 days	Poor pasture, no concentrates for 6 days	*25·0 ± 1·8 (4)	16·0 ± 1·3 (4)	−5
	13	Pregnant (twins) for 134 days	Poor pasture, no concentrates for 6 days	*21·5 ± 2·6 (4)	29·5 ± 4·0 (4)	−5
	14	Pregnant (single) for 132 days	Housed and given 150 g. of hay/day for 6 days	7·2 ± 1·0 (4)	23·8 ± 1·8 (4)	−9

* Concn. of plasma glucose.

Table 3) cannot be used directly for the computation of the carbon dioxide ratio, since the specific activity of expired carbon dioxide will depend partly on the specific activity of plasma acetate. The corresponding specific activities of plasma acetate for each experiment are therefore also shown in Table 3, and, by using these values, 'adjusted specific activities for carbon dioxide' have been computed. These are the values the specific activities of carbon dioxide would be expected to have were the plasma acetate in all cases to be 1 μc/m-mole. The adjusted specific activities of carbon dioxide have then been used to compute 'corrected' carbon dioxide ratios, as shown in the last column of Table 3. The mean corrected carbon dioxide ratios are: for non-pregnant or lactating animals, 1·58; for animals in early pregnancy, 1·66; for well-fed animals in late pregnancy, 1·74; for poorly fed animals in late pregnancy, 1·18. The difference between the last two values is significant at the 5 % level.

Turnover of citric acid-cycle acids. Weinman *et al.* (1957) have shown that it is possible to estimate the turnover of citric acid-cycle acids from the ratio of the specific activities of carbon dioxide produced when [1-^{14}C]acetate and [2-^{14}C]acetate respectively are administered. They calculate that, if the rate of entry of acetyl-CoA into the cycle is 1 m-mole/min. and the flow of acids into and out of the cycle is y m-mole/min., the carbon dioxide ratio is given by $(1+2y)$. Thus, from the mean carbon dioxide ratios given in Table 3, y will be about 0·37 m-mole/m-mole of acetyl-CoA oxidized by well-fed pregnant animals and 0·09 m-mole/m-mole of acetyl-CoA oxidized by under-fed animals. These values for y alone cannot be used to calculate the whole-body turnover of citric acid-cycle acids, unless the entry rate of acetyl-CoA into the cycle in all tissues is known. This value has not been measured directly but an approximation can be obtained by assuming that the major part of the animal's carbon dioxide output arises from the citric acid cycle. Column 4 of Table 4 indicates that the mean carbon dioxide output was 6·1 m-moles/min. for the well-fed pregnant animals and 8·1 m-moles/min. for the under-fed animals. As

Table 2. *Acetate-utilization rates in pregnant and non-pregnant sheep*

The values for plasma acetate concentration and for plasma acetate specific activity are the mean of three or four determinations during the period of constant plasma specific activity. (1) indicates results during the infusion of [1-^{14}C]acetate and (2) indicates results during the infusion of [2-^{14}C]acetate. The methods of infusion and of collecting and analysing samples are given in the text.

Condition of sheep	Sheep no.	Wt. (kg.)	Mean concn. of plasma acetate (m-moles/l.)	Infusion rate (μmc/min.)	Mean sp. activity of plasma acetate (μmc/m-mole)	Acetate-utilization rate (m-moles/min.)	Acetate-utilization rate (mg./min./kg.)	Group mean ± S.E.M. (mg./min./kg.)
Group A (non-pregnant)	1	85	(1) 0·86	580	142	4·1	2·9	
			(2) 0·63	580	214	2·7	1·9	
	2	71	(1) 0·75	580	215	2·7	2·3	
			(2) 0·71	540	208	2·6	2·2	2·44 ± 0·17 (6)
	3	70	(1) 0·79	570	196	2·9	2·5	
			(2) 1·07	570	172	3·3	2·9	
Group B (in early pregnancy)	4	92	(1) 0·70	570	161	3·5	2·1	
			(2) 0·51	573	182	3·1	2·0	
	5	81	(1) 0·81	537	247	2·2	1·6	
			(2) 0·52	535	293	1·9	1·4	2·08 ± 0·27 (8)
	6	91	(1) 0·59	577	241	2·4	1·6	
			(2) 0·54	588	267	2·2	1·5	
	7	75	(1) 0·95	580	166	3·5	2·8	
			(2) 0·79	565	126	4·5	3·6	
Group C (in late pregnancy; well-fed)	8	92	(1) 0·69	500	181	2·8	1·8	
			(2) 0·87	540	150	3·6	2·4	
	9	84	(1) 1·04	593	180	3·3	2·4	
			(2) 0·57	575	230	2·5	1·8	
	10	79	(1) 1·01	575	122	4·7	3·6	2·37 ± 0·23 (8)
			(2) 1·03	575	147	3·9	3·0	
	11	80	(1) 0·81	575	174	3·3	2·5	
			(2) 0·57	575	282	2·1	1·6	
Group D (in late pregnancy; under-fed)	12	86	(1) 0·56	575	439	1·3	0·9	
			(2) 0·60	575	347	1·7	1·2	
	13	90	(1) 0·55	595	397	1·5	1·0	
			(2) 0·47	593	340	1·7	1·1	1·00 ± 0·07 (6)
	14	77	(1) 0·40	550	645	0·9	0·7	
			(2) 0·59	565	414	1·4	1·1	

Table 3. *Carbon dioxide specific activities and carbon dioxide ratios of sheep infused with* [^{14}C]*acetate*

The specific activities of plasma acetate and of CO_2 in expired air were measured as described in the text. The values are the means of values obtained when plasma acetate specific activity was steady. (1) indicates results during infusion of [1-^{14}C]acetate and (2) results during infusion of [2-^{14}C]acetate. The CO_2 specific activities of column 5 are adjusted to a plasma acetate specific activity of 1 μc/m-mole to permit calculation of the specific activity ratios of column 6. The CO_2 ratio is defined in the text.

Condition of sheep	Sheep no.	Final sp. activity of CO_2 (μmc/m-mole)	Mean sp. activity of plasma acetate (μmc/m-mole)	Adjusted sp. activity of CO_2 (μmc/m-mole)	CO_2 ratio	CO_2 ratio: Group mean ± S.E.M.
Group A (non-pregnant)	1	(1) 13·2	142	93·0	1·88	1·58 ± 0·17 (3)
		(2) 10·6	214	49·6		
	2	(1) 19·2	215	89·3	1·27	
		(2) 14·6	208	70·2		
	3	(1) 25·3	196	129	1·59	
		(2) 14·0	172	81·5		
Group B (in early pregnancy)	4	(1) 31·0	247	125	1·84	1·66 ± 0·25 (4)
		(2) 20·0	293	68·3		
	5	(1) 28·2	161	175	2·04	
		(2) 15·6	182	85·6		
	6	(1) 19·2	241	79·6	1·87	
		(2) 11·4	267	42·6		
	7	(1) 12·0	166	72·1	0·91	
		(2) 10·0	126	79·5		
Group C (in late pregnancy; well-fed)	8	(1) 16·8	181	92·8	1·36	1·74 ± 0·14 (4)
		(2) 10·2	150	68·2		
	9	(1) 18·6	180	104	1·69	
		(2) 14·2	230	61·7		
	10	(1) 14·7	122	120	1·94	
		(2) 9·3	147	63·3		
	11	(1) 18·7	174	107	1·98	
		(2) 15·2	282	54·0		
Group D (in late pregnancy; under-fed)	12	(1) 25·2	645	39·1	0·98	1·18 ± 0·11 (3)
		(2) 16·6	414	40·2		
	13	(1) 20·8	397	50·8	1·34	
		(2) 12·8	340	37·6		
	14	(1) 22·9	439	52·2	1·23	
		(2) 12·8	347	42·6		

Table 4. *Production of carbon dioxide by sheep from* [^{14}C]*acetate*

The methods of measurement and calculation are given in the text. The percentage of CO_2 derived from acetate is the mean of values from the [1-^{14}C]acetate and [2-^{14}C]acetate infusions.

Condition of sheep	Sheep no.	Percentage of CO_2 from acetate	CO_2 output (m-moles/min.)	Acetate converted into CO_2 (m-moles/min.)	Percentage of acetate converted into CO_2
Group A (non-pregnant)	1	14·2	10·1	0·7	20·5
	2	15·9	5·6	0·45	17·0
	3	21·1	4·7	0·50	16·0
Group B (in early pregnancy)	4	19·3	7·7	0·73	22·0
	5	26·1	—	—	—
	6	12·3	8·8	0·54	23·5
	7	15·2	—	—	—
Group C (in late pregnancy; well-fed)	8	16·1	—	—	—
	9	16·5	—	—	—
	10	18·3	7·2	0·66	15·4
	11	16·2	5·1	0·41	15·2
Group D (in late pregnancy; under-fed)	12	7·9	7·1	0·28	18·8
	13	8·7	9·5	0·41	25·5
	14	9·4	7·7	0·36	31·0

each mole of acetyl-CoA entering the cycle produced 2 moles of carbon dioxide, acetyl-CoA oxidation becomes approximately 3·0 and 4·0 m-moles/min. for the well-fed and under-fed animals respectively.

By using the values for y given above the turnover of citric acid-cycle acids in all tissues is 1·1 m-moles/min. for group C and 0·27 m-mole/min. for group D sheep. Thus the findings suggest that the turnover of citric acid-cycle acids in late pregnancy is decreased in under-fed sheep to about one-quarter of that of well-fed sheep.

DISCUSSION

This work was undertaken to obtain information on the utilization of acetate and the turnover of citric acid-cycle components in pregnant sheep. The results show that the acetate-utilization rate in late pregnancy is about the same as that in non-pregnant females maintained under similar conditions. Since both groups of animals were making small weight increases, it is unlikely that the synthesis of depot fat from acetate would differ greatly. It therefore seems reasonable to conclude that the acetate-utilization rate of a single foetus is similar to the overall maternal rate of acetate utilization. This is in accordance with the observations of Pugh & Scarisbrick (1955), who found in sheep foetuses an average umbilical arteriovenous difference of about 1 mg./100 ml. of blood. This would correspond to an acetate-utilization rate of 1·16 mg./min./kg. for a 3 kg. foetus, which is unlikely to affect the overall acetate turnover rate of ewe and foetus when the small size of the foetus in relation to the ewe is taken into account. The percentage of carbon dioxide derived from oxidation of acetate is similar in non-pregnant animals, animals in early pregnancy and animals in late pregnancy when well fed. Although the results suggest that the proportion of acetate converted into carbon dioxide is greater in animals in early pregnancy, the findings are insufficient to assert this with certainty. Thus there is no firm evidence of any appreciable change in the pattern of acetate metabolism during normal pregnancy.

The acetate-utilization rate in poorly-fed pregnant sheep was about one-half of that of the non-pregnant and well-fed pregnant sheep. Exogenous (alimentary) acetate would certainly be less in these animals; but, as they were losing weight, the endogenous contribution would be expected to be greater (Annison & White, 1962), although it is clearly not great enough to compensate for the fall in alimentary absorption. The three animals in this group showed a high concentration of blood ketone bodies and a fall in that of blood glucose, but there were no symptoms of pregnancy toxaemia. Nevertheless, any changes in the metabolic pattern shown by these animals might indicate changes that occur in cases of pregnancy toxaemia before the development of clinical symptoms.

The biochemical lesion underlying pregnancy toxaemia has long been a source of speculation. It has been suggested (e.g. by Reid & Hogan, 1959) that the precipitating factor is a fall in the concentration of oxaloacetate leading to an interference with citric acid-cycle activity. Acetyl-CoA would thus tend to condense to acetoacetate and β-hydroxybutyrate. In the pregnant animal, the known heavy demand for hexose by the foetus could be responsible for the fall in oxaloacetate concentration in the maternal tissues.

Kalnitzky & Tapley (1958), however, found no decrease in the concentration of oxaloacetate in the liver of starved rats, and Ford (1963*b*) found no significant fall in the concentration of oxaloacetate in the liver or muscle of ketotic sheep compared with that of normal pregnant controls. In spite of these findings, it seemed possible that oxaloacetate turnover might be depressed in ketosis; therefore the whole-body turnover of citric acid-cycle acids has been calculated from both the carbon dioxide ratio and an estimate of the amount of acetate entering the cycle, in the manner suggested by Weinman *et al.* (1957). This calculation suggests that turnover fell in the under-fed ketonaemic sheep to about one-quarter of the turnover in the well-fed pregnant sheep. It is doubtful, however, whether this diminished turnover is related to ketonaemia. Thus carbon dioxide ratios as low as those in group D (Table 3) are shown by other sheep (nos. 2, 7 and 8). In addition, in other experiments (D. B. Lindsay & E. J. H. Ford, unpublished work), with ketonaemic animals with symptoms of pregnancy toxaemia, comparatively high carbon dioxide ratios have been obtained. Interference with citric acid-cycle function should affect the oxidation of metabolites. As shown in Table 4, although the percentage of carbon dioxide derived from acetate is decreased in poorly-fed animals in late pregnancy, this is simply because less acetate is available. The acetate presented to the tissues is oxidized as readily in this group as in other groups (Table 4).

SUMMARY

1. By using the continuous-infusion isotope-dilution method non-pregnant sheep were shown to utilize acetate at the rate of $2·44 \pm 0·17$ (6) mg./min./kg. (mean $\pm$ S.E.M., with the number of experiments in parentheses). The rate for sheep in early pregnancy was $2·08 \pm 0·27$ (8), for well-fed sheep in late pregnancy $2·37 \pm 0·23$ (8), and for under-fed sheep in late pregnancy $1·00 \pm 0·07$ (6).

2. The carbon dioxide ratio, i.e. the ratio of the specific activities of expired carbon dioxide during the infusion of [1-^{14}C]acetate and [2-^{14}C]acetate, respectively, was $1{\cdot}58 \pm 0{\cdot}17$ (3) for non-pregnant sheep, $1{\cdot}66 \pm 0{\cdot}25$ (4) for sheep in early pregnancy, $1{\cdot}74 \pm 0{\cdot}14$ (4) for sheep in late pregnancy, and $1{\cdot}18 \pm 0{\cdot}11$ (3) for under-fed sheep in late pregnancy. Calculations based on the last two ratios indicate that citric acid-cycle turnover in under-fed pregnant sheep is about one-quarter of the turnover found in well-fed pregnant sheep.

3. The output of carbon dioxide is similar in all four groups of animals, but the percentage of carbon dioxide derived from acetate is low in the poorly-fed sheep in late pregnancy. The amount of acetate converted into carbon dioxide is lower in these animals but, because total acetate utilization is low in this group, the acetate presented to the tissues is oxidized as readily as in the sheep of other groups.

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Studies on Carbohydrate-Metabolizing Enzymes

10. BARLEY β-GLUCOSIDASES*

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Although cereal extracts are well-known sources of amylolytic enzymes (see, for example, Hanes, 1937), the presence of β-glucosidases in such extracts is a more recent finding. Dillon & O'Colla (1950, 1951) reported that amorphous preparations of wheat β-amylase could also hydrolyse laminarin [a polymer of β-(1→3)-linked glucose residues], and Manners (1952) demonstrated the hydrolysis of cellobiose, salicin and laminarin by barley β-amylase preparations. The latter observations have now been extended, and the action of barley preparations on several substrates containing β-glucosidic linkages has been examined. Preliminary accounts of part of this work have been published (Manners, 1955; Anderson, 1958; Cunningham, 1961). During this period (1952–1961) the results of related investigations on barley β-glucosidases were published by Preece and co-workers (see, for example, Preece & Hoggan, 1956) and by Meredith and co-workers (see, for example, Bass & Meredith, 1955). These workers have used barley β-glucosan [an essentially linear polymer of glucose containing both β-(1→3)- and β-(1→4)-linkages] as the major substrate. However, the relative proportion of the two types of linkage is uncertain [methylation studies indicate about 50% of β-(1→3)-linkages whereas periodate-oxidation analysis suggests only 30% (see Aspinall & Telfer, 1954; Parrish, Perlin & Reese, 1960)] and the sequence of linkages has not been rigidly established. Moreover, studies by Parrish *et al.* (1960), confirmed by Cunningham & Manners (1961), have shown that certain 'laminarinase' preparations can, in fact, hydrolyse β-(1→4)-glucosidic linkages. In our studies we have used laminarin and cellodextrin (a water-soluble acid-degraded

* Part 9: Kjolberg & Manners (1963).