The difference in the level of peptidase activity in different clones of tea is apparently a genetic difference that may have considerable effect on the quality potential of a clone (Sanderson, 1964).

SUMMARY

1. The progressive increase in free amino nitrogen in tea shoot tips during withering was shown to be due to the activity of an endogenous tea peptidase system.

2. A suitable assay procedure was developed for the assay of this enzyme system in cell-free preparations of tea shoot tips.

3. The tea peptidase system showed maximal activity at 52° in 1 hr. incubations. The optimum pH was near 5.0.

4. The level of activity was markedly different in the various parts of the shoot tips and in different clones.

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Ketone-Body Synthesis in Relation to Age of Lambs

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The metabolism of butyrate by the rumen and omasum as well as by the liver of the adult sheep is known to result in the production of ketone bodies (Pennington, 1952). The pathway of synthesis is similar in rumen and omasum tissue (Hird & Symons, 1961) and in the liver of the rat (Hird & Symons, 1962). Although the physiological significance of ketone-body synthesis by any of these tissues is not yet known it is likely that such synthesis by tissues of the fore-stomachs would be related to the presence of fatty acids in the diet and their production from fermentative processes in the rumen.

The development of rumen function after the first few weeks of life is followed by increased concentrations of fatty acids in the rumen (Wardrop & Coombe, 1961) and it is therefore of interest to know whether the biochemical activity of this tissue shows changes in its metabolism of fatty acids. Accordingly, the capacity of the rumen epithelium to synthesize ketone bodies has been followed from the birth of the lamb through this dietary transition to full rumen function. Parallel studies on liver tissue from the same animals have also been made. Our results, where common types of experiments have been done, confirm the results

of Walker & Simmonds (1962) on the increase of ketone-body-forming capacity of rumen tissue with age.

MATERIALS AND METHODS

Materials. Impurities in carbon tetrachloride and in 2,4-dinitrophenylhydrazine give rise to a high blank value (extractable colour) in ketone-body estimations. The reagents were purified by the following method to give very low blank values. Carbon tetrachloride (A.R.; May and Baker Ltd., Dagenham, Essex) was shaken with 0.25 vol. of 2,4-dinitrophenylhydrazine (A.R.; British Drug Houses Ltd., Poole, Dorset) as a 0.1% (w/v) solution in 2N-HCl, and after separation was distilled; it was thus freed of reacting compounds. The 2,4-dinitrophenylhydrazine [0.1% (w/v) in 2N-HCl] was extracted five times with 0.2 vol. of redistilled carbon tetrachloride to free it of colour that would otherwise be extracted with the 2,4-dinitrophenylhydrazone of acetone.

Sodium butyrate was prepared from butyric acid (L. Light and Co. Ltd., Colnbrook, Bucks.) that had been redistilled before conversion into the sodium salt. The other reagents used were all of analytical grade.

Estimation of ketone bodies. Total ketone bodies and the percentage as β -hydroxybutyrate were estimated by the method of Michaels, Margen, Liebert & Kinsell (1951) as modified by Hird & Symons (1959). Conversion of ketone bodies into acetone was carried out in bottles as described, but with silicone-treated rubber stoppers (Esco Rubber Ltd., London, E.8) to overcome a variable blank contributed by the rubber caps previously used. The solution of the 2,4-dinitrophenylhydrazone of acetone in carbon tetrachloride was extracted finally with 0.5N-NaOH (10 ml. for 10 min.) before the extinction was read at 420 m μ .

Experimental animals. Corriedale lambs from a normal grazing flock were used in experiments on the change in metabolic activity with age. The ages of the animals in days were as follows, the numbers of animals being given in parentheses: 0-0.5 (6), 1 (3), 5 (2), 6 (1), 8 (1), 12 (1), 13 (4), 17 (3), 19 (2), 21 (1), 22 (2), 23 (2), 26 (1), 27 (3), 28 (1), 34 (1), 35 (1), 36 (1), 48 (1) and 63 (1). Group 2 (below) was also included in this group for the purpose of the statistical analysis presented in Tables 1-4. Four animals aged 92 (1), 93 (1), 94 (1) and 95 (1) days were used.

For dietary experiments, Border Leicester-Merino $\times$ Dorset Horn lambs were maintained on carefully controlled diets from birth. Body weights at the time of the experiment were approx. 40-50 lb. in these animals.

Group 1 (three animals). The diet consisted of 'milk' only. The 'milk' used was a mixture of dried skim milk, edible oil, calcium caseinate, glucose, lactose and essential vitamins and minerals with the following constitution: protein, 31%; carbohydrate, 44%; fat, 16%. Lambs were offered this diet twice daily from birth until slaughter.

Group 2 (four animals). The diet consisted of the 'milk' described above plus chaffed lucerne hay.

Group 3 (three animals). Animals were grazed for 62 days and then confined to a diet of 'milk' alone for a further 70 days.

The animals in these three groups were used for the collection of saliva in other experiments (Wilson, 1963), and before being slaughtered for the present purpose were anaesthetized with Nembutal for 2-3 hr.

Preparation of tissue. Animals were killed by severing the carotid arteries, and the rumen and a sample of liver were removed, washed with ice-cold 0.9% NaCl and placed in an ice bath. The preparation of tissue from the rumen and its incubation in Warburg vessels at 38° were carried out as given by Hird & Symons (1959). The medium used was Krebs-Ringer phosphate (Umbreit, Burris & Stauffer, 1957) made up without CaCl₂. It was found possible to strip the epithelium from the attached layers of muscle of the rumen tissue from animals of all ages. In certain comparative experiments, tissue with muscle layers attached was prepared by the method described by Walker & Simmonds (1962). Samples of liver were hand-sliced according to the method described by Deutsch (1936).

Analyses of results. Change in metabolic activity with age, birth to 63 days, has been studied by fitting polynomials to the results from the 38 Corriedale lambs. Polynomials are of the general form:

$$y = a + bx + cx^2 + \dots$$

where, in this particular case, y is the ketone-body production (or other dependent variable), x is the age, and a , b , c ... are regression coefficients, only those significant being retained. The results are presented in Figs. 1-9, together with the percentages of variance of the dependent variables accounted for by the equations. Any such curve displays the best available estimate of developmental trends for the particular variable concerned.

Major differences between these curves demand no formal tests of significance but, to assist the evaluation of smaller differences, the results of supplementary analyses of variance are presented in Tables 1-4. These analyses are of results from 16 of the 38 Corriedale lambs and from four of the ten Border Leicester-Merino $\times$ Dorset Horn lambs, i.e. those fed on a 'milk' and hay diet. Transformations of these results, before statistical analysis, were in general necessary, so as to equalize the experimental error variances for the five different tissue preparations being compared.

Results showing the effect of diet on metabolic activity were from the ten Border Leicester-Merino $\times$ Dorset Horn lambs, and the results of analyses of variance are presented in Tables 5 and 6. No transformations of results were essential to the comparisons being made in these experiments.

RESULTS

Oxygen consumption. Fig. 1 and Table 1 show that at birth the addition of butyrate and of glucose plus butyrate to rumen epithelium resulted in only a very slight increase in oxygen consumption, but that by 3 weeks the increase was marked and sustained over the whole experimental period. On the other hand, liver slices from newborn animals (Fig. 2 and Table 1) responded to butyrate and this response changed little with age.

Synthesis of ketone bodies. Fig. 3 and Table 2 show that the ability of rumen epithelium to make ketone bodies both in the presence and absence of added butyrate was very low at birth, but in the presence of butyrate increased considerably with age, reaching a maximum at about 8 weeks. Comparison of the Q_{O_2} values in the presence of added

butyrate (Fig. 1) and ketone-body production (Fig. 3) suggest that these changes with age are correlated. Fig. 3 also shows the effect of adding glucose to the medium containing butyrate. In the 0–1-day-old animals glucose stimulated ketone-body production slightly (Table 2) and invariably increased the percentage appearing as β -hydroxybutyrate during the first 4 weeks (Table 4). These results are similar to those reported by Hird & Symons (1959) for omasal tissue of the adult sheep, but the stimulation by glucose is less.

As an approximation it may be assumed that when the major ketone body is acetoacetate the conversion of 1 mol. of butyrate into acetoacetate will lead to the consumption of 1 mol. of oxygen. The results presented for rumen epithelium in Fig. 4 and Table 3 indicate that at birth less than 10% of the oxygen consumption was due to the conversion of butyrate into ketone bodies. This fraction, however, steadily increased with the age of the lamb, and after 30 days 60% or more of the oxygen consumed was used in this conversion. Even without added substrate, the metabolism of

endogenous substrates to acetoacetate accounted for a substantial proportion of the oxygen consumed. The presence of glucose with butyrate raised the ratio slightly at birth but thereafter its effect was not statistically significant (Table 3).

Fig. 5 and Table 2 show that liver tissue at birth was more active than rumen epithelium in producing ketone bodies, but here too the total production increased to a maximum during the first weeks of life. Under the conditions used *in vitro*, the ketone-body production by rumen epithelium on a dry-weight basis was considerably greater than that of the liver at the age of 4 weeks. Fig. 6 and Table 3 show that in terms of oxygen consumption the liver was less influenced by the conversion of added butyrate into acetoacetate than was the rumen

Fig. 1. Oxygen consumption by rumen epithelium as related to age of lambs. A, Glucose (15 mM) + butyrate (15 mM); B, butyrate (15 mM); C, no added substrate. Percentage variance accounted for by the polynomial was: A, 80.5%; B, 59.8%; C, 26.5%.

Fig. 2. Oxygen consumption by liver slices as related to age of lambs. A, Butyrate (15 mM); B, no added substrate. Percentage variance accounted for by the polynomial was: A, 4.6%; B, 38.0%.

Fig. 3. Total ketone-body production by rumen epithelium as related to age of lambs. Symbols and conditions are as for Fig. 1. Percentage variance accounted for by the polynomial was: A, 88.5%; B, 86.4%; C, 62.5%.

Fig. 4. Total ketone-body production by rumen epithelium as related to age of lambs. The results are expressed in terms of O_2 consumption. Symbols and conditions are as for Fig. 1. Percentage variance accounted for by the polynomial was: A, 89.4%; B, 94.9%; C, 52.3%.

epithelium, the conversion accounting for a maximum of about 40 % of the total oxygen consumed. Although the change in the ratio of ketone bodies formed to oxygen consumed in liver from birth to maximum activity was twofold compared with a

tenfold change in rumen tissue, the trend was similar.

Fig. 7 shows that, except in the newborn lambs (Table 4), acetoacetate (plus acetone) was quantitatively the most important ketone body, especi-

Table 1. *Effect of age on oxygen consumption by rumen epithelium and liver slices*

Experimental details are given in the text. The numbers of animals used were: birth, 6; 12-13 days, 5; 26-28 days, 5; 92-95 days, 4.

Age (days)	O ₂ consumption (μl./mg. dry wt. of tissue/hr.)			
	Birth	12-13	26-28	92-95
Treatment				
Rumen/no substrate	3.27	4.98	4.62	3.40
Rumen/butyrate	3.82	8.46	10.64	8.10
Rumen/butyrate + glucose	3.63	9.20	11.54	9.35
Liver/no substrate	7.82	8.14	6.72	6.05
Liver/butyrate	10.15	11.36	10.58	9.88
Differences for significance:				
<i>P</i> = 0.05	0.81	0.78	1.11	1.75
<i>P</i> = 0.01	1.10	1.08	1.53	2.45

Table 2. *Effect of age on total ketone-body production by rumen epithelium and liver slices*

Experimental details are given in the text. The results have been transformed to log(100 × total ketone-body production) for statistical analysis. The retransformed means for total ketone bodies are given in parentheses. The numbers of animals used were: birth, 6; 12-13 days, 5; 26-28 days, 5; 92-95 days, 4.

Age (days)	Total ketone-body production (μmoles)			
	Birth	12-13	26-28	92-95
Treatment				
Rumen/no substrate	1.26 (0.18)	2.12 (1.32)	2.26 (1.82)	1.95 (0.89)
Rumen/butyrate	1.66 (0.46)	2.80 (6.28)	3.15 (14.26)	3.10 (12.53)
Rumen/butyrate + glucose	2.12 (1.31)	2.84 (6.89)	3.21 (16.07)	3.13 (13.49)
Liver/no substrate	2.00 (1.01)	2.33 (2.14)	2.39 (2.44)	2.11 (1.29)
Liver/butyrate	2.66 (4.57)	2.97 (9.33)	2.97 (9.29)	2.91 (8.13)
Differences for significance:				
<i>P</i> = 0.05	0.15	0.10	0.10	0.16
<i>P</i> = 0.01	0.21	0.14	0.13	0.23

Table 3. *Effect of age on total ketone bodies produced/mole of oxygen by rumen epithelium and liver slices*

Experimental details are given in the text. The results have been transformed to log(100 × total ketone bodies produced/mole of O₂) for statistical analysis. The retransformed means for total ketone bodies produced/mole of O₂ are given in parentheses. The numbers of animals used were: birth, 6; 12-13 days, 5; 26-28 days, 5; 92-95 days, 4.

Age (days)	Total ketone bodies produced (moles/mole of O ₂)			
	Birth	12-13	26-28	92-95
Treatment				
Rumen/no substrate	0.44 (0.03)	1.07 (0.12)	1.25 (0.18)	1.07 (0.12)
Rumen/butyrate	0.77 (0.06)	1.52 (0.33)	1.78 (0.60)	1.85 (0.70)
Rumen/butyrate + glucose	1.22 (0.17)	1.53 (0.34)	1.80 (0.63)	1.81 (0.65)
Liver/no substrate	0.77 (0.06)	1.06 (0.12)	1.22 (0.17)	0.98 (0.10)
Liver/butyrate	1.31 (0.20)	1.57 (0.37)	1.60 (0.40)	1.56 (0.37)
Differences for significance:				
<i>P</i> = 0.05	0.14	0.12	0.06	0.12
<i>P</i> = 0.01	0.19	0.16	0.09	0.17

ally in the presence of added butyrate. However, 20 days after birth β -hydroxybutyrate increased and at 60 days represented about one-third of the total. The presence of glucose (Fig. 8) increased the proportion in the reduced form, an observation also made by Hird & Symons (1959). In liver tissue

(Fig. 9) β -hydroxybutyrate production exceeded acetoacetate production for the first 30 days.

It is apparent that there are changes associated with the age of the animals in both types of tissue. In general, also, where ketone-body production is low the proportion in the reduced form is higher, an observation to be expected if the available reduced coenzyme concentration remains constant at a low level. The addition of glucose (Fig. 8) would be expected to alter this concentration and there is a consistent corresponding increase in β -hydroxybutyrate.

Dietary experiments. Table 5 shows that, in each group, added butyrate stimulated oxygen consumption and produced a marked rise in ketone-body production over the no-substrate level. Animals kept on 'milk' alone for 92 days developed the capacity to make ketone bodies, but the total produced was less than half that observed in tissues obtained from animals given 'milk' and hay. On the other hand, animals first allowed to graze and then returned to a diet of 'milk' for a further

Fig. 5. Total ketone-body production by liver slices as related to age of lambs. Symbols and conditions are as for Fig. 2. Percentage variance accounted for by the polynomial was: A, 51.6%; B, 34.4%.

Fig. 6. Total ketone-body production by liver slices as related to age of lambs. Results are expressed in terms of O_2 consumption. Symbols and conditions are as for Fig. 2. Percentage variance accounted for by the polynomial was: A, 65.9%; B, 49.3%.

Fig. 7. Acetoacetate (plus acetone) and β -hydroxybutyrate production by rumen epithelium as related to age of lambs. In the presence of butyrate (15 mM): A, acetoacetate + acetone; B, β -hydroxybutyrate; and in the absence of added substrate: C, acetoacetate + acetone; D, β -hydroxybutyrate. Percentage variance accounted for by the polynomial was: A, 88.4%; B, 76.8%; C, 41.6%; D, 37.5%.

Table 4. Effect of age on the percentage of β -hydroxybutyrate produced by rumen epithelium and liver slices

Experimental details are given in the text. The results have been transformed to $\arcsin \sqrt{\%}$ for statistical analysis. The retransformed means for β -hydroxybutyrate production are given in parentheses. The numbers of animals used were: birth, 6; 12–13 days, 5; 26–28 days, 5; 92–95 days, 4.

Age (days)	β-Hydroxybutyrate produced (% of total ketone bodies)			
	Birth	12–13	26–28	92–95
Treatment				
Rumen/no substrate	76.2 (94.3)	25.8 (18.9)	43.9 (48.1)	19.8 (11.5)
Rumen/butyrate	51.2 (60.7)	16.0 (7.6)	27.5 (21.3)	26.2 (19.5)
Rumen/butyrate + glucose	62.5 (78.7)	37.8 (37.5)	42.7 (46.0)	36.4 (35.2)
Liver/no substrate	52.8 (63.5)	57.1 (70.5)	61.3 (76.9)	43.5 (47.4)
Liver/butyrate	50.9 (60.2)	45.9 (51.6)	47.8 (54.9)	40.2 (41.7)
Differences for significance:				
$P = 0.05$	10.5	6.8	7.8	14.2
$P = 0.01$	14.3	9.4	10.7	20.0

Fig. 8. Acetoacetate (+acetone) and β -hydroxybutyrate production by rumen epithelium in the presence of butyrate (15 mM) plus glucose (15 mM) as related to age of lambs. A, Acetoacetate + acetone; B, β -hydroxybutyrate. Percentage variance accounted for by the polynomial was: A, 89.2%; B, 75.6%.

Fig. 9. Acetoacetate (+acetone) and β -hydroxybutyrate production by liver slices as related to age of lambs. In the presence of butyrate (15 mM): A, acetoacetate + acetone; B, β -hydroxybutyrate; and in the absence of added substrate: C, β -hydroxybutyrate; D, acetoacetate + acetone. Percentage variance accounted for by the polynomial was: A, 28.4%; B, 48.9%; C, 32.9%; D, derived from C and Fig. 5 (curve B).

Table 5. *Effect of diet on respiration and ketone-body production by rumen epithelium in the presence of butyrate*

Tissue (250–300 mg. wet wt.) was incubated at 38° in 3.0 ml. of calcium-free Krebs–Ringer phosphate buffer at pH 7.4 containing butyrate (15 mM). The gas phase was O_2 . The results were calculated to 50 mg. dry wt./hr. The Q_{O_2} values are expressed in μ l./mg. dry wt./hr.

Treatment	No. of animals	Age (days)	No substrate				15 mM-Butyrate present			
			Q_{O_2}		Ketone bodies produced (μ mole)		Q_{O_2}		Ketone bodies produced (μ mole)	
1. 'Milk' only	3	92	2.6	—	0.45	—	4.8	—	5.5	—
2. 'Milk' and hay	4	93	3.4	—	0.90	—	8.1	—	12.6	—
3. Grazing followed by 'milk'	3	133	2.7	—	0.37	—	5.1	—	5.3	—
Differences:										
1 versus 2	—	—	0.8	—	0.45†	—	3.3*	—	7.1†	—
1 versus 3	—	—	—	0.1	—	–0.08	—	0.3	—	–0.2
Differences for significance:										
$P = 0.05$	—	—	1.1	1.2	0.25	0.27	2.5	2.7	3.5	3.7
$P = 0.01$	—	—	1.7	1.8	0.37	0.40	3.8	4.0	5.2	5.5

* Significantly different at the 5% level.

† Significantly different at the 1% level.

Table 6. *Effect of diet on respiration and ketone-body production by liver slices in the presence of butyrate*

Tissue (150–200 mg. wet wt.) was incubated at 38° in 3.0 ml. of calcium-free Krebs–Ringer phosphate buffer at pH 7.4 containing butyrate (15 mM). The gas phase was O_2 . The results were calculated to 50 mg. dry wt./hr. The Q_{O_2} values are expressed in μ l./mg. dry wt./hr. No differences were significant.

Treatment	No. of animals	Age (days)	No substrate				15 mM-Butyrate present			
			Q_{O_2}		Ketone bodies produced (μ mole)		Q_{O_2}		Ketone bodies produced (μ mole)	
1. 'Milk' only	3	92	6.0	—	1.75	—	8.1	—	6.5	—
2. 'Milk' and hay	4	93	6.0	—	1.36	—	9.9	—	8.2	—
3. Grazing followed by 'milk'	3	133	5.8	—	1.20	—	8.3	—	6.8	—
Differences:										
1 versus 2	—	—	0.0	—	–0.39	—	1.8	—	1.7	—
1 versus 3	—	—	—	–0.2	—	–0.55	—	0.2	—	0.3
Differences for significance:										
$P = 0.05$	—	—	1.4	1.5	1.38	1.47	2.6	2.8	2.9	3.1
$P = 0.01$	—	—	2.1	2.3	2.04	2.18	3.8	4.1	4.3	4.6

Table 7. *Total ketone-body production from butyrate by isolated rumen epithelium and whole rumen wall from adult sheep*

Narrow strips of tissue (250–300 mg. wet wt. of rumen epithelium or 500–600 mg. wet wt. of whole rumen wall) were incubated at 38° in 3.0 ml. of calcium-free Krebs-Ringer phosphate buffer at pH 7.4 containing butyrate (final concn. 15 mM). The gas phase was O₂. The results were calculated to 50 mg. dry wt. of rumen epithelium/hr. in each case.

Experimental conditions	Ketone bodies produced (μmoles)		Percentage decrease in ketone-body production
	Epithelium alone	Whole rumen wall	
Newly killed animals	5.1	2.9	43
	5.9	3.8	36
Abattoir material	3.4	2.5	26
	3.4	3.1	9
	3.5	1.9	46
	3.4	2.2	35

70 days lost some of their ability to synthesize ketone bodies from butyrate. Results with these animals were not statistically different from those with animals reared on 'milk' alone. Liver tissue (Table 6) responded much less to dietary changes. The results with epithelial tissue indicate that grazing is not essential for the development of the system responsible for the conversion of butyrate into ketone bodies, although it seems to add a stimulus to its further development.

Experiments on whole rumen wall. The activity of the system used by Walker & Simmonds (1962) was very much lower than that found in the present work, and accordingly the two preparations have been compared. Table 7 shows that detachment of the muscle layers from the epithelial tissue before incubation led to an increase in ketone bodies released into the medium. In further experiments where the muscle layers were removed at the end of the experiment it was found that the recovery of ketone bodies from this tissue by the method of Pennington (1952) accounted for only 16–33 % of the difference. It is possible that the missing ketone bodies were metabolized by the attached muscle, but where the detached muscle was incubated with the epithelial tissue from which it was derived there was no loss. A more likely explanation of the difference comes from observations on the behaviour of sheets of epithelial tissue in the presence of butyrate (Hird & Weidemann, 1964). These observations show that ketone bodies from butyrate are preferentially synthesized close to, and released from, the surface of the epithelial tissue normally attached to muscle. The greater activity of the system used in the present work is explicable in these terms.

DISCUSSION

As the carbon atoms of the ketone bodies are largely derived from added butyrate (Hird & Symons, 1959), it is apparent from the results presented that with rumen epithelium from the adult sheep the conversion of butyrate into ketone bodies accounts for a substantial proportion (up to 70 %) of the oxygen consumption. This tissue can thus obtain a major part of its energy supply from butyrate with ketone bodies as the terminal product. Useful energy contained in the end products (ketone bodies) is still available to other tissues.

However, the physiological significance of the metabolism of butyrate and longer-chain fatty acids to ketone bodies (Hird & Symons, 1961) is still obscure. Such metabolism could be regarded as providing a substantial proportion of the energy requirement of rumen epithelium; in addition it would lower the rate of entry of fatty acids, as such, from the ingesta into the circulation. However, there is little evidence that unrestricted entry of fatty acids would be deleterious, although it is known that injection of short-chain fatty acids results in hyperglycaemia (Ash, Pennington & Reid, 1959).

Liver tissue possesses considerable capacity to manufacture ketone bodies at birth when compared with rumen epithelium, and this is possibly related to the greater metabolic significance of the liver to the animal before birth. It is likely, however, that the rise in activity during the first 2 weeks might reflect the appearance of fatty acid-oxidizing enzymes in liver either at or just before birth. Baker & Meister (1950) have reported the absence of hexanoate and octanoate oxidation in homogenates of liver in the foetal rat. The smaller changes in ability to form ketone bodies associated with dietary fluctuations are probably explicable in terms of the relative isolation of the liver from dietary influences.

The changes reported above in the rumen epithelium of lambs are correlated with the increase in papillae size that is observed to occur with age and that has also been described on feeding short-chain fatty acids (including butyrate) to calves (Vair, Ward, Frandson & Flamboe, 1960; Sander, Warner, Harrison & Loosli, 1959). In the present investigation, a definite increase in enzyme activity, as well as increasing papillae size (see also Wilson, 1963), has been observed in animals given diets known to raise the concentration of the volatile fatty acids in the rumen. Papillae are known to be sparse and small when calves and lambs have been kept on all-milk diets (Warner, Flatt & Loosli, 1956; Wardrop, 1961; Wilson, 1963), and here also ketone-body-forming capacity has been found to be lower. Further, after an extended period of grazing,

large normal papillae were replaced by smaller papillae when the diet was changed to 'milk' alone, and this has also been observed with young calves (Harrison, Warner, Sander & Loosli, 1960) and sheep (Wilson, 1963). The results obtained in the present experiments and those of Walker & Simmonds (1962) are thus consistent with the hypothesis that the ketone-body-forming system is associated with, and changes with, papillae size.

The ketone-body-forming system has already shown signs of development before there is evidence of bacterial activity in the rumen. The results therefore are consistent with a component of the whole system being independent of dietary stimulation. A similar two-component system for intestinal alkaline phosphatase has been reported in the duodenum of the rat by Tuba & Robinson (1953).

SUMMARY

1. The metabolism of butyrate to ketone bodies by rumen epithelium and liver tissue from newborn and developing sheep has been investigated.

2. In the newborn lamb rumen tissue does not produce significant quantities of ketone bodies when incubated with added butyrate, but this activity increases rapidly with age, reaching a maximum at 5-8 weeks, depending on the substrates provided.

3. Ketone-body production by rumen epithelium from the mature lamb accounts for 70 % of the oxygen consumed in the presence of butyrate; in liver this value is 40 %.

4. When lambs are reared on milk diets, both oxygen consumption and ketone-body production from butyrate by rumen epithelium are present but are lower in comparison with normal activity.

5. Liver tissue is active in producing ketone bodies from added butyrate at birth, but total production and efficiency in terms of the oxygen used reach a maximum at 3 weeks of age, independently of the diet.

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