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Metabolic changes associated with intake by cows of complete diets containing straw and concentrates in different proportions

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1. Pelleted diets containing concentrates and 0, 200, 400 or 600 g chopped straw/kg were fed *ad lib.* for 5 h daily to four cows. Concentrations of various energy-yielding metabolites were measured in samples of rumen fluid and jugular blood taken before feeding and at intervals after food was offered.

2. After feeding, the pH of rumen fluid decreased rapidly and the total concentration of volatile fatty acids (VFA) increased; the changes were greatest in the diet containing no roughage. The changes were essentially complete by 135 min at approximately the time feeding stopped. Rumen lactate concentrations were always low and increased significantly only in cows given no roughage.

3. There were highly significant relationships between the peak rumen acetate concentration after a meal and the apparent digestibility of the dry matter of that meal, and the amount of material in the rumen at the end of a meal. The latter relationship resulted in there being no difference between treatments in the total amount of acetate present in the rumen after feeding.

4. In the blood, concentrations of acetate, propionate, β-hydroxybutyrate (BHB) and lactate all increased after feeding. The increase was prolonged and maximum values were rarely reached before 4–5 h. The highest concentrations of acetate and BHB were found in cows given 200 g roughage/kg and were twice as great as those in cows given no roughage; lower concentrations were found in cows given 400 or 600 g roughage/kg. This information, together with the rumen concentrations of acetate and butyrate, was interpreted as indicating an inhibition of VFA absorption from the rumen of cows given no roughage.

5. Plasma glucose concentrations decreased rapidly for 4 h after feeding, the decrease being greatest in cows given 200 g roughage/kg. Non-esterified fatty acid concentrations also decreased after feeding 200 and 0 g roughage/kg rations, but concentrations were not high at any time.

6. It is concluded that chemical changes in the rumen correspond to feeding behaviour much more closely than changes in blood and therefore any chemostatic regulation of food intake probably occurs at the former site, with acetate playing a major role. Monitoring energy balance at tissue level is likely to be mediated hormonally, with insulin an important factor.

In a previous report (Bines & Davey, 1970) it was shown that, as the proportion of roughage in the diet of the cow was reduced, there was a linear decrease in the importance of distension of the reticulo-rumen in the regulation of food intake. It was considered that, when an all-concentrate diet was given, and possibly also with diets containing a low proportion of roughage, the operative intake control mechanisms were probably dependent on concentrations of chemical substances within the gut or at some point following absorption from the gut.

There are a number of hypotheses concerning intake regulation, some relevant to mammalian species in general, others unique to ruminants. In the absence of firm experimental evidence to the contrary, it is reasonable to assume that some generalized mechanism of regulation of energy balance may exist which applies to both ruminant and non-ruminant species. Such a mechanism would probably be multifactorial and would involve the neuroendocrine system. Since the unweaned ruminant has digestive processes essentially similar to those of non-ruminants, the existence of a common mechanism regulating energy balance seems likely. In the mature ruminant, peripheral aspects of the regulatory mechanism probably exist which are unique to this class of animal; these could make allowance for its unique digestive system.

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The experiment described in this paper was designed to define the nature of the chemostatic mechanism regulating intake by cows of highly digestible diets, and its relationship to known physical mechanisms.

EXPERIMENTAL

Procedure

A balanced 4×4 Latin square experiment was used to compare the following diets each given to four non-lactating cows:

Diet	Barley straw (g/kg)	Concentrate (g/kg)
60R	600	400
40R	400	600
20R	200	800
0R	0	1000

Each 5-week experimental period consisted of: 14 d for adjustment to the diet; 10 d during which voluntary food intake, digestibility, breakdown of cotton thread in the rumen and mean retention times of undigested food residues were measured; 3 d during which blood and rumen samples were collected; 3 d recovery; 3 d on which behaviour was recorded; 2 d on which the amount of material in the reticulo-rumen was measured.

Full details of the cows, their housing and the complete diets used have been given previously (Bines & Davey, 1970).

Rumen samples

On days 25–27 of each experimental period, rumen samples were collected 15 min before the cows were fed and at 60, 135, 225, 300 and 390 min after food was placed before the cows. Samples were withdrawn from a point in the lower half of the rumen contents immediately below the fistula by gentle suction through a filter held in place by a 1 kg weight. The pH of the sample was measured immediately and samples were then stored at −20°. Subsequent analysis for the total concentration and molar proportions of volatile fatty acids (VFA) was by gas–liquid chromatography (Sutton & Johnson, 1969). Lactic acid was determined by the method of Elsden & Gibson (1954). Samples collected 60 min after feeding were not analysed.

Blood samples

On days 25–27 of each experimental period, blood samples were collected 60 and 15 min before the cows were fed and at 60, 135, 225, 300 and 390 min after food was placed before the cows. Samples were withdrawn from a cannula established in the jugular vein on the 24th day. The samples were heparinized and immediately centrifuged to separate cells from plasma. A portion of the plasma was deproteinized for measurement of organic acids (Ramsey, 1963) and further portions were assayed for non-esterified fatty acids (NEFA) (Baird, Black & Faulkner, 1967), and for glucose using a commercial kit (Boehringer Corporation (London) Ltd, London).

RESULTS

Physical measurements

These have been given in detail previously (Bines & Davey, 1970). For the diets 60R, 40R, 20R, 0R intakes of dry matter (DM) were 10·73, 11·25, 10·71 and 7·47 kg/d respectively; intakes of digestible energy (DE) were 109·6, 122·6, 135·1 and 109·6 MJ/d respectively. These compare with a maintenance requirement of about 70 MJ/d DE for a 532 kg cow.

Table 1. *pH and total volatile fatty acid (TVFA) concentrations in rumen fluid of cows given* ad lib. *access to complete diets containing straw and concentrates in different proportions*†

	Period after feeding (min)					
	−15	60	135	225	300	390
Diet						
pH						
60R	7·0	6·7	6·6	6·4	6·4	6·5
40R	7·1	6·6	6·3	6·0	6·0	6·1
20R	7·0	6·4	5·9	5·5	5·5	5·6
0R	7·0	5·9	5·4	5·3	5·2	5·3
SE of diet means‡ 0·07						
SE of 'times' means§ 0·06						
			diets × times***			
TVFA (mmol/l)						
60R	61·4	—	81·3	84·3	81·2	83·2
40R	51·4	—	88·1	92·0	94·0	91·9
20R	43·6	—	108·6	114·6	114·5	106·2
0R	54·3	—	154·9	156·6	152·4	125·3
SE of diet means 4·34						
SE of 'times' means 2·92						
			diets × times***			

*** $P < 0.001$.
† For details, see p. 568.
‡ SE between diets at the same or different times.
§ SE between times for the same diet.

Rumen samples

Before the cows were fed, pH values were about 7·0 and total VFA (TVFA) concentrations were invariably low; TVFA in cows receiving diet 20R were significantly lower ($P < 0.01$) than in cows given diet 60R (Table 1). After the cows were fed, pH decreased rapidly and concentrations of TVFA increased rapidly during the first 135 min, but after that there was little change for 4 h. The extent of the change in both measurements increased with increasing concentration of the diet.

The concentrations of individual VFA and lactic acid in rumen fluid (Table 2) also changed most during the first 135 min after feeding, and the magnitude of these changes increased with increasing concentration of the ration. Immediately before feeding, the concentration of acetate was highest with diet 60R and decreased as the proportion of concentrate in the diet increased, with similar, low values for diets 20R and 0R. Concentrations of the other acids before feeding did not differ significantly between diets. After feeding, concentrations of all VFA in rumen fluid of cows given the diets containing roughage were similar. Exceptionally high values, averaging 14·2 mmol/l, for lactate in one cow on diet 40R were discarded and missing values calculated. Lactate was higher at 225 min in cows given diet 0R than in cows on the other diets. There were no other differences between treatments. Highest concentrations of all the acids were found in cows given diet 0R, although similar butyrate levels were also found with diet 20R. Other values for diet 20R were intermediate between those on the two high-roughage diets and the all-concentrate diet. There was a highly significant ($P < 0.001$) positive linear relationship between the peak rumen acetate concentration after a meal and the apparent digestibility of the DM of that meal (Fig. 1). This is in contrast to the negative correlation between the total amount of material in the rumen at the end of a meal and the apparent digestibility of DM, shown previously (Bines & Davey, 1970). Accordingly, the relationship between peak rumen acetate concentration and the amount of material in the rumen at the end of a meal was also examined (Fig. 2) and was found to be very highly significant ($P < 0.001$).

Table 2. *Concentrations (mmol/l) of volatile fatty acids and lactic acid in the rumen fluid of cows given* ad lib. *access to complete diets containing straw and concentrates in different proportions*†

	Period after feeding (min)				
	−15	135	225	300	390
Diet					
Acetate					
60R	44·5	51·9	53·6	51·5	54·3
40R	36·4	52·0	56·1	57·8	57·1
20R	27·4	60·5	64·4	65·2	62·7
0R	28·9	81·0	80·7	78·4	64·4
SE of diet means‡ 3·24					
SE of 'times' means 2·37					
		diets × times***			
Propionate					
60R	9·6	17·0	17·1	16·6	16·5
40R	8·6	18·8	17·8	17·5	16·2
20R	8·7	22·3	22·5	22·5	20·6
0R	14·7	44·8	47·4	47·3	40·2
SE of diet means 3·50					
SE of 'times' means 1·22					
		diets × times***			
Butyrate + isobutyrate					
60R	6·2	11·3	12·4	12·2	11·5
40R	5·2	15·9	16·7	17·2	17·1
20R	6·0	21·1	22·9	22·0	19·0
0R	7·5	21·3	21·4	20·2	15·7
SE of diet means 2·00					
SE of 'times' means 0·96					
		diets × times***			
Valerate + isovalerate					
60R	1·1	1·2	1·2	1·0	1·0
40R	1·3	1·5	1·5	1·5	1·6
20R	1·6	4·6	4·8	4·7	3·8
0R	2·8	7·1	6·8	6·0	4·6
SE of diet means 0·65					
SE of 'times' means 0·32					
		diets × times***			
Lactate					
60R	0·1	0·5	0·4	0·3	0·1
40R	0·2	1·6	0·9	0·7	0·4
20R	0·2	1·6	0·8	0·7	0·4
0R	0·4	2·1	2·1	1·1	1·1
SE of diet means 0·42					
SE of 'times' means 0·40					
		diets × times NS			

NS, not significant. *** $P < 0.001$. † For details, see p. 568.
‡ SE between diets at the same or different times.
§ SE between times for the same diet.

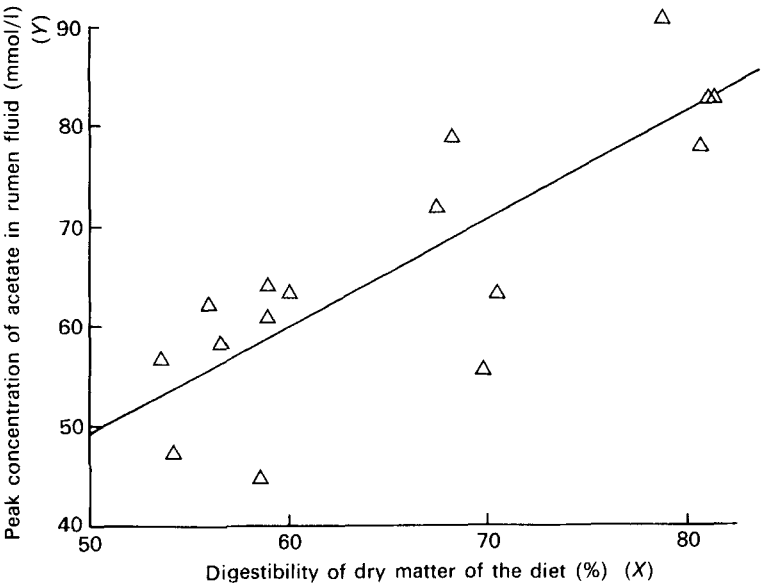

Fig. 1. The relationship between the peak concentration of acetate in rumen fluid and the apparent digestibility of the dry matter of the diet when complete diets containing straw and concentrates in different proportions were offered *ad lib.* to four cows. $Y = 1{\cdot}0684^{***}X - 4{\cdot}20$. $r = 0{\cdot}82^{***}$.

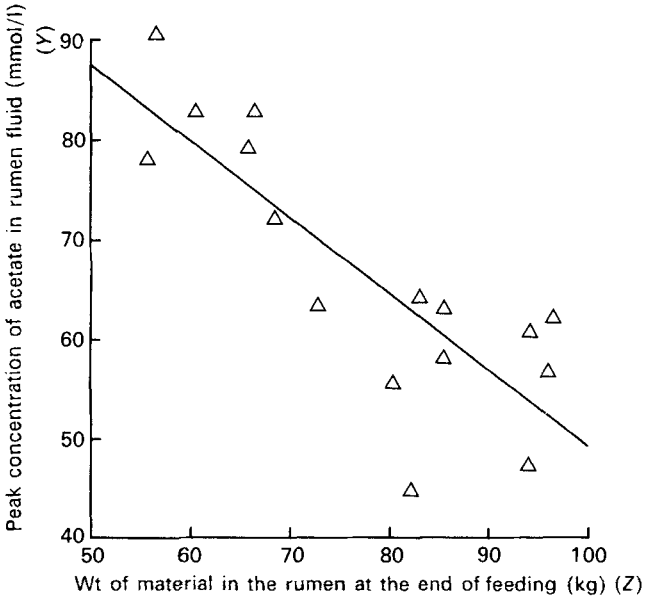

Fig. 2. The relationship between the peak concentration of acetate in rumen fluid and the weight of digesta in the reticulo-rumen at the end of a meal when complete diets containing straw and concentrates in different proportions were offered *ad lib.* to four cows. $Y = 126{\cdot}32 - 0{\cdot}7723^{***}Z$. $r = -0{\cdot}82^{***}$.

Table 3. *Calculated† total amounts (mmol) of volatile fatty acids in rumen fluid of cows before and after* ad lib. *access to complete diets of concentrates and straw in different proportions‡*

Period after feeding (min) ...	Acetate		Propionate		Butyrate		Valerate	
	−15	300	−15	300	−15	300	−15	300
Diet								
60R	2536	4060	545	1314	350	954	61·5	74·8
40R	1797	4252	423	1288	258	1276	63·5	109·0
20R	1079	4031	334	1392	236	1376	64·3	282·8
0R	1236	4170	607	2474	319	1078	120·3	318·5
SE of 'times' means§	175·4		81·7		122·6		25·21	
SE of diet means‖	193·9		135·6		135·9		32·84	
Diets × times	*		**		NS		*	

NS, not significant. * $P < 0.05$. ** $P < 0.01$.
† For details, see p. 572. ‡ For details, see p. 568.
§ SE between times for the same diet. ‖ SE between diets at the same or different times.

The amounts of VFA present in the rumen immediately before feeding and at the end of the 5 h feeding period were calculated by multiplying the concentrations of the acids in rumen fluid by the amounts of water present in the rumen, at those times. The amount of water in the rumen was calculated as the difference between the weights of total digesta and digesta DM present in the rumen (Bines & Davey, 1970; Table 5). The results are presented in Table 3.

Blood samples

The concentrations of organic acids in blood plasma are shown in Table 4. Before feeding, these were similar on all treatments and for all acids except lactate, which was significantly ($P < 0.01$) higher in cows given diet 60R. After feeding, concentrations of all acids measured in plasma increased although none of the increases was significant ($P > 0.05$) in cows given diet 60R. In contrast to the situation in the rumen, this increase in acid concentration was prolonged, with maximum concentrations rarely observed before 4–5 h after feeding. The notable exceptions to this generalization were propionate and lactate with the two high-roughage diets and β-hydroxybutyrate (BHB) with diet 60R. In all instances where maximum concentrations were observed 4 h or later after feeding, the diurnal variation was much greater than for those compounds which reached their maximum concentration earlier.

The greatest contrast between blood and rumen concentrations of acids after feeding was seen in acetate and BHB concentrations in cows given diets 20R and 0R. In the rumen, peak acetate concentration was higher and butyrate concentration the same on diet 0R compared to 20R. In blood, however, peak concentrations of acetate and BHB were at least twice as great in cows given diet 20R as in cows given diet 0R. Peak propionate and lactate concentrations in plasma were similar for both these treatments even though peak rumen concentration of propionate was twice as great with 0R as with 20R.

To permit further statistical evaluation of the observations on plasma acetate, values for individual cows in each period were plotted graphically, and curves were drawn in by hand. From these curves, values were read off for absolute peak, relative peak (absolute peak − prefeeding value), time of peak after feeding and rate of increase in concentration from the time of feeding to the peak; means of these values are shown in Table 5. Significant ($P < 0.05$) differences between treatments occurred in all measurements made except time taken to reach peak concentration. The other three measurements were all greatest for diet 20R; peak concentration being significantly ($P < 0.05$) greater than on diets 0R and 60R, and relative peak concentration and rate of increase in concentration being significantly ($P < 0.05$) greater than for all the other diets.

Table 4. *Concentrations (mmol/l) of organic acids in the plasma of cows given* ad lib. *access to complete diets containing straw and concentrates in different proportions*†

	Period after feeding (min)						
Diet	−60	−15	60	135	225	300	390
Acetate							
60R	0·69	0·60	0·64	0·90	1·06	1·06	0·85
40R	0·52	0·52	0·87	0·93	1·42	1·72	1·20
20R	0·33	0·53	0·70	1·46	2·37	2·83	1·98
0R	0·35	0·38	0·63	1·08	1·33	1·36	1·01
SE of diet means‡ 0·229							
SE of 'times' means§ 0·174							
			diet × time***				
Propionate							
60R	0·08	0·06	0·13	0·10	0·10	0·11	0·11
40R	0·08	0·08	0·15	0·12	0·13	0·13	0·11
20R	0·11	0·11	0·11	0·16	0·24	0·17	0·14
0R	0·08	0·11	0·13	0·21	0·19	0·24	0·18
SE of diet means 0·025							
SE of 'times' means 0·024							
			diet × time NS				
β-Hydroxybutyrate							
60R	0·22	0·23	0·43	0·39	0·37	0·43	0·37
40R	0·22	0·20	0·46	0·57	0·83	0·79	0·64
20R	0·19	0·24	0·45	0·93	1·31	1·82	1·78
0R	0·15	0·20	0·42	0·70	0·86	0·77	0·78
SE of diet means 0·015							
SE of 'times' means 0·014							
			diet × time***				
Lactate							
60R	0·60	0·51	0·61	0·57	0·53	0·56	0·51
40R	0·35	0·45	0·73	0·70	0·74	0·74	0·65
20R	0·27	0·42	0·48	0·89	1·10	0·92	0·92
0R	0·35	0·49	0·55	0·93	1·05	0·98	0·73
SE of diet means 0·086							
SE of 'times' means 0·081							
			diet × time**				

NS, not significant. ** $P < 0·01$. *** $P < 0·001$. † For details, see p. 568.
‡ SE between diets at the same or different times. § SE between times for the same diets.

Table 5. *Changes in acetate concentrations in plasma of cows given* ad lib. *access to complete diets containing roughages and concentrates in different proportions*†

Diet ...	60R	40R	20R	0R	Least significant difference
Peak concentration after feeding (mmol/l)	1·13	1·83	2·94	1·46	1·26
Difference betweeen peak and prefeeding concentrations (mmol/l)	0·56	1·26	2·46	1·04	1·21
Time of peak concentration (min after feeding)	266	288	273	257	48
Rate of increase in concentration (mmol/l per min)	0·0022	0·0043	0·0088	0·0040	0·0037

† For details, see p. 568.

Table 6. *Regressions* (df = 14) *of dry matter* (DM) *intake* (y_1), *digestible energy* (DE) *intake* (y_2) *and amount of* DM *digested in the rumen* (y_3) *on concentration of acetate in the plasma or rumen fluid before feeding, postfeeding maximum, and change in concentration between maximum and prefeeding in cows given* ad lib. *access to complete diets containing straw and concentrates in different proportions*†

Significance of regression

	y_1	y_2	y_3
Plasma acetate			
Prefeeding (x_1)	‡	NS	NS
Maximum (x_2)	NS	NS	NS
Change (x_3)	NS	NS	NS
Rumen acetate			
Prefeeding (z_1)	NS	NS	*
Maximum (z_2)	**	NS	NS
Change (z_3)	**	NS	‡

Details of significant regressions

	y_1	z_2	z_3
Mean ± SE	9·85 ± 0·433	66·3 ± 3·32	32·0 ± 4·70
Range	6·51–12·30	44·6–90·8	6·4–63·0
b		−0·094** ± 0·0242	−0·062** ± 0·0181
Intercept		16·05*** ± 1·64	11·85*** ± 0·667
Variance accounted for (%)		48	42

	y_3	z_1	z_3
Mean ± SE	4·56 ± 0·214	34·3 ± 2·06	32·0 ± 4·70
Range	3·02–6·01	19·8–49·1	6·4–63·0
b		−0·060* ± 0·0227	0·023‡ ± 0·0106
Intercept		6·60*** ± 0·800	3·84*** ± 0·389
Variance accounted for (%)		28	19

b, Regression coefficient.
† For details, see p. 568.
NS, not significant. ‡ $P < 0·1$. * $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$.

Regressions were calculated between various measures of intake (DM intake, DE intake, amount of DM digested in the rumen) and the concentrations of acetate in plasma and rumen fluid before feeding, at peak concentration post-feeding, and the change in concentration after feeding (Table 6). Tests for parallelism and coincidence were carried out between cows within treatments and between treatments within cows. These did not reveal any discrepancies compared with the over-all regressions, and hence only the latter are given in Table 6. None of the relationships involving plasma acetate was significant ($P > 0·05$). DM intake was significantly ($P < 0·01$) related to both absolute and relative peak concentrations of acetate in rumen fluid and the concentration of acetate in rumen fluid before feeding was significantly ($P < 0·05$) related to the amount of DM digested in the rumen.

Interesting contrasts between diets in the concentration of glucose and NEFA in plasma were also apparent (Table 7). Glucose concentrations did not vary between treatments before feeding, but decreased rapidly after feeding, the extent of the decrease being least for diet 60R and greatest for 20R. Generally, minimum concentrations were not observed until 4 h after feeding, although they occurred earlier with diet 60R, where the decrease was least. In contrast, NEFA concentrations did not differ significantly between treatments

Table 7. *Concentrations of glucose and non-esterified fatty acids (NEFA) in the plasma of cows given* ad lib. *access to complete diets containing straw and concentrates in different proportions*†

	Period after feeding (min)						
	−60	−15	60	135	225	300	390
				Glucose (mmol/l)			
Diet							
60R	3·87	3·98	3·63	3·49	3·59	3·72	3·80
40R	4·05	4·14	3·72	3·37	3·09	3·14	3·33
20R	3·94	3·93	3·63	2·92	2·52	2·52	3·22
0R	4·10	4·18	3·85	3·22	3·21	3·18	3·64
SE of diet means‡ 0·135							
SE of 'times' means§ 0·101							
				diet × time***			
				NEFA (μmol/l)			
60R	158	166	127	121	96	104	97
40R	158	149	116	114	90	111	106
20R	191	193	147	108	110	104	120
0R	175	186	142	97	98	103	121
SE of diet means 19·7							
SE of 'times' means 17·2							
				diet × time NS			

NS, not significant. *** $P < 0{\cdot}001$.
† For details, see p. 568.
‡ SE between diets at the same or different times.
§ SE between times for the same diet.

either before or after feeding, but, compared to prefeeding values, there was a significant ($P < 0{\cdot}05$) decrease after feeding in cows given diets 0R and 20R.

DISCUSSION

It is likely (Bines & Davey, 1970) that physical factors were of major importance in limiting intakes of the two diets containing the highest proportions of roughage. The objective of the present paper was to seek possible explanations for the large difference in intake between diets 20R and 0R where physical factors were unlikely to have played a large role. Since animals do not consume more energy than they can utilize, the amount of energy consumed as diet 20R cannot have been greater than the total daily amount the cow could utilize. Therefore, the amount of energy consumed as diet 0R must have been less than the daily amount the cow could utilize. This difference would have been less apparent if the frequency of feeding were increased and if the cows had had a longer period of access to food (Bines & Davey, 1970), but, during the limited period of access available in this experiment, expression of maximum intake must have been prevented largely by a chemical mechanism. Thus, it is necessary to examine contrasts between these two diets, in the concentrations of blood and rumen metabolites.

It has been shown (Bines, 1968) that diurnal variations in the concentrations of various energy-yielding metabolites in the blood of the cow are much greater when concentrates are given than when the diet consists of hay. In the present study, the largest differences in plasma metabolites between diets 0R and 20R after feeding were seen in concentrations of acetate and BHB which were twice as high with diet 20R as with diet 0R. It is thus highly unlikely that either of these factors operated to inhibit intake of diet 0R. Plasma concentrations of propionate and lactate reached very similar maximum values when either diet was given.

This would be expected if either of these acids was functioning as a signal substance in the regulation of food intake, but, as these peak values were not observed until at least 225 min after food was offered and eating had stopped after 154 min on diet 0R and 168 min on diet 20R (Bines & Davey, 1970), it seems unlikely that the concentration of either of these acids in blood played a direct role in causing cessation of eating.

Infusion of similar amounts of acetate into the blood and the rumen has shown that the concentration of the acid in the rumen was the more important in relation to regulation of food intake (Baile & Mayer, 1968). Numerous other experiments have shown that the injection of VFA into the rumen depresses intake in ruminants (see Bines, 1971). In the rumen of our cows, most changes after feeding were essentially complete by 135 min, a period of time much closer to that at which the cows stopped eating the two high-concentrate diets. Comparing diets 0R and 20R showed that the former usually produced a more extreme situation in the rumen than the latter; pH was lower and TVFA higher when diet 0R was given. This was accompanied by higher concentrations of acetate, propionate, valerate and lactate. Peak butyrate concentrations were similar for the two diets and could, therefore, possibly have controlled intake, although Baile & Mayer (1969) found that butyrate was considerably less effective in inhibiting food intake than acetate or propionate when injected into the rumen. Rumen lactate can probably be eliminated from the present discussion in view of the much higher level encountered in one cow given diet 40R than with either of the high-concentrate diets. Acetate and propionate both reached substantially higher concentrations when diet 0R was given than when the cows received diet 20R. If critical concentrations of these acids were not exceeded when diet 0R was given, one would have anticipated that intake of diet 20R would have continued until blood levels were similar to those observed when diet 0R was given. Thus it appears unlikely that concentrations of these acids could have operated alone to restrict intake of diet 20R.

There was a significant negative linear relationship between the total amount of digesta in the rumen after a meal and the digestibility of the diet (Bines & Davey, 1970). This contrasts with the positive relationship between peak rumen acetate concentration and digestibility of the diet now reported. Therefore, it is possible that, over the range of digestibilities examined in this experiment, both rumen distension and rumen acetate concentrations provided satiety signals which were summed to give a similar total effect (Table 3) regardless of the diet fed, and that this total effect determined the amount of food consumed. Thus there was a significant relationship between the peak concentration of acetate and the total amount of digesta in the rumen at the end of a meal. Harding & Leek (1972) have reported that certain receptors in the rumen wall are sensitive to both mechanical stimuli and chemical stimuli, including VFA; this would facilitate an additive effect of distension and VFA concentration. However, it should be noted that Baile, Mayer & McLaughlin (1969) found that an increase in acetate concentration was more effective in reducing intake than increasing the total acetate content without an increase in concentration. Certainly, a role in intake regulation for both acetate and propionate in the rumen has been clearly established by Baile & Mayer (1969) for sheep given a single diet. The possible additivity of physical and chemical satiety signals, over a range of diets, has not previously been examined, to our knowledge.

The relative concentrations of organic acids in rumen fluid and blood plasma in this experiment contrast with those reported by Simkins, Suttie & Baumgardt (1965). In their experiments, blood acetate concentration was directly proportional to rumen acetate concentration and satiety occurred when the two levels reached maxima. In the present experiment regressions of plasma acetate concentration on rumen acetate concentration were calculated for prefeeding levels, maximum postfeeding levels and for the change between the two levels; none of these regressions was significant. Furthermore, in the present

experiment, maximum concentrations of acids in blood plasma were not reached for a period after satiety was reached when cows were given diets oR and 20R, the diets on which chemostatic regulation of intake was likely to be of greatest importance. Another discrepancy between the present work and that of Simkins *et al.* (1965) is seen in the absolute levels of acids in blood and rumen fluid. Their maximum rumen acetate level of 652 mg/100 ml is equivalent to 109 mmol/l which contrasts with the highest value observed in the present work of 53·4 mmol/l with a diet containing a similar proportion of concentrate. Even allowing for the difference in intake, the contrast is still great, particularly in relation to the maximum plasma concentrations of acetate which would have been approximately 1 mmol/l in the work of Simkins *et al.* (1965), assuming a packed cell volume of approximately 0·40, and was a little higher than this in the present experiment when a similar diet was given. Cows given diet 20R in the present experiment tolerated maximum plasma acetate levels probably three times as great as those reported by Simkins *et al.* (1965) so it seems unlikely that this factor was of any great significance in controlling intake in the latter work.

In the present experiment, the contrast between diets 20R and oR in the relative maximum acetate concentrations of plasma and rumen fluid is also of interest. Thus, in spite of much higher peak concentrations of acetate in the rumen of cows given diet oR, plasma concentrations were only about half those of cows given diet 20R. Annison (1965) concluded that concentration gradient between rumen fluid and blood was the most important factor determining the rate of absorption of VFA from the rumen. It seems likely, in view of the higher rumen concentration and lower plasma concentration, admittedly in peripheral blood, that the concentration gradient would have been greater when diet oR was given and this would have been expected to give rise to higher plasma acetate concentrations with this diet than with diet 20R. If, as is claimed (Aafjes, 1967), lowered rumen pH enhances acetate absorption, this should also have contributed to a higher plasma acetate concentration in cows given diet oR. Even if allowance is made for the reduced volume of rumen contents at the end of a meal in cows given diet oR relative to that in cows given 20R, the total amount of acetate in the rumen was at least as great in cows given oR as in cows given 20R (Table 3). This may indicate a marked short-term inhibition of acetate absorption from the rumen when diet oR was given. It is clear that the process of absorption from the rumen is not fully understood and it may have played a significant role in the present work.

Satiety signals emanating from the rumen can be only part of a complete system of intake regulation. Control of food intake in animals receiving a concentrated diet adequate in all nutrients, is essentially a control of energy balance (Bines, 1971). Since utilization of energy occurs in the tissues of the animal, it is at this level that energy balance must be maintained, rather than within the rumen. Thus, signals must be derived from the tissues which indicate the relative balance between supply and utilization of energy at that level, and an integration of the signals from tissues and from the rumen will be required to complete the system. It has been suggested (Thye, Warner & Miller, 1970) that the level of NEFA in plasma may play a role in indicating energy balance at tissue level and concentrations of NEFA before feeding were found to be directly related to the amount of energy consumed at the subsequent meal. In the present experiment, concentrations of NEFA before feeding (Table 7) were not obviously related to subsequent energy intakes especially when diet oR was given. Thus a more complex monitoring of energy balance must be sought.

The changes after feeding in the concentration of glucose in plasma (Table 7) may shed some light on this. As the concentration of the diet increased, there were increases in the concentration of propionate in both rumen fluid and blood plasma. Propionate provides

one of the major sources of glucose in ruminant animals (Lindsay, 1970), yet over the range 60R to 20R plasma glucose concentrations were inversely related to blood and rumen propionate levels, and, even though glucose was higher on diet 0R than on 20R, it was still substantially lower than on 60R in spite of the very much higher propionate levels. This suggests that, in spite of the increased gluconeogenesis from propionate which is likely to have occurred after a meal of concentrates, there is an even greater rate of removal of glucose from the bloodstream, presumably by deposition as glycogen. This implies a considerable endocrine involvement, particularly of hormones such as insulin. A considerable increase in plasma insulin concentration occurs after a meal (Trenkle, 1970; Hove & Blom, 1973; Bines & Hart, unpublished), and it has been suggested that intraruminal infusions of propionate, which depress intake, may do so by causing a release of insulin (Jones, 1972; Bhattacharya & Alulu, 1975). Injections of insulin into goats (Baile & Mayer, 1968) and sheep subcutaneously or intravenously at an unspecified site (Muller & Colenbrander, 1970) did not affect food intake. However, we have seen no reports of the effects of injection of insulin at a site close to the liver, which has been suggested (Jones, 1972) as a likely site for the involvement of insulin in the regulation of food intake.

A complex picture emerges in which extremes of the rumen environment, particularly acetate concentration and physical distension, appear to be balanced against tissue energy requirement, probably subject to endocrine monitoring. Under certain undefined circumstances, an inhibition of acetate absorption from the rumen may lead to an excessive accumulation of this acid in the rumen, causing an abnormally low intake of an all-concentrate diet at any given meal. Comparison of present observations with parallel measurements in lactating cows may help to elucidate the critical levels.

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REFERENCES

Aafjes, J. H. (1967). *Z. Tierphys. Tierern. Futtermittelk.* **22**, 69.
Annison, E. F. (1965). In *Physiology of Digestion in the Ruminant*, p. 185. [R. W. Dougherty, editor]. Washington, DC: Butterworths.
Baile, C. A. & Martin, F. H. (1971). *J. Dairy Sci.* **54**, 897.
Baile, C. A. & Mayer, J. (1968). *J. Dairy Sci.* **51**, 1490.
Baile, C. A. & Mayer, J. (1969). *Am. J. Physiol.* **217**, 1860.
Baile, C. A., Mayer, J. & McLaughlin, C. L. (1969). *Am. J. Physiol.* **217**, 397.
Baird, J. D., Black, M. W. & Faulkner, D. E. (1967). *J. clin. Path.* **20**, 905.
Bhattacharya, A. N. & Alulu, M. (1975). *J. Anim. Sci.* **41**, 225.
Bines, J. A. (1968). *Proc. Nutr. Soc.* **27**, 15A.
Bines, J. A. (1971). *Proc. Nutr. Soc.* **30**, 116.
Bines, J. A. & Davey, A. W. F. (1970). *Br. J. Nutr.* **24**, 1013.
Elsden, S. R. & Gibson, Q. H. (1954). *Biochem. J.* **58**, 154.
Harding, R. & Leek, B. F. (1972). *J. Physiol., Lond.* **223**, 32*P*.
Hove, K. & Blom, A. K. (1973). *Acta endocr., Copenh.* **73**, 289.
Jones, G. M. (1972). *Can. J. Anim. Sci.* **52**, 207.
Lindsay, D. B. (1970). In *Physiology of Digestion and Metabolism in the Ruminant*, p. 438. [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
Muller, L. D. & Colenbrander, V. F. (1970). *J. Anim. Sci.* **31**, 145.
Ramsey, H. A. (1963). *J. Dairy Sci.* **46**, 480.
Simkins, K. L., Suttie, J. W. & Baumgardt, B. R. (1965). *J. Dairy Sci.* **48**, 1629.
Sutton, J. D. & Johnson, V. W. (1969). *J. agric. Sci., Camb.* **73**, 459.
Thye, F. W., Warner, R. G. & Miller, P. D. (1970). *J. Nutr.* **100**, 565.
Trenkle, A. (1970). *J. Nutr.* **100**, 1323.

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