Effects on plasma insulin of intermittent infusions of propionic acid, glucose or casein into the alimentary tract of non-lactating cows maintained on a liquid diet

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1. An experiment was conducted using three non-lactating cows completely maintained by infusions of volatile fatty acids into the rumen, and casein into the abomasum. Plasma insulin responses to propionic acid, glucose or casein were recorded. Further information was obtained using protein-free infusions.

2. When part of the propionic acid was infused into the rumen in a twice-daily 3 h dose and the remainder infused continuously with acetic and butyric acids and casein, there were large increases in the concentrations of propionic acid and insulin in the jugular blood. When glucose, corresponding in energy to that supplied by the intermittent propionic acid infusions was similarly infused, the plasma levels of glucose and insulin were increased. Glucose appeared to stimulate a greater increase in insulin than did propionic acid. Casein infused into the abomasum in intermittent doses produced a rise in plasma insulin, but smaller than that observed with propionic acid or with glucose.

3. The protein-free infusion was characterized by a lower concentration of insulin in the blood plasma, a reduction in plasma urea and free amino nitrogen and unchanged plasma glucose.

Glucose, volatile fatty acids (VFA) and amino acids (AA) are generally considered to be potential stimuli for insulin secretion in the ruminant. There are various reports of intravenous infusion of glucose or VFA having produced elevation in plasma insulin concentration (Manns & Boda, 1967; Horino *et al.* 1968). Similar rises were not observed when these nutrients were infused into the abomasum and rumen respectively, so as to ensure more normal absorption and metabolism (Sterm *et al.* 1970; Bassett, 1972).

The present experiment was designed to measure plasma insulin and metabolites in cattle when glucose, propionic acid or casein were infused as intermittent doses or continuously into the rumen or abomasum. Information on metabolites in the blood plasma of ruminants maintained on protein-free diets is not easy to interpret, since protein of rumen microbial origin is still digested and absorbed in the small intestine. A study was made when the animals were maintained on a protein-free infusion.

EXPERIMENTAL

Animals and treatments

Three non-lactating Friesian cows, each fitted with a rumen cannula and with an abomasal catheter, were used. Their initial and final average live weights were 517 and 576 kg respectively. They were infused as described by MacLeod *et al.* (1982). No solid food was offered. The infusion room temperature was maintained at approximately 22° and the level of infusion was calculated to be approximately 1·5 × maintenance energy from an estimated requirement of 0·450 MJ/kg live weight$^{0.75}$ per d. The cows were allocated to a randomized sequence of six treatment periods, each of 7 d duration. When increased amounts of VFA or different mixtures were required for consecutive treatments, the change was effected gradually over 2 d.

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Infusion procedures

Continuous VFA and casein infusions. A mixture of VFA was infused into the rumen and a casein solution into the abomasum over periods of 24 h. The molar proportions of the VFA were 0·65 for acetic acid and 0·175 each for propionic and butyric acids.

Intermittent doses of propionic acid. A proportion of the propionic acid equivalent to 0·15 of the daily energy supplied by all the VFA was infused into the rumen in intermittent doses of 3 h duration over the periods 11.00–14.00 hours and 23.00–02.00 hours. The remainder of the propionic acid together with acetic and butyric acids was continuously infused as a mixture of VFA in the molar proportions 0·74, 0·06 and 0·20 for acetic, propionic and butyric acids respectively.

Continuous glucose infusion. Glucose in an amount corresponding to the energy supplied by propionic acid given as intermittent doses was infused continuously into the abomasum. The VFA infused into the rumen had the same molar proportions as that used during continuous VFA and casein infusion and the amounts infused were reduced to balance the energy given as glucose.

Intermittent infusions of glucose. The same amount of glucose as that given during continuous glucose infusion was infused into the abomasum in two doses only, timed as for the doses of propionic acid.

Intermittent infusions of casein. Casein solution, equivalent in energy value to the infusions of propionic acid and glucose, was similarly infused into the abomasum in two separate doses. The remaining casein was continuously infused in the normal manner.

Casein-free infusion. During this period no casein was infused and the amounts of VFA were increased to maintain a level of infusion almost isoenergetic with the other treatments.

Measurements

Plasma samples. Blood was withdrawn through a catheter inserted in the jugular vein on the day before sampling. Blood samples were withdrawn on the last 2 d of each period. The samples were removed at two-hourly intervals between 08.00 and 20.00 hours and were assumed to be representative of the complete daily infusion cycle. From 11.00 to 14.00 hours, blood samples were taken half-hourly. The samples were collected in heparinized tubes and placed in an ice-bath for up to 30 min until centrifuged at 1670 ***g*** for 20 min.

Chemical analysis. The plasma samples were analysed for insulin by radioimmunoassay (Bassett & Thorburn, 1971), for glucose (Trinder, 1969), for free NH_2-N (Palmer & Peters, 1969) and for urea (Marsh *et al.* 1965). Samples obtained at 11.00, 12.00, 13.00, 14.00 and 16.00 hours from the continuous VFA infusion, intermittent doses of propionic acid and of glucose treatments, were analysed for VFA by a freeze-transfer and gas–liquid chromatographic technique. The method used was a modification of that described by Ribeiro (1979) based on the procedure of Pethick *et al.* (1981). In brief, 2 ml plasma were added to 2 ml 2 M-citric acid and 0·2 ml ^{14}C-labelled acetic acid (a transfer marker containing about 4000 counts/min and 3 nM-acetate in 0·2 ml) in a flask which was rotated in liquid N_2 to produce a frozen film. The flask was quickly connected to a vacuum pump and to a receiving tube containing 50 μl 1 M-sodium hydroxide which was immersed in liquid N_2. Overnight VFA and water sublimated gradually from the contents of the flask to freeze in the receiving tube. The sample thus transferred was freeze-dried. Immediately before analysis it was dissolved in 50 μl metaphosphoric acid (80 g/l), and 50 μl 20 mM-hexanoic acid were added as a quantitative chromatographic internal standard. After centrifugation, 50 μl were taken for measurement of ^{14}C activity to determine the efficiency of transfer of VFA (usually 60–70 %) and 0·3 μl was injected into a gas–liquid chromatograph column for measurement of VFA. Standards and appropriate reagent blanks were similarly treated. It was assumed

Table 1. *Daily amount of infusates and gross energy of infusates administered to non-lactating cows*

(Quantities in parentheses are those given as intermittent infusions)

	Volatile fatty acids (kg)			Casein (kg)	Glucose (kg)	Gross energy (MJ)
	Acetic	Propionic	Butyric			
Continuous infusion	2·23	0·74	0·88	0·50	—	81·69
Propionic acid: intermittent infusion	2·29	0·74 (0·50)	0·91	0·50	—	83·33
Glucose: continuous infusion	1·90	0·63	0·75	0·52	0·66	82·23
Glucose: intermittent infusion	1·88	0·62	0·74	0·50	0·67 (0·67)	81·16
Casein: intermittent infusion	2·17	0·72	0·86	0·52 (0·46)	—	80·39
Casein-free infusion	2·53	0·84	1·00	—	—	79·15

that each VFA was transferred with the same efficiency as acetic acid, although in fact this may have overestimated the efficiency of transfer of propionic and butyric acids by about 10 and 15% respectively (Ribeiro, 1979).

Statistical analysis

The experiment was designed and analysed as a randomized block with animals corresponding to blocks. Replicate samples were taken on two consecutive days for each animal and each treatment. The means of those samples were analysed. The values relating to insulin concentrations were analysed using a square-root transformation.

RESULTS

The amounts of VFA, glucose and casein actually infused are given in Table 1. The average values for insulin, glucose, free NH_2-N and urea in the plasma over the thirteen sampling times are given in Table 2. Mean values for three samples obtained before the infusions (08.00, 10.00 and 11.00 hours) and the values at 14.00 hours corresponding to the end of the infusions are also shown. The changes in concentration of insulin, glucose, free NH_2-N and urea are given in Fig. 1. For convenience, the six treatments were divided into two groups. The basal treatment, continuous infusion of VFA and casein, is included in both groups in the figure.

Insulin. Plasma insulin concentration was relatively constant during the continuous VFA and casein infusions, continuous glucose infusion and casein-free infusion. When casein was infused intermittently, insulin concentration tended to rise gradually during the infusions, reaching about 48 mU/l during the last sampling times of 13.30 and 14.00 hours, but none of the values differed significantly from those observed during the continuous VFA and casein infusions.

With intermittent infusions of propionic acid, a steady increase in plasma insulin was observed. At 14.00 hours, the time at which the intermittent infusion was stopped, the concentration was significantly different ($P < 0·01$) from that observed on continuous infusion. Insulin concentrations were back to basal levels 2 h after the intermittent infusions had ceased.

Continuous infusion of glucose did not elevate plasma insulin to levels significantly above those seen when glucose was not infused. Intermittent infusions of glucose produced similar trends to those of propionate but with two main differences. First, the concentration

Table 2. *Plasma concentration of insulin, glucose, free amino-nitrogen and urea given as the average concentration of the three sampling times before the intermittent infusions (pre-infusion), the concentration at 14.00 hours (end of the infusion) and the average concentration of all the samples (mean)**

	Insulin (mU/l)			Glucose (mg/l)			Free NH_2-N (mg/l)			Urea (mg/l)		
	Pre-infusion	14.00 hours	Mean	Pre-infusion	14.00 hours	Mean	Pre-infusion	14.00 hours	Mean	Pre-infusion	14.00 hours	Mean
Continuous VFA infusion	25·7	24·8	28·1	685	648	669	24	26	25	99	100	99
Propionic acid: intermittent infusion	17·3	89·5	37·0	661	626	670	27	25	27	110	91	104
Glucose: continuous infusion	25·0	32·5	31·2	696	698	713	27	27	27	97	98	98
Glucose: intermittent infusion	20·1	120·0	44·2	668	987	752	26	23	25	93	95	95
Casein: intermittent infusion	23·9	48·9	30·6	677	608	665	21	33	26	106	104	105
Casein-free infusion	22·4	25·7	24·4	674	670	674	19	20	20	39	41	39
SED (10 df)	3.42†	16·24†	4·82†	30·4	40·7	26·3	1·3	2·0	1·5	8·4	6·5	7·3

VFA, volatile fatty acids; SED, standard error of difference.

* For details of infusions, see p. 140 and Table 1.

† SEM, levels of significance were obtained using a square-root transformation.

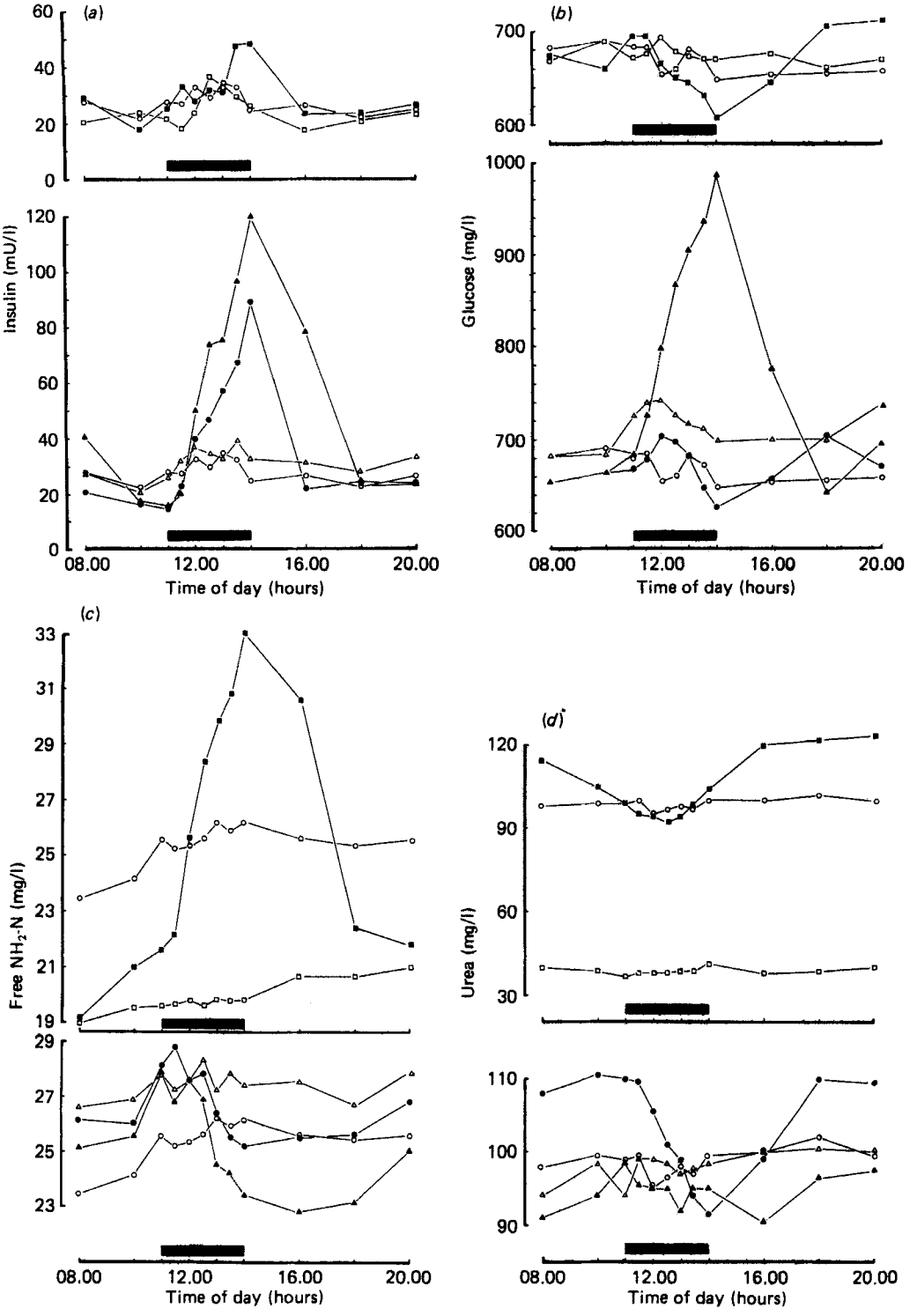

Fig. 1. Changes in plasma concentrations of (*a*) insulin, (*b*) glucose, (*c*) free amino-nitrogen and (*d*) urea of cows maintained by intragastric infusions given as a continuous infusion of volatile fatty acids and casein (○), with intermittent infusion of propionic acid (●), as a continuous glucose infusion (△), with intermittent infusion of glucose (▲), as a casein-free infusion (□) or with intermittent infusion of casein (■). ▬, Duration of the intermittent infusions. For details of infusion, see p. 140 and Table 1.

at the end of the intermittent infusions of glucose was greater (120·0 mU/l) than that with propionic acid (89·5 mU/l). Second, plasma insulin was still very high 2 h after the infusions of glucose had ceased and it took a further 2 h for the levels to return to a basal concentration.

Glucose. The concentration of plasma glucose remained constant during continuous VFA and casein-free infusions. Plasma glucose did not alter during the first half hour of the intermittent infusions of casein but subsequently there was a trend for a decrease, reaching a value of 608 mg/l by 14.00 hours. Plasma glucose increased from 661 mg/l before the intermittent infusions of propionic acid to 704 mg/l 1 h after their start; the concentration then decreased and reached the value of 626 mg/l by the end of the infusion at 14.00 hours. None of those values differed significantly from the corresponding values of the continuous VFA and casein infusions. When glucose was infused intermittently, plasma glucose increased from 668 mg/l at the start to 987 mg/l by the end of the infusion. The level was still high at 776 mg/l 2 h later. The concentration of 987 mg/l at 14.00 hours differed significantly ($P < 0·001$) from the control value of 648 mg/l on the continuous VFA and casein infusions.

Free NH_2-N. During the continuous VFA and casein, the casein-free and the continuous glucose infusions, the concentration of plasma free NH_2-N did not fluctuate to any great extent. With intermittent infusions of casein, however, the concentration increased from a basal level of 21 to 33 mg/l by the end of the infusion at 14.00 hours and differed significantly ($P < 0·01$) from the values obtained on continuous infusion of VFA and casein. At 2 h after the infusion the concentration was still high, and it took a further 2 h to return to the basal level. With intermittent infusions of propionic acid there was a trend for plasma free NH_2-N to show a period of high concentration corresponding to the beginning of the infusions followed by a decline. This pattern was more clearly seen when intermittent infusions of glucose were given. The average concentration of plasma free NH_2-N over the 12 h observation was significantly lower ($P < 0·01$) during the casein-free infusion than during the continuous VFA and casein infusion.

Urea. Variations in plasma urea were not large over the 12 h periods of observation except during the intermittent infusions of casein and of propionic acid. In the former case before the intermittent infusions, the concentrations of urea tended to decrease from 08.00 to 11.00 hours and reached low values at mid-infusion. It then rose progressively to plateau at 16.00 hours. With intermittent infusions of propionic acid the urea concentration remained high during the initial 3 h pre-intermittent infusion phase. The infusion was then accompanied by progressively falling levels of urea. Subsequently blood urea levels recovered to their pre-intermittent infusion levels within 4 h. The concentrations of urea during the intermittent infusions of casein and propionic acid did not differ significantly from the corresponding values of the continuous VFA and casein infusions but the average plasma urea concentration was significantly lower ($P < 0·001$) during the casein-free infusion than during the continuous VFA and casein infusions.

VFA. The concentrations of VFA in plasma are given in Table 3. There were no large changes in the five samples taken during the continuous VFA and casein infusions and the intermittent infusions of glucose whereas the concentration of all three acids rose during the intermittent infusions of propionic acid. The extent of the increase was greatest for propionic acid which ranged from 0·01 mmol/l before the intermittent infusions to 0·21 mmol/l by the end of the infusion at 14.00 hours. The corresponding values for acetic acid were 1·42 and 2·18 mmol/l and for butyric acid 0·01 and 0·04 mmol/l. The concentrations at 14.00 hours were significantly higher than the corresponding values on continuous VFA and casein infusions ($P < 0·05$ for acetic and butyric acids, $P < 0·01$ for propionic acid). Fig. 2 summarizes the changes in plasma insulin, glucose and propionic acid when the animals were intermittently infused with propionic acid or glucose.

Table 3. *Concentration (mmol/l) of volatile fatty acids in the blood plasma of cows given either continuous or intermittent infusions of propionic acid or glucose during 3 h**

		Control (continuous)	Propionic acid (intermittent infusion)	Glucose (intermittent infusion)	SED (4 df)
		Concentration of acetic acid			
Immediately before intermittent infusion (11.00 hours)		1·58	1·42	1·18	0·201
Start of each infusion (h)	1	1·64	1·69	1·37	0·148
	2	1·81	1·80	1·26	0·272
	3	1·72	2·18	1·05	0·156
Post-infusion (h)	2	1·84	1·67	1·14	0·164
		Concentration of propionic acid			
Immediately before intermittent infusion (11.00 hours)		0·02	0·01	0·03	0·004
Start of each infusion (h)	1	0·03	0·04	0·03	0·005
	2	0·03	0·07	0·03	0·013
	3	0·03	0·21	0·03	0·024
Post-infusion (h)	2	0·03	0·05	0·03	0·010
		Concentration of butyric acid			
Immediately before intermittent infusion (11.00 hours)		0·02	0·01	0·01	0·003
Start of each infusion (h)	1	0·02	0·03	0·02	0·007
	2	0·02	0·03	0·02	0·006
	3	0·02	0·04	0·02	0·006
Post-infusion (h)	2	0·02	0·02	0·02	0·003

SED, standard error of difference.
* For details of infusion, see p. 140 and Table 1.

DISCUSSION

Insulin responses to different nutrients

The differences in patterns of the insulin response curves, the heights of the peaks and the times needed to return to initial concentrations suggest that different mechanisms were involved in stimulation of the pancreas.

Propionic acid. The concentration of plasma insulin increased immediately after the intermittent infusion of propionic acid began and continued to rise throughout the infusion. There was little change in the concentration of plasma glucose. The difference between the two response curves suggests that the increase in plasma insulin was not mediated via glucose. The concentration of propionic acid in the peripheral plasma increased significantly during the intermittent infusions of propionic acid (Fig. 2). Manns *et al.* (1967) showed that intravenous infusions of propionic or butyric acids resulted in increased insulin concentrations in jugular blood, suggesting that these acids may stimulate insulin secretion. Bines & Hart (1984) studied the role of propionic acid in the control of insulin secretion. They infused a mixture of VFA into the rumen of cows given 3 kg hay and serially omitted one of the acids from the mixture. They observed that the omission of only propionic acid resulted in a major reduction in insulin concentration indicating that 'priopionate and only propionate is a major stimulant of insulin secretion in the bovine' (Bines & Hart, 1984).

A problem, however, arises with jugular infusions in that the plasma concentration may rise more abruptly and to a higher peak than when similar quantities are absorbed from the gut when the nutrients must traverse the gut wall and liver before reaching the pancreas.

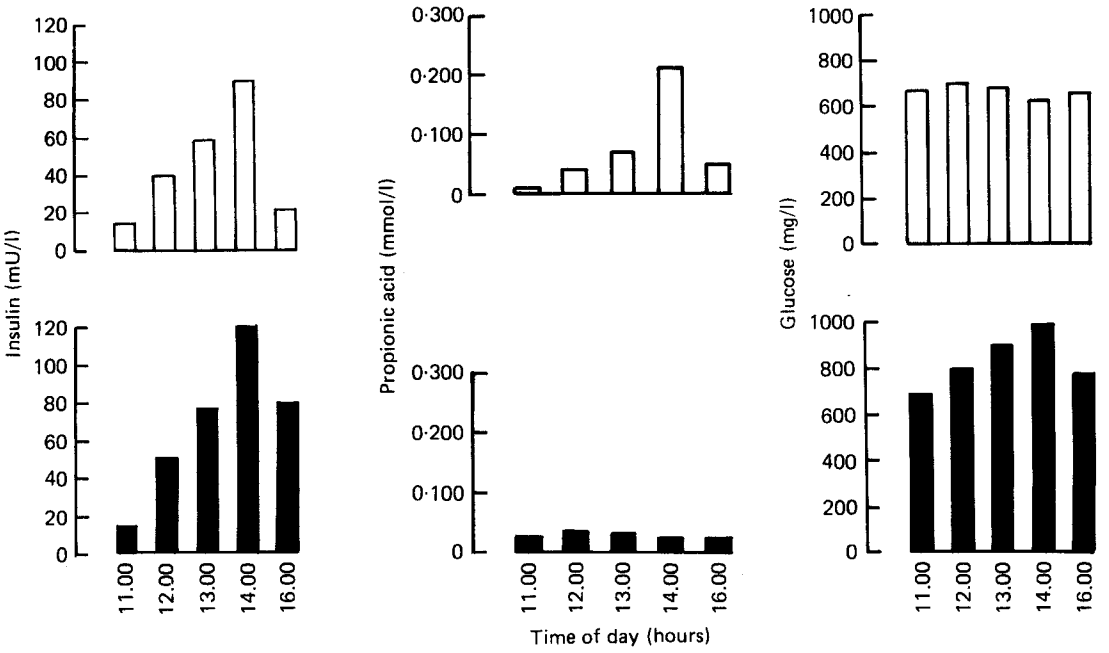

Fig. 2. Changes in plasma concentration of insulin, propionic acid and glucose when propionic acid (□) or glucose (■) was infused intermittently. For details of infusions, see p. 140 and Table 1.

The concentrations of propionic and butyric acids in the peripheral circulation of ruminants are generally low under normal feeding practice but the situation may differ in lactating dairy cows given large amounts of concentrate over short periods at milking time. In the present experiment so much propionic acid may have been absorbed that the capacity of the gut wall and liver to metabolize it seems to have been exceeded, leading to a substantial rise in its concentration in peripheral blood. During the intermittent infusions, jugular propionic acid concentration rose from a pre-infusion level of 0·01 mmol/l to over 0·21 mmol/l at the end of the infusion. It is suggested that the high propionic acid concentration served as the stimulus for insulin secretion. However, this does not necessarily imply a direct effect of propionic acid on the pancreas. Propionic acid may have induced glucagon release which in turn would have stimulated insulin secretion either directly or via its effects on hepatic glycogenolysis and gluconeogenesis (Bassett, 1972). Although glucagon measurements were not made in the present study, the finding that glucose tended to decrease following the intermittent infusions of propionic acid suggested that glucagon secretion was not stimulated. Propionic acid may also have stimulated gastrointestinal hormone release (Trenkle, 1972) or acted on hepatic neuro receptors (Anil & Forbes, 1984) which may then have caused insulin secretion. When propionic acid was infused intermittently there was a small rise in plasma butyric acid and since butyric acid is a powerful stimulus to insulin secretion (Manns *et al.* 1967) some of the response may have originated from the increase in butyric acid concentration.

Glucose. Plasma insulin concentration increased when glucose was infused intermittently, as it did with propionic acid. There were however differences, principally in the greater size and elevation of the insulin response to glucose. The response curves for plasma glucose also differed in that the pattern of plasma glucose mirrored that of insulin when glucose was infused intermittently while little change was evident in plasma glucose when propionic acid

was infused. The insulin response to intermittent infusions of glucose was attributed to a direct effect of the elevated plasma concentration of glucose on the pancreas.

Casein. The insulin response to intermittently infused casein differed from the responses to intermittent infusions of glucose and propionic acid in that there was a time-delay and the amplitude of response was less. The response to casein was apparently not due to an increased gluconeogenesis from AA since there was a simultaneous decline in plasma glucose levels. The concentration of free NH_2-N was increased during the intermittent infusions of casein suggesting that some AA may have stimulated the pancreas. Little information is available about the effect of AA on insulin production in the ruminant but Hertelendy *et al.* (1969) and Chew *et al.* (1984) intravenously infused arginine and Davis (1972) infused arginine, leucine and phenylalanine, resulting in increased plasma insulin.

Changes in free NH_2-N and urea

When casein was infused intermittently, the concentration of free NH_2-N in plasma increased to high values at the end of the infusions. By contrast urea concentration was low and it was not till 2 h after the end of the infusions that the concentration of urea was maximal. The present observations are different from the results reported by Williams & Smith (1975) in which plasma AA and urea concentrations decreased soon after the morning feed of calves given milk. The increase in plasma free NH_2-N in our infused animals is similar to that found in simple-stomached animals and could be associated with the relatively large amount of protein infused in the abomasum over a short period of time, while in calves offered a milk feed the concentration of N in the small intestine is reasonably constant and not greatly affected by feeding (Mylrea, 1966). There are various ways in which AA are metabolized, such as gluconeogenesis or incorporation into peptides and proteins. In the present experiment an increase in the incorporation of AA into peptides and proteins appeared unlikely since no large demands were expected from non-lactating animals. The decrease in plasma glucose during the intermittent infusions of casein indicated that gluconeogenesis did not appear to have been of great importance. The most important effect seemed to be the increased concentration of free AA. The high urea concentration observed 2 h after the intermittent infusions of casein may have resulted from either a higher rate of catabolism of AA or a lower rate of AA synthesis. The declining plasma urea from 08.00 to 12.30 hours suggests a reduction in catabolism of AA or an increase in their synthesis since little protein was being infused during this pre-infusion period.

The concentration of free NH_2-N was high at the beginning of the intermittent infusions of glucose and propionic acid. It then decreased and remained low until 2 or 4 h after the end of the infusions. Decreases in plasma concentration of AA were also reported by Mercer & Miller (1982) in sheep which were given diets supplemented with urea, fishmeal or ground-nut meal and infused into the rumen with a mixture of VFA. In the present experiment, the extent of the decrease in free NH_2-N was smaller with intermittent infusions of propionic acid than with those of glucose. There were corresponding increases in insulin but the peak of insulin was smaller and the concentration returned to the basal level in a shorter time with the intermittent infusions of propionic acid. The reduction in free NH_2-N during the infusions may have been due to an effect of insulin on AA, facilitating the movement of AA into body reserves as suggested by the results of Call *et al.* (1972) and Brockman *et al.* (1975).

Change in plasma metabolites during the casein-free infusion

When compared with the continuous infusion, the casein-free infusion was characterized by a lower plasma concentration of insulin (13%), reductions in plasma urea (61%) and free NH_2-N (20%) and unchanged plasma glucose. In a situation of limited N availability, it is likely that the animals reduced their catabolism of protein, utilizing protein from their tissues to maintain basal NH_2-N metabolism. The large reduction in plasma urea concentration in a situation where gut microbial protein synthesis from recycled urea was virtually eliminated indicated how far the cows were able to reduce the catabolism of AA.

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