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THE ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN THE CONTROL OF PANCREATIC ENDOCRINE RESPONSES TO MILK INGESTION IN THE CALF

By S. R. BLOOM,* A. V. EDWARDS and R. N. HARDY

*From the Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG, and the *Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W.12*

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SUMMARY

1. Pancreatic endocrine reponses to ingestion of milk have been investigated in conscious unweaned calves, 3–5 weeks after birth. Passage of gastric content from abomasum to small intestine was prevented by means of a cannula placed in the duodenum adjacent to the pylorus and food was witheld for at least 22 h in order to deplete liver glycogen.

2. Under these conditions ingestion of milk was followed by a prompt rise in the concentrations of pancreatic glucagon, PP and gastrin in the arterial plasma but the usual rises in plasma glucose and insulin concentration were absent.

3. Evidence was obtained to show that absorption of glucose from the small intestine occurs sufficiently rapidly to account for the initial rise in plasma glucose concentration after feeding in normal animals. However, the rise in plasma glucagon concentration was sufficient to contribute to alimentary hyperglycaemia by promoting hepatic glycogenolysis in calves with abundant liver glycogen.

4. None of the neuroendocrine responses to ingestion of milk was affected by prior section of the splanchnic nerves whereas each was blocked by atropine (0·2 mg/kg), showing that all depend upon muscarinic, parasympathetic rather than sympathetic activity, in the absence of extraneous stress.

INTRODUCTION

The autonomic nervous system mediates the release of pancreatic hormones in response to numerous stimuli in the normal conscious animal. The evidence now available suggests that both the sympathetic and parasympathetic innervation influence the rates at which insulin and glucagon are released from the pancreas under resting conditions but that PP release, which is increased by vagal stimulation, is unaffected by sympathetic stimulation (Bloom, Edwards & Hardy, 1978; Edwards & Bloom, 1978). Pancreatic endocrine responses to non-specific stresses such as hypoxia or exercise appear to depend upon activation of the sympathetic innervation (Bloom *et al.* 1977, 1978; Järhult, Holst & Ingemansson, 1977), whereas vagal fibres provide the efferent pathway for pancreatic neuroendocrine responses to specific glycaemic stimuli such as moderate hypoglycaemia or 2-deoxyglucose (Bloom, Edwards & Vaughan, 1974; Bloom *et al.* 1978).

Much of the work that has led to these conclusions has been carried out in the young calf, in which feeding causes pronounced changes in heart rate and blood pressure due to increased sympathetic efferent activity (Bloom, Edwards, Hardy, Malinowska & Silver, 1975), as has also been described in the lamb and the kid (Bloom *et al.* 1975; Harding, Johnson, McClelland, McLeod & Whyte, 1978). The present experiments were undertaken to investigate the extent to which the changes in the concentrations of pancreatic hormones in the blood, that occur in response to ingestion of milk in the calf, are mediated via autonomic pathways. Secondary effects arising from the passage of gastric content to the intestine have been avoided by means of a cannula inserted into the proximal duodenum immediately below the pylorus.

Certain of these results have been published previously in a preliminary form (Edwards & Bloom, 1978).

METHODS

Animals

The experiments were carried out on pedigree Jersey calves obtained from local farms. The animals were kept in the Laboratory Animal House and habituated to drinking milk from a bucket for at least one week before experiments were performed. Particular care was taken to ensure that the animals used were in a healthy condition and had not recently suffered from scour.

Operative and experimental procedures

Preparatory surgery was carried out under halothane anaesthesia. Polyethylene catheters (Portex PP190) were inserted into the saphenous arteries and so positioned that the tips lay in the abdominal aorta. The free ends were secured in a plastic wash-bag and sutured to the right flank. One catheter was used subsequently for blood pressure recording and the other for collection of arterial blood samples. A J-shaped glass cannula was inserted into the proximal duodenum immediately below the pylorus in such a way as to divert the gastric content to a receptacle attached to the side of the animal. A second cannula was inserted into the duodenum just below the first to enable duodenal infusion of gastric content as desired. When required, both splanchnic nerves were cut immediately below the diaphragm. Recovery from operation was rapid and the animals were normally able to stand 10 min after anaesthesia was discontinued.

Experiments were performed on the day following operation and food was withheld during the intervening period. In each case a bucket containing 4 pints of warm milk was presented to the animal without warning in order to preclude anticipatory responses. Drinking invariably began immediately and the milk was consumed within 1–2·5 min, at which time the bucket was removed from sight. Samples of arterial blood were collected at intervals before feeding, 30 sec after feeding began and at the time the bucket was removed. Further samples were taken 2·5, 5, 7·5, 10, 20, 30, 45, 60, 90, 120, 180 and 210 min after the end of feeding.

Blood pressure records

Aortic blood pressure and heart rate were recorded by means of a Beckman miniature blood pressure transducer connected to a small U.H.F. transmitter, the signal from which could be picked up on an adjacent receiver and was recorded on a Devices M19 recorder. Both transducer and transmitter were stored in the plastic wash-bag and both cardiovascular parameters were monitored continuously. The transmitter and receiving equipment were constructed by Mr P. L. Joyce in the Physiological Laboratory.

Analytical procedures

Arterial blood samples were collected into heparinized tubes containing aprotonin (Trasylol: Bayer) 1000 K.i.u./ml blood, centrifuged at +4 °C immediately and the plasma removed and

stored at −20 °C. Glucagon was measured by a radioimmunoassay using antiserum relatively specific for pancreatic glucagon, which was C-terminal reacting (Assan & Slusher, 1972). This assay reacted less than 5% with glucagon-like immunoreactivity of enteric origin. Insulin (Albano, Ekins & Turner, 1972), gastrin (Bloom, 1974) and pancreatic polypeptide (Adrian, Bloom, Bryant, Polak, Heitz & Barnes, 1977) were measured by conventional immunoassays. Glucose and galactose were estimated enzymatically. The haematocrit was also determined. At the conclusion of each experiment small pieces of liver were removed for glycogen analysis (Edwards, 1971). In several experiments tritiated glucose (D-[1−^{3}H]glucose: Radiochemical Centre, Amersham) was dissolved in milk to provide a test-feed (1 mCi in 850 ml. warm milk). Plasma and samples of evaporated plasma reconstituted with water, were deproteinized with trichloracetic acid (0·8 ml. plasma: 0·08 ml. 55% TCA) and centrifuged. The supernatant (0·25 ml.) was added to 5 ml. liquid scintillator (KL 354; Nuclear Enterprises Ltd, Edinburgh) and counted in a Nuclear Enterprises 8312 liquid scintillation counter. Correction was made for quenching by means of an internal standard. Statistical analyses were made according to the methods of Snedecor & Cochran (1967).

RESULTS

Effects of autonomic blockade on responses to feeding in normal calves

The role of the autonomic innervation in the genesis of pancreatic endocrine responses to feeding was investigated in unweaned calves 3–5 weeks after birth. Normally, ingestion of 1·7 l warm milk (3·5 pints) causes an abrupt rise in the concentration of both pancreatic glucagon and insulin in the plasma, together with an associated rise in plasma glucose concentration (Bloom *et al.* 1975). These initial responses to feeding are transient; maximal plasma glucose and insulin concentrations occur 15–30 min after the feed and the glucagon response is still more evanescent (Fig. 1). Each of these initial responses was effectively abolished in calves with cut splanchnic nerves, that were given atropine (0·2 mg/kg) 10 min before feeding, even though the characteristic cardiovascular changes which occurred during ingestion (tachycardia and hypertension) were not significantly diminished. Following autonomic blockade, no change in plasma glucose concentration occurred for 20 min, or in glucagon and insulin concentration for 60 min after feeding (Fig. 1). After this prolonged period of quiescence there was a steady rise in all three parameters to coincident peaks at 120 min. Thus, each of these responses to feeding in the calf is greatly delayed by autonomic blockade. However, the effects of feeding were not directly comparable in these two groups of animals as the mean plasma glucose concentration immediately before the ingestion was significantly lower in the experimental group (autonomic blockade: $3·8 \pm 0·3$ mmol/l; control: $5·6 \pm 0·3$ mmol/l; $P < 0·01$). Initial mean plasma insulin concentration was also much lower in the experimental than in the control group, although the difference did not happen to achieve statistical significance (autonomic blockade: 15 ± 5 pmol/l; control: 209 ± 89 pmol/l; $P > 0·05$).

Mean plasma pancreatic polypeptide (PP) concentration rose extremely rapidly during feeding in normal calves and had risen by a peak increment of $112·4 \pm 35·4$ pmol/l at the end of the feed, from an initial concentration of $67·0 \pm 10·7$ pmol/l Thereafter it declined steadily and had returned to within the resting range after 30 min. The rapidity of this response, in which the concentration of the hormone more than doubles within 2 min, strongly suggests that it is neurally mediated. This contention is supported by the finding that the PP response was completely abolished by autonomic blockade (Fig. 2). In contrast, the changes in mean plasma

gastrin concentration during the first 60 min after feeding in these two groups of calves were closely similar and provide no indication of any autonomic involvement in gastrin release under these conditions (Fig. 2).

Visual inspection of the fore-stomachs under general anaesthesia at the end of each experiment invariably revealed the presence of a large clot of milk in the

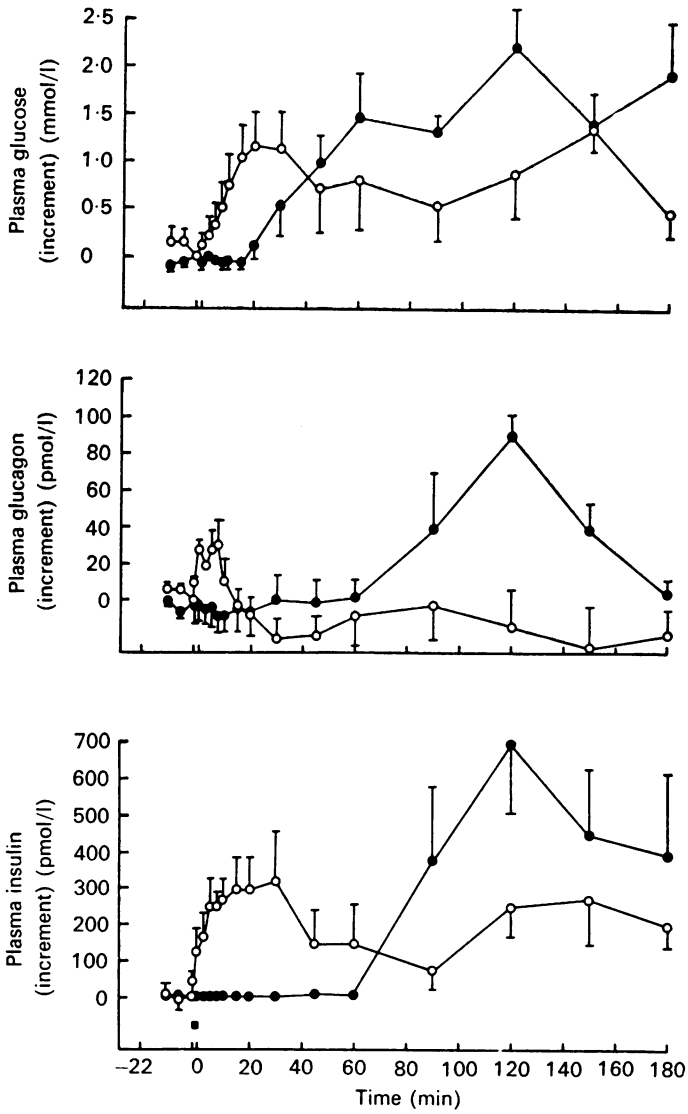

Fig. 1. Comparison of the changes in arterial plasma glucose, glucagon and insulin concentration in response to feeding in 3–5 week old calves given 1·7 l warm milk at the signal bar. ○, normal controls (n = 7; modified from Bloom *et al.* 1975). ●, calves with cut splanchnic nerves given atropine (0·2 mg/kg) 10 min before feeding (n = 4). Vertical bars: S.E. of each mean value. Absolute values before feeding as follows, with controls in brackets. Glucose: 3·8 ± 0·3 mmol/l, (5·6 ± 0·3 mmol/l). Glucagon: 35 ± 17 pmol/l, (54 ± 14 pmol/l). Insulin: 15 ± 5 pmol/l, (209 ± 89 pmol/l).

abomasum, with virtually none in either the reticulum or rumen. It was therefore concluded that passage of the ingested milk to the abomasum via the oesophageal groove had not been significantly inhibited by autonomic blockade.

These results show that the initial release of pancreatic glucagon, insulin and PP during feeding, together with the associated rise in plasma glucose concentration, depend upon the integrity of the autonomic nervous system but do not elucidate the underlying mechanism. This might be neuroendocrine; alternatively, the difference in basal plasma glucose concentration or delayed gastric emptying, due to autonomic blockade could account for the differences between these two groups

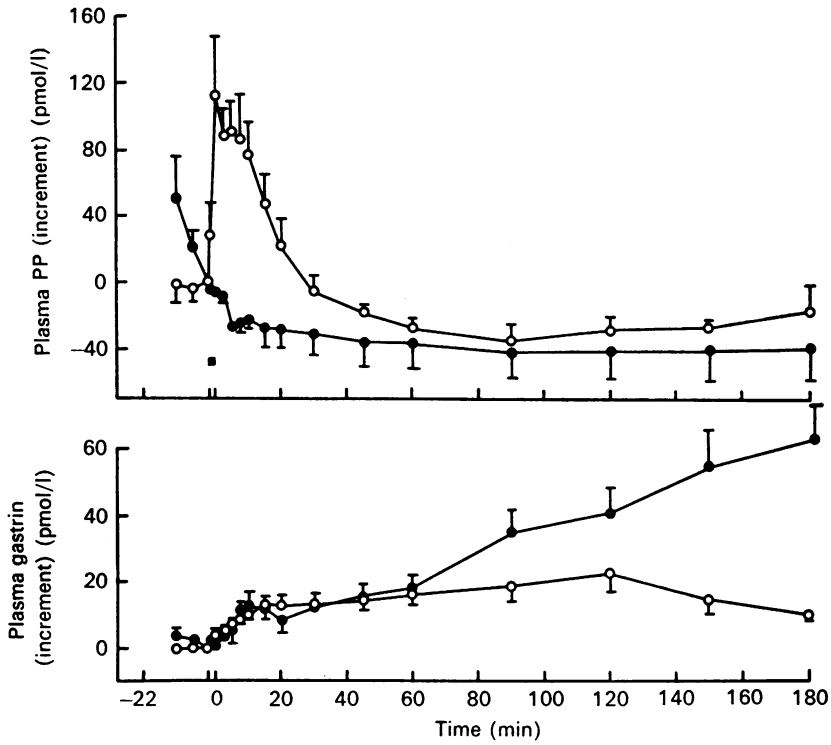

Fig. 2. Comparison of the changes in arterial plasma gastrin and PP concentration in response to feeding in 3–5 week old calves given 1·7 l warm milk at the signal bar. ○, normal controls (n = 7). ●, calves with cut splanchnic nerves given atropine (0·2 mg/kg) 10 min before feeding (n = 4). Vertical bars: S.E. of each mean value. Absolute values before feeding as follows, with controls in brackets. PP: 60 ± 18 pmol/l, (67 ± 10 pmol/l). Gastrin: 10·9 ± 2·0 pmol/l (10·0 ± 3·7 pmol/l).

of animals. These possibilities were investigated by examining the responses of calves in which a glass cannula had been placed in the proximal duodenum in such a way that effluent gastric content would be diverted to some external receptacle. In this way responses to ingestion of food and gastric digestion could be studied in isolation from any consequences of intestinal absorption.

Responses to feeding in calves with duodenal cannulae

In these experiments gastric content was collected as it emerged from the duodenal cannula and the rate of gastric emptying was estimated gravimetrically. Food was witheld for at least 22 h before each experiment, by which time gastric emptying had virtually ceased. When offered the test-meal, consisting of 1·7 l. warm milk in a bucket, each animal started to drink without hesitation and completed the meal within 1·5–2 min. The cardiovascular changes during ingestion were indistinguishable from those observed in normal animals except that the rise in blood pressure in the calves with cut splanchnic nerves was slightly smaller.

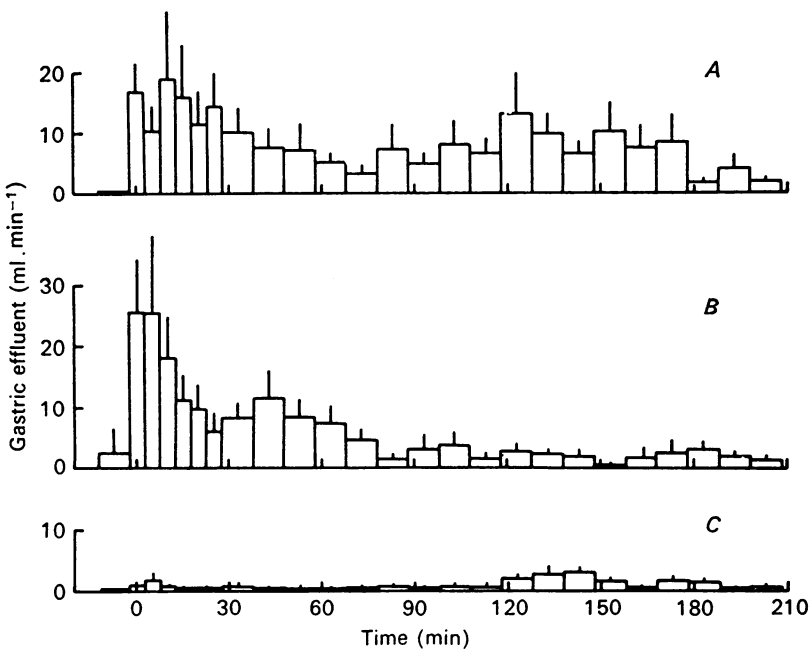

Fig. 3. Comparison of the rates of gastric emptying in 3–5 week old calves, with duodenal cannulae placed in the duodenum immediately below the pylorus, in response to feeding 1·7 l milk between −2 and 0 min. *A*: normal controls ($n = 8$). *B*: cut splanchnic nerves ($n = 7$). *C*: atropine (0·2 mg/kg; $n = 8$). Vertical bars: S.E. of each mean value.

In the control group gastric emptying began 30–60 sec after drinking started and a maximal rate (18·8 ± 11·0 ml/min) was recorded 8–13 min later; the flow then fell steadily to a nadir (3·2 ± 1·4 ml/min) at 70 min. Thereafter, a second, more prolonged phase of emptying occurred which was maximal at 120 min and virtually complete at 210 min (Fig. 3). By this time the total volume of gastric content collected from each animal (1·54 ± 0·2 l) was approximately equal to the volume of milk consumed (1·7 l). The pattern of gastric emptying in calves with cut splanchnic nerves differed from the control group in that maximal rates were achieved almost immediately when the animals started to drink (25·6 ± 6·6 ml/min, −2 to +3 min; 25·4 ± 7·6 ml/min, +3 to +8 min) and then steadily diminished (Fig. 3). The second phase of gastric emptying was abolished by section of the splanchnic nerves, consequently

the mean total volume of gastric content collected during the course of each experiment (1·2 ± 0·1 l) was less than that in the control animals. Administration of atropine (0·2 mg/kg) strongly inhibited both phases of gastric emptying and the mean total volume collected was reduced to 0·22 ± 0·03 l. in these animals (Fig. 3).

The rise in plasma glucose concentration which normally occurs during the initial phase of gastric emptying, was absent in calves with duodenal cannulae, in which the passage of gastric content to the intestines was prevented, as was the rise

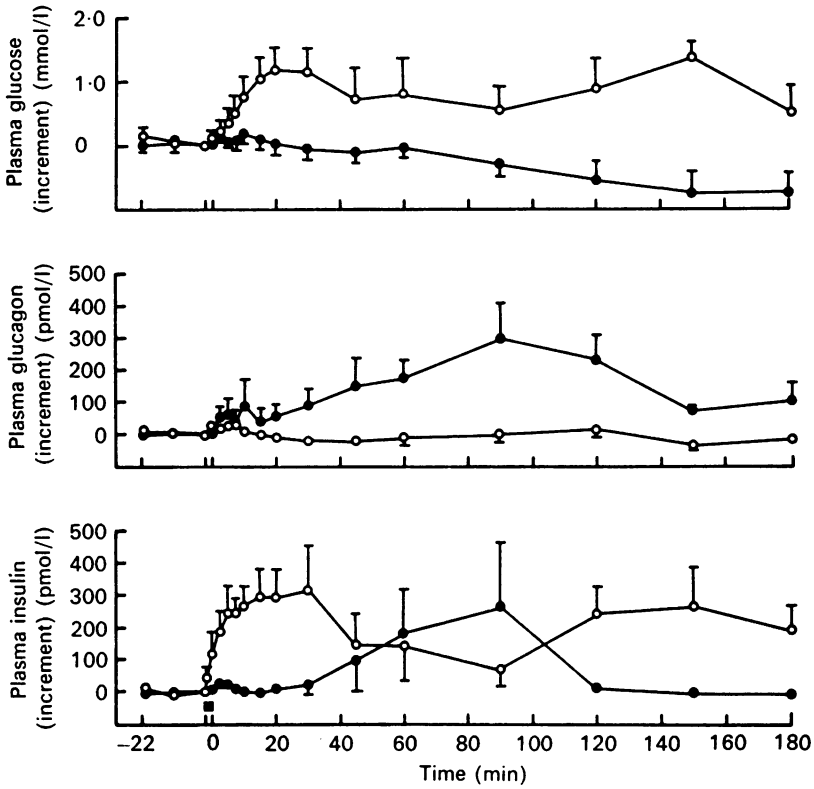

Fig. 4. Comparison of the changes in arterial plasma glucose, glucagon and insulin concentration in response to feeding in 3–5 week old calves given 1·7 l warm milk at the signal bar. ○, normal calves (n = 7; modified from Bloom *et al.* 1975). ●, calves with duodenal cannulae (n = 7). Vertical bars: S.E. of each mean value. Absolute values before feeding as follows, with controls in brackets. Glucose: 4·0 ± 0·4 mmol/l, (5·6 ± 0·3 mmol/l). Glucagon: 43 ± 23 pmol/l, (54 ± 14 pmol/l). Insulin: 36 ± 4 pmol/l, (209 ± 89 pmol/l).

in plasma insulin concentration (Fig. 4). Calves with duodenal cannulae resembled the calves with cut splanchnic nerves given atropine (Fig. 1) in that both the initial glucose and insulin values (glucose: 4·0 ± 0·4 mmol/l; insulin: 36 ± 4 pmol/l) were much lower than those of the normal controls (glucose: 5·6 ± 0·3 mmol/l; insulin: 209 ± 89 pmol/l). However, whereas the glucagon response to ingestion was abolished by autonomic blockade, mean plasma glucagon concentration rose to even higher values in the cannulated animals than in the control group during the

first 10 min after feeding (Fig. 4). Thereafter there was a slow rise in the concentration of both glucagon and insulin in the plasma of calves with duodenal cannulae, to coincidental peaks at 90 min. The extent of the two responses was closely similar (glucagon: 298 ± 107 pmol/l; insulin: 268 ± 195 pmol/l); the glucagon: insulin molar ratio was unaffected and there was no significant change in mean plasma glucose concentration.

The absence of the initial rise in plasma glucose concentration in response to feeding and its relative constancy thereafter, in calves with duodenal cannulae, may have resulted in part from depletion of liver glycogen. Each of these animals was found to have an extremely low concentration of glycogen in the liver at the

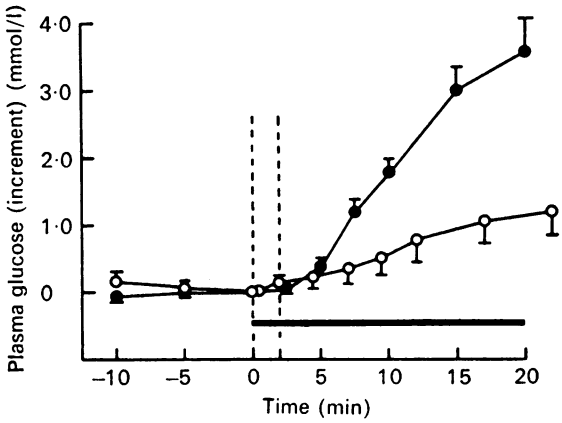

Fig. 5. Comparison of the changes in mean plasma glucose concentration in normal 3–5 week old calves, in response to feeding 1·7 l warm milk (○, n = 7; modified from Bloom *et al.* 1975), with those during intraduodenal infusions of gastric content (●, n = 4). Each intraduodenal infusion comprised the whole of the gastric effluent collected from the same calf, during the first 20 min after a feed of 1·7 l warm milk 3 hr previously. Vertical bars: S.E. of each mean value. Vertical hatched lines: duration of feeding. Horizontal bar: duration of infusion.

conclusion of the experiment (< 8·0 mg/g), food having been withheld for at least 22 h before the test-meal. It may also have arisen because intestinal absorption of glucose was prevented. This question was investigated further by establishing the latency and extent of the hyperglycaemic response to intraduodenal infusions of gastric content. These experiments were carried out in calves provided with two duodenal cannulae. The proximal cannula was used for collection of gastric content, which was later infused into the duodenum via the distal cannula. In each case the whole of the gastric content, collected during the first 20 min after feeding, was infused into the duodenum over a 20 min period, at a steady rate, 3 h later. Comparison of the rise in mean plasma glucose concentration during intraduodenal infusions of gastric content with that which occurs in normal calves, during the first 20 min after a feed, shows that glucose is absorbed sufficiently rapidly to account for the initial hyperglycaemic response in normal calves. Both responses were found to have roughly the same latency (about 2 min) and the extent of the rise in mean plasma glucose concentration was substantially greater than that after a normal meal (Fig. 5).

In addition, the extent to which glucose might be absorbed from the abomasum was assessed by comparing the changes in plasma radioactivity of normal calves and calves with duodenal cannulae, when each was fed 1 mCi [^{3}H]glucose dissolved in 850 ml warm milk. Closely similar changes in plasma radioactivity occurred in each of the normal calves and the results of a typical experiment are shown in Fig. 6. Plasma samples were counted before and after they had been evaporated to dryness, in order to distinguish plasma [^{3}H]glucose from total plasma ^{3}H and the results are expressed as counts/min. ml plasma (CPM/ml). Plasma [^{3}H]glucose rose rapidly

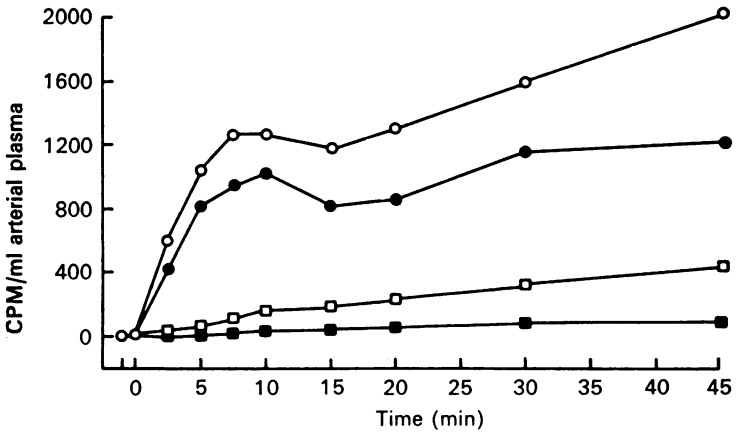

Fig. 6. Comparison of the changes in radioactivity of the arterial plasma following the ingestion of 1 mCi [^{3}H]glucose in 850 ml. warm milk, in a 3–5 week old calf, in which intestinal absorption was prevented by means of a duodenal cannula (squares), with those in a normal calf of the same age (circles). Open symbols: total plasma ^{3}H. Closed symbols: plasma [^{3}H]glucose.

to an initial peak at 10 min and the shape of the curve was reminiscent of that for plasma glucose (Fig. 1), allowing for the fact that the volume of milk consumed was only half that of the standard feed. In contrast, there was virtually no rise in plasma [^{3}H]glucose in calves in which passage of the tracer from abomasum to small intestine was prevented (Fig. 6). Estimations of the concentration of glucose and galactose in the gastric content in these experiments showed that there was a small but significant increase in the concentration of both hexoses with time, showing that some lactose is digested in the abomasum, albeit slowly. It was also observed that the increase in galactose concentration invariably exceeded that of glucose, presumably because glucose is preferentially metabolized by the abomasal mucosa. It is concluded that this process is responsible for the slow but steady rise in total plasma ^{3}H that occurred in calves with duodenal cannulae (Fig. 6).

Effect of autonomic blockade on the responses to ingestion of milk

The extent to which the initial endocrine responses to ingestion of food depend upon the integrity of the autonomic nervous system was assessed by investigating the effects of autonomic blockade on calves in which intestinal digestion and absorption were prevented by means of a duodenal cannula. As stated previously, the initial transient increases in plasma glucose and insulin concentration after

feeding fail to occur in calves with these duodenal cannulae (Fig. 4), whereas the glucagon response is unaffected (Figs. 4 and 7). Prior section of both splanchnic nerves produced no significant change in any of these responses to ingestion of milk. In contrast, the rise in plasma glucagon concentration was completely blocked by atropine (Fig. 7). Mean initial plasma glucose concentrations were closely similar in these two groups (controls: 4·0 ± 0·4 mmol/l; atropinized animals: 4·5 ± 0·4 mmol/l) showing that the pancreatic islets were exposed to the same basal level of

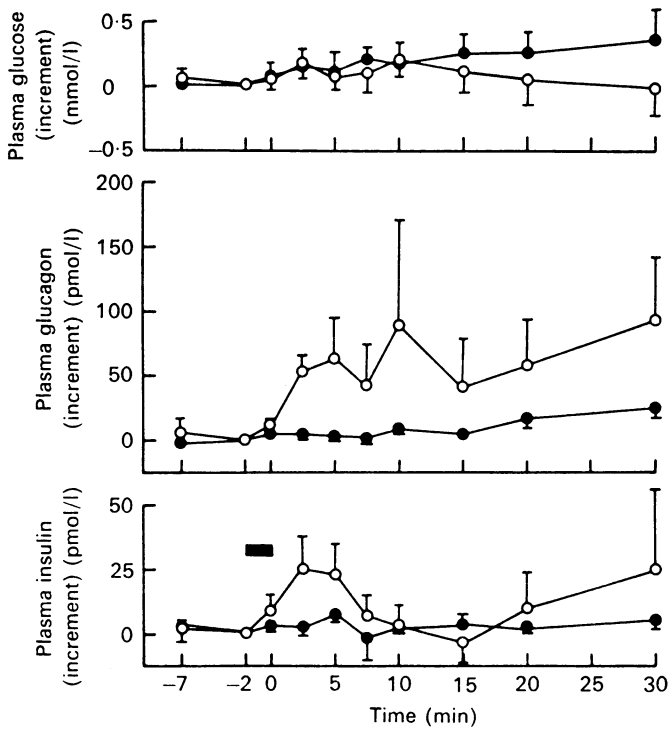

Fig. 7. Comparison of the changes in mean plasma glucose, glucagon and insulin concentration in 3–5 week old calves with duodenal cannulae, in response to feeding 1·7 l warm milk at the signal bar. ○, controls (n = 6). ●, calves given atropine 10 min before feeding (0·2 mg/kg; n = 7). Vertical bars: S.E. of each mean value. Absolute values before feeding as follows, with controls in brackets. Glucose: 4·5 ± 0·4 mmol/l, (4·0 ± 0·4 mmol/l). Glucagon: 21 ± 8 pmol/l, (43 ± 23 pmol/l). Insulin: 18 ± 4 pmol/l, (36 ± 4 pmol/l).

glycaemic stimulation or inhibition. Neither the presence of the glucagon response (in the control group) nor its absence (in the atropinized group) seemed to influence plasma glucose.

Ingestion of the standard meal was associated with a rapid rise in plasma gastrin concentration in control calves with duodenal cannulae. The mean concentration rose at a steadily declining rate, from an initial value of 31·3 ± 3·1 pmol/l, to an initial peak incremental value of 39·8 ± 9·5 pmol/l at 7·5 min and then more gradually to a second peak incremental value of 72·5 ± 9·3 pmol/l at 120 min (Fig. 8). This response was substantially greater than that which occurred in normal control

animals of the same age (Fig. 2), in which the corresponding incremental values were 8·6 ± 1·9 pmol/l (7·5 min) and 21·8 ± 5·2 pmol/l (120 min), and the differences between the mean values for these two groups of animals were all statistically significant after 5 min ($P < 0·01$). An even more abrupt rise in plasma gastrin concentration occurred in calves with duodenal cannulae after section of the splanchnic nerves. In these animals the mean value was found to have increased from 45·2 ± 18·8 pmol/l at time = 0, by 40·3 ± 22·3 pmol/l at the end of the feed (2 min) and to an initial peak incremental value of 51·9 ± 20·7 pmol/l 5 min later, although the differences between the two groups were not statistically significant at any stage (Fig. 8). Administration of atropine (0·2 mg/kg) 10 min before feeding strongly suppressed the initial rapid rise in plasma gastrin concentration in calves with

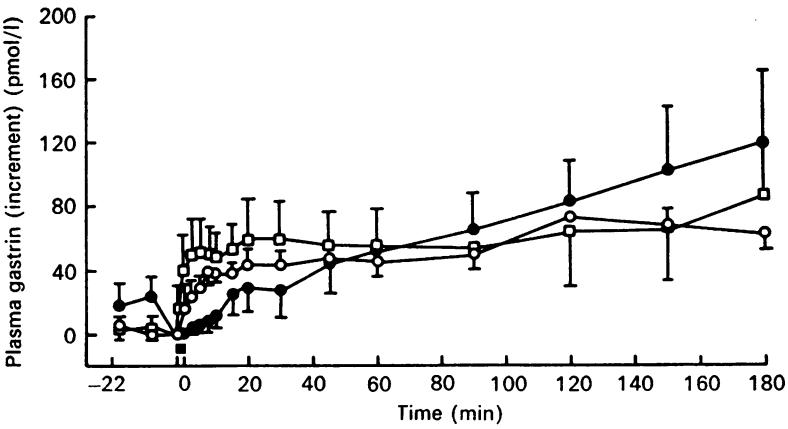

Fig. 8. Comparison of the changes in mean plasma gastrin concentration in response to feeding in 3–5 week old calves with duodenal cannulae given 1·7 l warm milk at the signal bar. ○, controls ($n = 6$). □, cut splanchnic nerves ($n = 5$). ●, atropine (0·2 mg/kg; $n = 7$). Vertical bars: S.E. of each mean value. Absolute values before feeding as follows. Controls: 31·3 ± 3·1 pmol/l Cut splanchnic nerves: 45·2 ± 18·8 pmol/l Atropine: 51·4 ± 24·4 pmol/l.

duodenal cannulae and led to a steady, roughly linear, increase in the mean values over the subsequent 3 hr (Fig. 8). The results in calves with duodenal cannulae are consistent with the classical view that the parasympathetic innervation to the pyloric antrum plays an important part in the control of gastrin release during the cephalic and gastric phases of digestion and to which our study of the effects of autonomic blockade in normal calves provided no clue (Fig. 2).

The question as to whether sympathetic or parasympathetic stimulation leads to release of PP during feeding was examined by comparing the responses of calves with duodenal cannulae after either atropine (0·2 mg/kg) or splanchnic nerve section. In the group with cut splanchnic nerves the mean arterial plasma PP concentration rose abruptly during feeding, the peak incremental values coincided with cessation of drinking, as in normal calves (Figs. 2 and 9) and returned rapidly to base line thereafter. This effect was completely blocked by the administration of atropine 10 min before feeding (Fig. 9). Closely similar changes in plasma PP concentration occurred in calves with duodenal cannulae, whether or not the splanchnic nerves

were cut, and they were always about half as great as those observed in the normal controls (Fig. 2). It is therefore concluded that release of PP during ingestion is probably initiated by a vagal muscarinic mechanism and that it is normally potentiated in some way by the passage of gastric content from the stomach to the duodenum.

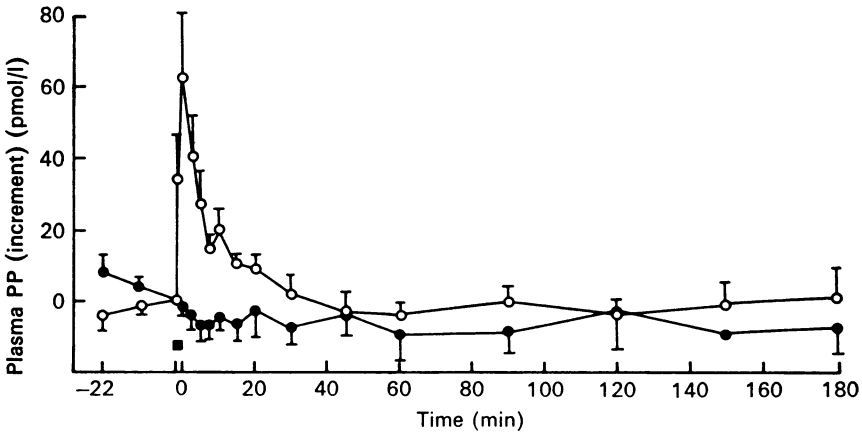

Fig. 9. Comparison of the changes in mean plasma PP concentration in response to feeding in 3–5 week old calves with duodenal cannulae given 1·7 l warm milk at the signal bar. ○, cut splanchnic nerves (n = 4). ●, atropine (0·2 mg/kg; n = 5). Vertical bars: S.E. of each mean value. Absolute values before feeding as follows. Cut splanchnic nerves: 41·8 ± 6·9 pmol/l. Atropine: 33·0 ± 4·1 pmol/l.

DISCUSSION

The results of these experiments provide direct evidence that the increase in sympathetic efferent activity during feeding in the unweaned calf, which is manifested by such pronounced hypertension and tachycardia (Bloom *et al.* 1975), has a negligible effect on the release of pancreatic hormones. When intestinal digestion and absorption were prevented, by the presence of a duodenal cannula, in calves with depleted reserves of glycogen in the liver, the rise in plasma glucose concentration which normally occurs after feeding was averted. Consequently, any secondary effects which might have arisen from alimentary hyperglycaemia were eliminated. Under these conditions the initial rise in plasma insulin concentration after feeding was almost completely suppressed and section of both splanchnic nerves was without apparent effect. The initial rise in plasma glucagon concentration did not differ significantly in calves with duodenal cannulae from that in normal calves but neither was it diminished by section of the splanchnic nerves. Further, no evidence has been obtained to suggest that the sympathetic system affects the rate at which PP is released during feeding. Gastrin release, in response to ingestion of milk, may have been enhanced in calves with cut splanchnic nerves, but these results are equivocal and may well have been due to some non-specific effect, such as a greater blood flow through the antral mucosa after sympathetic denervation. In view of the sensitivity of the pancreatic α cell to stimulation via the sympathetic innervation (Bloom, Edwards & Vaughan, 1973) and the susceptibility of the β cell to α adreno-

ceptor inhibition (Bloom & Edwards, 1975, 1978) the finding that intense sympathetic activity can occur without apparently affecting the release of either glucagon or insulin was unexpected. We consider that the most likely explanation is that, in the absence of extraneous stress, activation of the sympathetic system during ingestion is restricted to those pathways subserving exclusively cardiovascular functions.

In calves with duodenal cannulae, through which the abomasal effluent was collected, gastric emptying was initiated within 30–60 sec after drinking started and a maximal rate was quickly achieved, as has been described by Ash (1964). The initial phase of emptying subsided roughly exponentially, in accordance with observations by numerous workers in various species, including the unweaned calf (Marbaix, 1898; Hunt & Spurrell, 1951; Bell & Razig, 1973), and the total volume of gastric effluent collected during the next 3·5 h after feeding was roughly equal to the volume of the test-meal. It therefore seems unlikely that gastric emptying was significantly inhibited by the presence of a cannula in the duodenum or the passage of gastric effluent through it. The fact that the rate of gastric emptying is influenced by the composition of the duodenal content (Hunt, 1959; Bell & Mostaghini, 1975; Bell & Watson, 1976) raises the possibility that it may have been modified in some way in calves in which the gastric effluent was denied access to the duodenum. Bell and Mostaghini (1975) found no difference in the rate of abomasal emptying between calves in which the effluent passed through the duodenum and those in which it passed out through an open duodenal cannula, but used water rather than milk. They concluded from this study, using re-entrant cannulae in the duodenum 'that an open duodenal cannula does not cause any marked variation in emptying of the abomasum when compared to the rate of emptying in the normal viscus...', but restricted their study to cannulated animals and present no data regarding normal animals. However, the question is inconsequential so far as the rapid responses to ingestion are concerned, which the present experiments were designed to elucidate.

The route through which ingested milk is propelled to the abomasum in the unweaned ruminant is complex and involves a coordinated sequence of reflexes, whereby the reticulo-ruminal sac is by-passed (Comline & Titchen, 1951). In the present experiments it was established that the efficient delivery of milk to the abomasum was unimpaired by atropinization, as could be predicted from the radiological study of Newhook & Titchen (1974). Nevertheless, each of the endocrine responses that were found to follow the ingestion of milk within 10 min, and could be demonstrated while intestinal digestion and absorption were prevented, namely release of pancreatic glucagon, PP and initially gastrin, were blocked by atropine at a dose of 0·2 mg/kg. It is therefore concluded that each depends upon the integrity of some parasympathetic, muscarinic mechanism, which is clearly distinguishable from those that are solely concerned with the mechanical conveyance of the ingested milk to the abomasum.

The normal rise in plasma insulin concentration disappeared almost completely when gastric effluent was denied access to the small intestine, as did the rise in plasma glucose concentration. Hyperglycaemia, consequent upon intestinal absorption, provides an effective stimulus to insulin release in normal animals and this effect is known to be potentiated by the release of gastric inhibitory peptide (GIP)

from the jejunum, at least in primates (Dupré, Ross, Watson & Brown, 1973; Turner, Etheridge, Jones, Marks, Meldrum, Bloom & Brown, 1974). Simultaneous elimination of both effects therefore provides a plausible explanation for the absence of the insulin response to feeding in calves with duodenal cannulae. However, it leaves in abeyance the further question of the extent to which reflex vagal activity normally predisposes the β cell to respond to intestinal stimuli. In atropinized calves without duodenal cannulae (Fig. 1) the normal rise in plasma glucose concentration was delayed by roughly 20 min, presumably due to inhibition of gastric emptying, but the insulin response was delayed for much longer (60 min), suggesting that the pancreatic β cells were refractory to alimentary hyperglycaemia in the presence of atropine. Thereafter the concentration of both glucagon and insulin rose steadily and achieved coincident peaks at the time that the second phase of gastric emptying is maximal, in calves with duodenal cannulae (120 min). This pattern suggests that some factor which stimulates secretion of both glucagon and insulin is eventually released from the small intestine. However, the delayed release of insulin might also be attributed to the fact that the initial plasma glucose concentration was well below normal in these animals and a precise definition of the interactions of all the various insulinotropic factors must await the results of future studies.

The speed with which gastric emptying is initiated and lactose is then hydrolysed in the small intestine is such that intestinal absorption of glucose is detectable within 2 min after the animals start to drink. The rate of absorption of glucose subsequently fully accounts for the observed rise in plasma glucose concentration, in accord with Steffens' observations in conscious rats fed carbohydrate-rich meals (Steffens, 1969). The initial glucagon response caused no rise in plasma glucose concentration in calves in which intestinal absorption of glucose was prevented, but the reserves of liver glycogen were depleted in these animals. The extent of the rise in plasma glucagon concentration ($63{\cdot}0 \pm 48{\cdot}5$ pmol/l at 5 min) was comparable with that which occurs during moderate insulin hypoglycaemia in calves of the same age ($62{\cdot}5 \pm 12{\cdot}3$ pmol/l, $n = 4$, Bloom *et al.* 1974) and which is likewise blocked by atropine. It also exceeded the rise in plasma glucagon concentration observed in anaesthetized dogs, in response to intramesenteric infusions of glucagon at a dose of 5 $ng.kg^{-1}$ min^{-1} for 10 min ($46{\cdot}3 \pm 7{\cdot}9$ pmol/l, $n = 4$, Bloom & Edwards, 1975) and which caused a rise in arterial plasma glucose concentration of between 1·4 and 1·9 mmol/l. Thus, release of pancreatic glucagon, in response to vagal stimulation during ingestion of milk, may be expected to initiate the observed rise in plasma glucose concentration in calves with abundant liver glycogen.

In order to establish the extent to which neurally mediated endocrine responses occur in the conscious animal during feeding it is necessary to separate the effects attributable to ingestion from subsequent gastrointestinal events as far as possible. This is well illustrated by the changes in plasma gastrin concentration that occurred in the various groups of calves used in the present study. Complete autonomic blockade, in otherwise normal calves, produced no significant change in the pattern of gastrin release and gave no indication of any reflex vagal component such as that which has been demonstrated in the dog (Walsh & Grossman, 1975). But when the passage of gastric effluent to the intestine was prevented, the rise in plasma gastrin

concentration after feeding was greatly enhanced. Moreover, administration of atropine (0·2 mg/kg) to these animals suppressed gastrin release for several minutes, thereby revealing the effect of the muscarinic stimulus during the initial phase of gastrin release.

The most clear-cut neuroendocrine response to feeding was found to be the release of PP. When intestinal digestion and absorption were prevented this response was mediated entirely via the parasympathetic innervation. In normal calves the plasma PP concentration rose more rapidly and achieved higher values than in calves with duodenal cannulae, thus indicating additional enteric stimulation. In human subjects both vagal and enteric stimuli have been implicated in the release of PP after a meal (Schwartz, Stadil, Chance, Rehfeld, Larsson & Moon, 1976; Adrian, Bloom, Besterman, Barnes, Cooke, Russell & Faber, 1977) although intravenous nutriments do not appear to influence PP release (Adrian *et al.* 1977) and the relative importance of the two pathways is not yet established. Interpretation of these studies in vagotomized patients is complicated by the fact that the enteric stimulus depends upon effective gastric emptying, which in turn depends upon the integrity of the parasympathetic innervation to the gastrointestinal tract. Enteric hormones that have been found to exert a direct stimulatory effect on the release of PP from the isolated canine pancreas include caerulein (a cholecystokinin/pancreozymin analogue), GIP and gastrin (Adrian, Bloom, Hermansen & Iversen, 1978). Of these caerulein, which is the most potent, has been shown to produce a substantial rise in plasma PP concentration when infused intravenously in man (Adrian *et al.* 1978). Thus, cholecystokinin/pancreozymin (CCK/PZ) seems to be the most likely candidate for the role of enteric PP stimulant at the present time. Effects that have been attributed to PP include broad antagonism to CCK/PZ (inhibition of enzyme output from the pancreas and gall-bladder contraction) and either stimulation or inhibition of gastric acid secretion depending on the dose (Lin, Chance & Evans, 1973). In man only the effects on the pancreas and biliary tree appear to be of physiological significance (Adrian, Greenberg, Besterman, McCloy, Chadwick, Marnes, Mallinson, Baron, Alberti & Bloom, 1978). It has recently been shown that administration of PP to hyperphagic obese mice reduces food intake and suppresses weight gain (Malaisse-Lagae, Carpentier, Patel, Malaisse & Orci, 1977) which suggests that the hormone may play a part in the control of food intake. The speculation that release of PP might signal satiety derives some support from the fact that peak levels coincide with cessation of feeding even when, as in the calf, ingestion is completed within 2 min.

All the neuroendocrine responses to feeding in the calf, that have been investigated in the present study have been found to be mediated by the parasympathetic rather than the sympathetic innervation. In this respect they resemble the pancreatic endocrine responses to other glycaemic stimuli such as hypoglycaemia (Bloom *et al.* 1974) and 2-deoxyglucose (Bloom *et al.* 1978). These findings support the general conclusion that pancreatic endocrine responses to vegetative stimuli are mediated via the parasympathetic innervation and that these may be modified by sympathetic activity under conditions of stress.

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