Factors affecting the voluntary intake of food by sheep

3. The effect of intravenous infusions of gastrin, cholecystokinin and secretin on motility of the reticulo-rumen and intake

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1. Sheep given ground and pelleted lucerne hay (*Medicago sativa*) *ad lib.* were infused intravenously with pentagastrin, secretin, cholecystokinin (CCK) and its analogues to assess their effects on motility of the reticulo-rumen and on food intake. In the latter experiments the animals were deprived of their diet for periods of up to 6 h to induce hunger and the infusions were made before and during 3–10 min periods of feeding.

2. Pentagastrin, an analogue of gastrin, depressed intake by 35–50% ($P < 0.05$) when it was infused at 9 μg/kg per h during 30 min of feeding. The threshold may however be below 1 μg/kg per h as this dose decreased intake of 12–17%. The frequency of reticular contractions decreased by 13, 35, 39 and 44% when 1, 3, 9 and 27 μg pentagastrin/kg per h respectively was infused ($P < 0.025$).

3. Secretin depressed food intake 38% after 30 min ($P < 0.025$) when 8 Clinical Units (CU)/kg per h was infused but the threshold could be less than this dose since 0.5 CU/kg per h depressed intake by 12%. Contraction amplitude but not frequency decreased at 8 CU/kg per h.

4. CCK produced a 39% decrease in intake during the first 10 min of feeding ($P < 0.05$) and the threshold was between 5 and 15 Ivy Dog Units (IDU) or 425 and 1276 pmol/kg per h. The frequency of reticular contractions was not affected by 1.7 IDU/kg per h but it was depressed 21 and 63% by 5 and 15 IDU/kg per h. Octapeptide at 1.5 and 3 μg (1312 and 2624 pmol)/kg per h depressed intake by 11 and 43% respectively after 10 min (not significant) and 1.5 μg/kg per h depressed motility by 39% ($P < 0.01$). Ceruletide at 810 ng (599 pmol)/kg per h depressed intake by 31% (not significant) after 10 min and decreased motility by 52% ($P < 0.05$). The threshold dose for ceruletide on intake appeared to be about 90 ng or 66 pmol/kg per h which is considerably less than that for CCK or octapeptide.

5. The biological significance of gastrointestinal hormones as signals of satiety in normal sheep is not known since doses of pentagastrin and CCK that suppressed intake also interfered quite markedly with motility. However there is good reason to suspect that elevated concentrations of gastrin and CCK in blood of parasitized sheep may account at least in part for their symptoms of rumen atony and reduced food intakes.

Interest is being shown in gastrin, secretin and cholecystokinin as signals of satiety since their concentrations in venous blood increase during a meal in human beings (gastrin: Wyllie *et al.* 1972; Gedde-Dahl, 1975; Fritsch *et al.* 1976; Woussen-Colle *et al.* 1977; cholecystokinin: Harvey *et al.* 1973; Johnson & McDermott, 1973; secretin: Schaffalitzky De Muckadell & Fahrenkrug, 1978) and in animals with simple stomachs (gastrin: Debas *et al.* 1976; Svensson *et al.* 1976; secretin: Kim *et al.* 1979). Secretin release was often observed in the more distant past in response to an infusion of acid into the duodenum but not by meals (Rhodes *et al.* 1976; Boden *et al.* 1978) partly because the assays used were not as sensitive as those of Schaffalitzky De Muckadell & Fahrenkrug (1978) and Kim *et al.* (1979).

Injections of cholecystokinin (CCK) have depressed the intake of food by rats (Gibbs *et al.* 1973; Antin *et al.* 1975; Maddison, 1977; Antin *et al.* 1978; Mueller & Hsiao, 1979), mice (Strohmayer *et al.* 1976), monkeys (Gibbs *et al.* 1976), dogs (Sjödin, 1972), rabbits (Houpt & Anika, 1977), human beings (Sturdevant & Goetz, 1976) and sheep (Baile & Grovum, 1974; Grovum, 1977*a*, *b*) but not in domestic fowl (Snapir & Glick, 1978). However CCK did not reduce intake in rats when it was injected at the start of spontaneous

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meals (Glick *et al.* 1971) and Mineka & Snowdon (1978) found that CCK injections initially depressed food intake by rats but with repeated testing on successive days the effect disappeared and there was no difference between CCK and normal saline (9 g sodium chloride/l). Secretin injections were without effect on food intake in the rat (Glick *et al.* 1971; Gibbs *et al.* 1973) but secretin and pentagastrin (an analogue of gastrin with similar activity) did decrease intake by sheep (Grovum *et al.* 1974; Grovum, 1977*a*, *b*).

The research now reported indicated that intravenous infusions of pentagastrin, secretin and CCK depressed food intake by hungry sheep.

EXPERIMENTAL

Sheep and surgery

Cross-bred Suffolk wethers were used in all experiments. Sheep nos. 1, 2, 3, 5 and 14 which were used in all experiments except Expt 4(*b*) weighed 45 ± 1 kg at the beginning of the experiments and 62 ± 2 kg at the end. Sheep nos. 2, 3, 4, 5 and 15 used in Expt 4(*b*) weighed 45 ± 1·7 kg. The animals were treated for internal parasites with subcutaneous injections of a broad-spectrum anthelmintic (Tramisol; Cyanamid). External parasites when evident were killed with a dusting powder containing Malathion. The animals were fitted with rumen cannulas and were allowed to recover for 14 d before the experiments commenced.

Housing, feeding and diets

The sheep were held in a room which was always illuminated. Ground and pelleted lucerne (*Medicago sativa*) hay (g/kg: 947 dry-matter, 144 (nitrogen × 6·25), 81 ash, 400 acid-detergent fibre, 27 fat) was available *ad lib.* except during the experimental sessions in Expt 1 and during the periods of deprivation immediately before the experimental sessions in Expts nos. 2, 3, 4 and 5. Water was available continuously.

Measurement of motility of the reticulum

A polyethylene tube (up to 600 mm in length, 6 mm i.d., 9 mm o.d.) with a toy balloon secured on one end and a rubber stopper on the other was directed through a rumen cannula into the reticulum and held in place by pushing the stopper into the cannula. A vinyl tube (1 m in length, 8 mm i.d., 11 mm o.d.) was fitted securely over the tubing protruding from the sheep and connected to a glass 'T' piece having a three-way stopcock attached to its side arm. Another vinyl tube (4 m in length, 5 mm i.d., 7 mm o.d.) connecting the remaining outlet of the 'T' piece and a strain gauge transducer was filled with water to 100 mm below the 'T' piece which was fixed securely to the side of the metabolism cage. The transducer was mounted on a trolley and pressures in the reticulum were recorded after 5 ml air was introduced into the balloon through the stopcock. The volume of air did not by itself produce pressure inside the balloon. Artifact due to movement of the animal was minimal with this set up and biphasic and triphasic pressure waves during mixing and rumination cycles of motility confirmed that the balloon was in the reticulum. The portion of the polyethylene tube inside the balloon had to be perforated with holes because without these, the tip of the tube was frequently covered by the balloon and pressure changes could not be recorded.

General protocol

During Expt 1 food was withdrawn while hormones were infused. In Expts nos. 2, 3, 4 and 5 the schedule of feeding was as follows except that when ceruletide was used, intakes were not measured after the infusion was stopped. Fresh food and water were made available between 08.00 and 09.00 hours. The sheep were deprived of food but not water from 09.00 to 13.56 hours (deprivation period was 296 min in this instance). Treatments were imposed (cholecystokinin was infused intravenously for example) before feeding, 13.31–13.56 hours and during feeding, 13.56–14.30 hours. Food intakes were recorded at 0–10 min, 13.56–14.06

hours; 10–20 min, 14.08–14.18 hours and 20–30 min, 14.20–14.30 hours. Infusions were stopped at 14.30 hours. Food intakes were measured at 30–40 min, 14.33–14.43 hours.

The treatments were imposed according to a 5 × 5 Latin-Square design in all experiments except Expt no. 1 but the deprivation period varied from 160 to 395 min.

Expt 1. *Motility of the reticulum*

The effect of intravenous infusions of gastrointestinal hormones on motility of the reticulum was used as a biological assay to assess their physiological significance as signals of satiety. A more direct method would have been to measure concentrations of the hormones by radioimmunoassay in serum from normal sheep and those infused with hormone but these assays were not available for use in sheep.

Part A. The motility of the reticulum of sheep nos. 1, 2, 3, 5 and 14 was recorded while equal volumes (0·6 ml/min) of either normal saline (control) or pentagastrin in solution (1, 3, 9 and 27 μg/kg per h) was infused into the jugular vein for periods of 30 min. Control records were obtained before any of the pentagastrin infusions were made. Pentagastrin, a synthetic peptide with all the activities of gastrin (Walsh & Grossman, 1975) was used rather than gastrin because it was readily available and was relatively inexpensive. Rehfeld & Larsson (1979) have also shown that in the gut the predominant form of gastrin is the tetrapeptide from its carboxy terminal. The lot number of the pentagastrin supplied by Ayerst Laboratories was IBFD-UA. The last 10 min segments from the control and the treatment periods were analysed for number of biphasic contractions and for amplitude of the last reticular contraction of each set.

Part B. The procedures described in part A were followed except that secretin (Karolinska Institute, Sweden; lot nos. 17561 and 17141) was infused at 8 Clinical Units (CU)/kg per h for 18 min.

Part C. The procedures described in part A were followed except that CCK (Karolinska Institute, Sweden; lot nos. 27551, 27631 and 27612) was infused at 1·7, 5 and 15 Ivy Dog Units (IDU)/kg per h for periods ranging from 15 to 20 min. The chemical was supplied in vials.

Part D. The procedures described in part A were followed except that ceruletide (gift from Farmitalia Laboratories, Milan, Italy; lot no. TF 17789) was infused at 270 and 810 ng/kg per h for 21 min. Ceruletide, originally called cerulein, is a decapeptide having the activity of cholecystokinin (Bertaccini *et al.* 1968).

Part E. The procedures described in part A were followed except that octapeptide of CCK (gift from Dr M. A. Ondetti, Squibb Institute, Princeton, New Jersey, USA; lot no. B # UTA-860-H/TJ-5) was infused at 1·5 μg/kg per h for 18–21 min. The octapeptide is present in the gut (Dockray, 1977) and it was shown to have the activities of cholecystokinin but to be more potent than CCK on a molar basis in causing the gall bladder to contract (Ondetti *et al.* 1970).

Expt 2. *Pentagastrin and food intake*

Part A. Five sheep deprived of food for 317 min were infused intravenously with pentagastrin (lot 2197-TE) at 0 (normal saline control), 1, 3, 9 and 27 μg/kg per h for 25 min before feeding commenced and for 34 min while food intakes were measured. After the infusion was stopped food intakes were recorded during another 10 min feeding period. The treatments were imposed according to a 5 × 5 Latin-Square design and the pentagastrin was diluted with normal saline to make the rates of infusion of fluid similar in the five treatments. The infusion rates of pentagastrin were scaled from 27 down to 1 μg/kg per h because 27 μg/kg per h markedly inhibited motility of the reticulo-rumen in all sheep (Expt 1) whereas 1 μg/kg per h had only a slight effect.

Part B. The experiment described in part A was repeated except that the sheep were

deprived of food for only 160 min and the lot no. of pentagastrin was (L) IBFD-UA. This trial assessed whether shortening the deprivation period would increase the depressing effect of pentagastrin on intake as was noted in part A.

Expt 3. *Secretin and food intake*

Five sheep deprived of food for 322 min were infused as described for Expt 2 except that pure porcine secretin (3500 CU/mg; Karolinska Institute, Sweden) was administered at 0, 0·13, 0·5, 2 and 8 CU/kg per h. Secretin did not markedly affect motility of the reticulo-rumen (Expt 1) so the physiological significance of secretin on food intake can only be judged from the finding that 0·47–0·63 CU secretin/kg per h infused into the portal vein of sheep approximately doubled bile flow and 5 CU/kg per h tripled it (Heath, 1970; Pass & Heath, 1976). There was no indication that bile flow was maximal at their highest rates of infusion.

Expt 4. *CCK and food intake*

Part A. Five sheep deprived of food for 296 min were infused as described in Expt 2 except that porcine CCK (Karolinska Institute, Sweden) from a bulk source (15% pure) and from vials (lot 275112) was administered at 0, 0·56, 1·7, 5 and 15 IDU/kg per h. The largest rate of infusion was set partly because of economy and partly because it markedly inhibited motility of the reticulo-rumen (Expt 1) in most of the sheep. Faustini *et al.* (1973) reported that 66 ng CCK/kg per min, which is equivalent to 11·88 IDU/kg per h assuming 3000 IDU CCK/mg, markedly inhibited motility of the reticulo-rumen of sheep but did not cause the gall bladder to contract. An infusion of 0·96 μg or 2·88 IDU/kg per h also induced a half-maximal secretion of protein by the pancreas of dogs (Debas & Grossman, 1973).

Part B. Five sheep deprived of food for 273 min were infused as described in Expt 2 except that ceruletide (lot TF/17789; Farmitalia, Milan, Italy) from vials was administered at 0, 30, 90, 270 and 810 ng/kg per h. The largest dose rate markedly inhibited motility of the reticulum in three of the five sheep (Expt 1). However Faustini *et al.* (1973) caused inhibition with as little as 6 ng/kg per h perhaps because their animals had less than 24 h to recover from surgery. The gall bladder also contracted within 60–90 sec of infusing ceruletide intravenously at 60 ng/kg per h.

Part C. Five sheep deprived of food for 285 min were infused as described in Expt 2 except that octapeptide of CCK obtained in bulk from Squibb was administered at 0, 0·38, 0·75, 1·5 and 3·0 μg/kg per h. Infusing 1·5 μg/kg per h in Expt 1 increased the interval between successive reticular contractions substantially but did not alter their amplitude. The effect of 3 μg/kg per h on motility was not tested due to a limited supply of the chemical.

Expt 5. *Interaction between reticular distension and pentagastrin*

It is generally agreed that satiety probably results from the converging influence of several factors on the central mechanisms controlling intake. However the experimental evidence for this is lacking. Grovum (1978) demonstrated in separate experiments that reticular and abomasal distension depressed food intake by hungry sheep but that the individual depressing effects were not additive when various combinations of distension were applied simultaneously. The present experiment was done to ascertain if the depressing effects of reticular distension (Grovum, 1978) and pentagastrin (Expt 2) on food intake by sheep were additive. Distension up to 800 ml depressed intake in a rectilinear manner but with pentagrastrin the threshold for effect appeared to be near or slightly below 1 μg/kg per h.

A Graeco Latin Square was used which allowed combinations of reticular distension (treatments A–E) and pentagastrin (treatments 1–5) given below to be used experimentally to assess the individual effects of distension, pentagastrin, sheep and period of the experiment in an analyses of variance. Reticular distension: B, no reticular distension (no

probe in place); A, 0 ml distension (probe in place); E, 267 ml distension; D, 533 ml distension; C, 800 ml distension. Pentagastrin: 4, normal saline infused intravenously (0·5 ml/min); 3, pentagastrin infused intravenously, 3 μg/kg per h; 2, pentagastrin infused intravenously, 9 μg/kg per h; 5, pentagastrin infused intravenously, 27 μg/kg per h; 1, pentagastrin infused intravenously 81 μg/kg per h.

Combinations of treatments

Sheep . . .	1	2	3	4	5
Period					
1	A_1	B_3	C_5	D_2	E_4
2	B_2	C_4	D_1	E_3	A_5
3	C_3	D_5	E_2	A_4	B_1
4	D_4	E_1	A_3	B_5	C_2
5	E_5	A_2	B_4	C_1	D_3

The experimental procedure (p. 185) was followed except that (*a*) the deprivation period was 395 min, (*b*) the pentagastrin (lot (L) ICQF-UG) infusion commenced 25 min before feeding, (*c*) the reticulum was distended with warm water in a balloon secured to a polyethylene tube just before feeding time (*d*) after 30 min of feeding, the pentagastrin infusion was stopped, the balloons were removed from the reticulum over a period of 15 min and the animals were allowed free access to food for an additional 30 min. The placement of the balloons in the reticulum was always verified before the animals were fed by recording biphasic and triphasic pressure waves associated with the mixing and the regurgitation of stomach contents.

RESULTS

Expt 1. *Motility of the reticulum*

The effect of various gastrointestinal hormones and analogues on the frequency and amplitude of contractions of the reticulum is summarized in Table 1. Pentagastrin significantly decreased the frequency of contractions in a dose related manner but the amplitude of the contractions were suppressed only at the highest rate of infusion. The motility was eliminated completely for approximately 5 min on the average when infusions at the two higher rates commenced. Secretin decreased amplitude slightly. All compounds having CCK activity markedly inhibited frequency but not amplitude of contractions. The inhibition of frequency increased in relation to the dose tested.

Expt 2. *Pentagastrin and food intake*

Food intake for 30 min during the infusion of 9 and 27 μg pentagastrin/kg per h was depressed ($P < 0{\cdot}05$) whether the deprivation period was 317 min (Table 2) or 160 min (Table 3). In both instances the threshold for effect may have been below 1 μg/kg per h since intakes at this dose rate were lower than the corresponding control values. At all infusion rates tested, the relative depressions in intake in 30 min were greater for the 160 min deprivation period than for the 317 min period. In neither experiment was there an immediate recovery of intake after the infusions were stopped (Tables 2 and 3).

Expt 3. *Secretin and food intake*

Food intake was depressed ($P < 0{\cdot}025$) during the infusion of 8 CU secretin/kg per h for all feeding periods except 0–10 min (Table 4). The threshold for effect may have been less than 0·5 CU/kg per h since intakes at this dose rate were lower than the control values during all but one of the feeding periods. The apparent compensatory feeding that occurred when the infusion at 8 CU/kg per h was stopped did not attain significance ($P > 0{\cdot}05$).

Table 1. *Expt* 1. *The effect of various gastrointestinal hormones and analogues infused intravenously on motility of the reticulum in sheep*

(The frequency of biphasic contractions and the average amplitude of the last contraction of each set is reported for the last 10 min of the infusion period)

Peptide	Infusion rate (/kg per h)	*n*	Frequency (nos./10 min)				Amplitude (mm Hg)				I Incidence	
			Control (1)	Infusion (2)	Statistical significance of difference	(2)÷(1)	Control (3)	Infusion (4)	Statistical significance of difference	(4)÷(3)	(min)	(%)
Pentagastrin (μg)	1	7	13	11·3	****	0·87	18·68	16·54	NS	0·89	0	0
	3	8	13·13	9·6	**	0·65	18·27	14·78	NS	0·84	0	0
	9	10	13·5	8·2	****	0·61	17·81	19·34	NS	1·15	5·3	40
	27	9	13·22	7·4	****	0·56	21·97	18·71	*	0·83	4·9	56
Secretin (CU)	8	5	13·0	12·2	NS	0·94	21·08	17·34	****	0·82	0	0
Cholecystokinin (IDU)	0·56	2	13·5	13·5	NS	1·01	29·82	32·6	NS	1·09	0	0
	1·7	5	13·6	13·4	NS	0·99	23·41	26·94	NS	1·18	0	0
	5	5	12·6	10·0	**	0·79	25·99	24·65	NS	0·93	0	0
	15	5	13·2	5·2	***	0·37	21·83	15·36	NS	0·73	4·8	40
Ceruletide (ng)	270	5	12·2	10·8	NS	0·89	28·56	29·49	NS	1·07	0	0
	810	5	11·8	5·8	*	0·48	30·19	19·53	NS	0·71	0	0
Octapeptide (μg)	1·5	5	13·8	8·4	***	0·61	24·65	19·95	NS	0·79	0	0

NS, not significant; I, the interval at the start of the infusions during which motility was completely eliminated; CU, Clinical Units; IDU, Ivy Dog Units.
* $P < 0·05$, ** $P < 0·025$, *** $P < 0·01$, **** $P < 0·005$.

Table 2. *Expt* 2. *Effect of infusing pentagastrin intravenously on food intake* (g) *by sheep after a deprivation period of* 317 min

(Mean values for five sheep)

Period after deprivation (min)	Analyses of variance of 5 × 5 Latin Square: Rate of infusion (μg/kg per h) 0	1	3	9	27	Statistical significance of differences	SE† (g)
0–10	222	208	180	144	158	*	18
10–20	101	99	66	73	65	NS	14
0–20	323[a]	307[ab]	245[ab]	217[b]	224[b]	**	23
20–30	47	18	65	22	32	NS	13
0–30	370[a]	325[ab]	311[ab]	239[b]	256[b]	*	26
			Infusion stopped				
30–40	51	30	18	19	35	NS	13

[a, b] Mean values with different superscripts differed significantly by a sequential variant of the Q method; NS, not significant.

* $P < 0.05$, ** $P < 0.025$.

† $\sqrt{\frac{\text{Error mean square}}{5}}$.

Table 3. *Expt* 2. *Effect of infusing pentagastrin intravenously on food intake* (g) *by sheep after a deprivation period of* 160 *min*

(Mean values for five sheep)

Period after deprivation (min)	Analyses of variance of 5 × 5 Latin Square: Rate of infusion (μg/kg per h) 0	1	3	9	27	Statistical significance of differences	SE† (g)
0–10	156	140	116	95	123	NS	15
10–20	93	61	73	50	60	NS	12
0–20	249	201	189	145	183	NS	22
20–30	43	40	30	4	6	NS	11
0–30	292[a]	241[ab]	220[ab]	149[b]	190[b]	**	24
			Infusion stopped				
30–40	17	10	6	18	29	NS	5

[a, b] Mean values with different superscripts differed significantly by a sequential variant of the Q method; NS, not significant.

** $P < 0.025$.

† $\sqrt{\frac{\text{Error mean square}}{5}}$.

Expt 4. *CCK and food intake*

Part A. Food intake was depressed ($P < 0.05$) in the 0–10 min feeding period by infusing 15 IDU CCK/kg per h (Table 5). This depression relative to the control value was 39% but depressions of similar magnitude after 20 (38%) and 30 min of feeding (28%) failed to attain significance ($P > 0.05$). The threshold for effect appeared to be between 5 and 15 IDU/kg per h since intakes for 5 IDU/kg per h were similar to the control values. Significant

Table 4. *Expt* 3. *Effect of infusing secretin intravenously on food intake* (g) *by sheep after a deprivation period of* 322 *min*

(Mean values for five sheep)

Period after deprivation (min)	Analyses of variance of 5 × 5 Latin Square: Rate of infusion (CU/kg per h) 0	0·13	0·5	2	8	Statistical significance of differences	SE† (g)
0–10	214	202	175	178	147	NS	15
10–20	77^{ab}	138^{a}	95^{ab}	71^{ab}	48^{b}	**	16
0–20	291^{ab}	341^{a}	270^{ab}	249^{ab}	195^{b}	**	26
20–30	60^{a}	37^{ab}	35^{ab}	23^{b}	24^{b}	**	7
0–30	351^{a}	378^{a}	305^{ab}	273^{ab}	218^{b}	**	28
			Infusion stopped				
30–40	23	34	25	39	74	NS	18

[a, b] Mean values with different superscripts differed significantly by a sequential variant of the Q method; NS, not significant; CU, Clinical Units.

** $P < 0{\cdot}025$.

† $\sqrt{\frac{\text{Error mean square}}{5}}$.

Table 5. *Expt.* 4. *Effect of infusing cholecystokinin intravenously on food intake* (g) *by sheep after a deprivation period of* 296 *min*

(Mean values for five sheep)

Period after deprivation (min)	Analyses of variance of 5 × 5 Latin Square: Rate of infusion (IDU/kg per h) 0	0·56	1·7	5	15	Statistical significance of differences	SE† (g)
0–10	210^{a}	201^{a}	202^{a}	205^{a}	125^{b}	*	18
10–20	86	91	100	94	52	NS	18
0–20	287	302	301	299	177	NS	31
20–30	22	43	37	45	44	NS	12
0–30	309	345	339	344	221	NS	32
			Infusion stopped				
30–40	55^{ab}	30^{b}	82^{a}	34^{b}	48^{b}	****	9

[a, b] Mean values with different superscripts differed significantly by a sequential variant of the Q method; NS, not significant; IDU, Ivy Dog Units.

* $P < 0{\cdot}05$, **** $P < 0{\cdot}005$.

† $\sqrt{\frac{\text{Error mean square}}{5}}$.

differences in intakes were observed after the infusions were stopped but their meaning is difficult to interpret.

Parts B and C. The depressions in food intake observed when 810 ng ceruletide and 3·0 μg octapeptide of CCK/kg per h were infused were substantial but they failed to attain significance (Tables 6 and 7; $P > 0{\cdot}05$). After 10, 20 and 30 min of feeding, the depressions amounted to 31, 29 and 26% for ceruletide and 43, 37 and 39% for octapeptide respectively. These values were similar to those for CCK when it was infused at 15 IDU/kg per h. These

Table 6. *Expt.* 4. *Effect of infusing ceruletide intravenously on food intake (g) by sheep after a deprivation period of 273 min*

(Mean values for five sheep)

Period after deprivation (min)	Analyses of variance of 5 × 5 Latin Square: Rate of infusion (ng/kg per h) 0	30	90	270	810	Statistical significance of differences	SE† (g)
0–10	267	235	238	215	184	NS	18
10–20	68	104	79	51	53	NS	12
0–20	335	339	317	276	237	NS	26
20–30	35	14	27	33	36	NS	9
0–30	370	353	344	310	273	NS	30

NS, not signigicant. † $\sqrt{\frac{\text{Error mean Square}}{5}}$.

Table 7. *Expt* 4. *Effect of infusing octapeptide of CCK intravenously on food intake (g) by sheep after a deprivation period of 273 min*

(Mean values for five sheep)

Period after deprivation (min)	Analyses of variance of 5 × 5 Latin Square: Rate of infusion (μg/kg per h) 0	0·38	0·75	1·5	3·0	Statistical significance of differences	SE† (g)
0–10	197	196	193	175	112	NS	20
10–20	95	90	103	79	71	NS	17
0–20	293	286	296	254	183	NS	35
20–30	61	78	52	29	34	NS	12
0–30	354	365	349	284	218	NS	39
			Infusion stopped				
30–40	34	34	44	50	75	NS	14

NS, not significant. † $\sqrt{\frac{\text{Error mean square}}{5}}$.

effects for ceruletide and octapeptide appear to be biologically significant because they fit well into extended dose response curves for these peptides (unpublished information). The thresholds for effect appear to be approximately 90 ng ceruletide/kg per h and between 0·75 and 1·5 μg/kg per h for octapeptide.

Expt 5. *Interaction between reticular distension and pentagastrin*

Both reticular distension and pentagastrin tended to depress intake (Table 8) but the effects were not significant ($P > 0{\cdot}05$). The greatest intake depression with pentagastrin occurred when it was infused at 9 μg/kg per h. Compensatory feeding was not observed in the experiment after the balloons were removed from the reticulum and the infusion of pentagastrin was terminated. When sheep and period effects were removed from the 0–30 min

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Table 8. *Expt 5. Effect of combinations of reticular distension and of pentagastrin infusions on the intake of food (g) by sheep deprived of pelleted alfalfa for 395 min*

(Mean values for five sheep)

Time after deprivation (min)	Analyses of variance of 5 × 5 Graeco Latin Square												
	Distension (ml)					Statistical significance of differences	Pentagastrin (μg/kg per h)					Statistical significance of differences	SE† (g)
	0 (no probe)	0 (probe)	267	533	800		0 (saline)‡	3	9	27	81		
0–10	247	216	161	136	122	NS	197	180	137	193	175	NS	73
0–20	312	300	205	195	167	NS	273	245	185	255	233	NS	88
0–30	378	357	258	216	199	NS	325	298	219	286	280	NS	101
Compensatory feeding with balloons removed from reticulum and infusion stopped													
30–40	117	80	139	146	146	NS	129	94	165	102	139	NS	109
30–50	136	90	170	219	180	NS	160	133	231	132	139	NS	123
30–60	160	118	205	285	191	NS	197	173	262	188	139	NS	140
Total intakes during the experiment													
0–60	539	475	463	501	390	NS	522	472	481	473	419	NS	116

NS, not significant. † $\sqrt{\frac{\text{Error mean square}}{5}}$. ‡ 9 g sodium chloride/l.

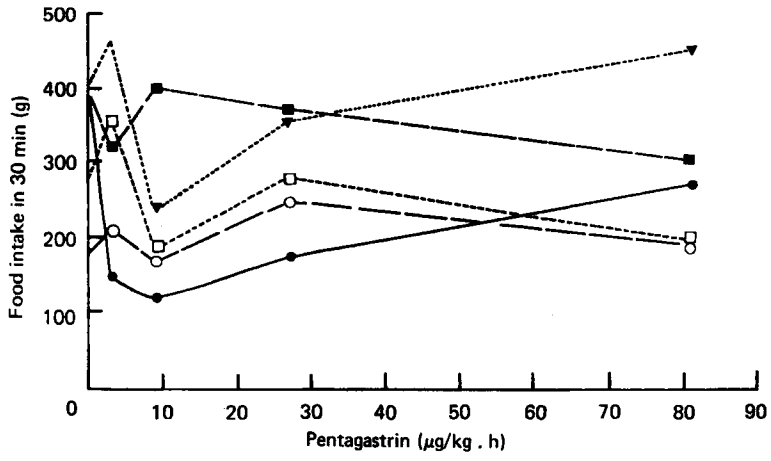

Fig. 1. Expt 5. Effect of combinations of reticular distension (ml) and pentagastrin infusions (μg/kg per h) on the intake of food by sheep deprived of their diet for 395 min. ▲ .... ▲, Normal (no probes) ■ – – – ■, no distension (probes in place); □ – – – □, 267 ml distension; ○ —— ○, 800 ml distension; ● —— ●, 533 ml distension. The values were collected according to a 5 × 5 Graeco Latin Square design and the period and sheep effects were removed by subtracting from each observation the period mean and the sheep mean and adding back twice the value of the general mean. Each point represents the value from only one sheep.

intakes, (observation–sheep mean–period mean + 2 general mean) the values were plotted (Fig. 1) to demonstrate that pentagastrin did not have any consistent effect on intake at any level of reticular distension except that 9 μg/kg per h depressed intake relative to normal saline in all instances except when there was just a probe in the reticulum. However this effect was not significant.

DISCUSSION

The results of these experiments which were published briefly elsewhere (Grovum, 1977*a*, *b*) demonstrated that continuous intravenous infusions of gastrin, secretin and CCK can depress food intake by hungry sheep. Single injections of pentagastrin and CCK have previously been reported to do this also (Baile & Grovum, 1974; Grovum *et al.* 1974) but secretin was without effect (Baile & Grovum, 1974). Most of this discussion will be devoted to the biological significance of the findings now reported but firm statements cannot be made until the normal concentrations of these hormones in the systemic circulation of sheep are compared to those produced during the infusions. The effects of the hormones on motility of the reticulo-rumen contributes in part to these judgements of biological significance because a depression of food intake is probably unphysiological if the associated motility pattern has ben disturbed markedly or completely abolished. An error in judgement could be made in the comparison however because the experiments on motility were done on animals at rest. It is well known that motility of the reticulo-rumen is stimulated markedly by feeding and it is possible that motility during a meal is relatively difficult to suppress with homone infusions. Thus a given rate of hormone infusion needed to suppress intake could possibly look better biologically if motility were studied during a meal than if the motility were studied in animals at rest. The error, if any, would favour conservatism. The rate of hormone infusion needed to depress intake has also been related to dose response information for other functions of these peptides. It is also not known as yet if another feeding regimen would allow the anorectic properties of the hormones to be observed with lower rates of hormone infusion.

Expt 1. *Motility of the reticulum*

The inhibition of reticular motility by pentagastrin, CCK and ceruletide is a confirmation of work of Carr *et al.* (1970), Ruckebusch (1971) and McLeay & Titchen (1970, 1975) with pentagastrin and Faustini *et al.* (1973) and Wilson *et al.* (1976) with CCK and ceruletide. However the experiment extends their findings for pentagastrin and CCK by showing that the effects were related to dose. Secretin slightly depressed the amplitude of reticular contractions but did not alter their frequency which is contrary to the results of Bruce & Huber (1973). The reason for this discrepancy is not known as the secretin used was obtained from the same source in both experiments. It is possible that the inhibitory effects of pentagastrin, cholecystokinin, ceruletide and octapeptide on intake were mediated through a disruption of motility of the reticulo-rumen but this argument would not apply to secretin.

Expt 2. *Pentagastrin and food intake*

The important question to be answered is whether the infusion of approximately 1 μg pentagastrin/kg per h, which approximates the threshold value for the suppression of food intake, falls within the physiological range of gastrin activity in the sheep. The amplitude of reticular contractions was affected little by the rates of infusion used but at 1, 3 and 9 μg/kg per h the frequencies of contractions were decreased 13, 35 and 39% from normal. The effect at 1 μg/kg per h was minimal but motility was still interfered with so the question of biological significance remains. Bell *et al.* (1977) claimed that 0·6–1·8 μg/kg per h was within but on the high side of the physiological range when they described the inhibitory influences of pentagastrin on gastric emptying in the calf. Hamilton *et al.* (1976) who produced similar results in man with 1·2–2·4 μg/kg per h cited evidence that 3 μg/kg per h produces results identical with endogenously-released gastrin. They also stated that antral motility was not stimulated until 0·6 μg/kg per h was infused but that it was maximal at 6 μg/kg per h. Thus they claimed that their infusion rates were within the physiological range. In another study, a dose of only 0·7 μg/kg per h was required for half-maximal secretion of hydrochloric acid in man (Corazziari *et al.* 1978). A subcutaneous injection of 3 μg pentagastrin/kg in sheep produced a marked increase in acid secretion from a fundic pouch over a period of 1 h which was similar to that produced by teasing the sheep with food or feeding but it was substantially less than that induced with a subcutaneous injection of 40 μg histamine acid phosphate/kg (McLeay & Titchen, 1975). This suggests that an intravenous infusion of 1 μg pentagastrin/kg per h may not be unphysiological in sheep. One extremely interesting observation reported by McLeay & Titchen (1975) is that antrectomy increased the intake of chopped lucerne hay in two sheep. If this could be repeated in a larger number of animals it would indicate that gastrin may have an inhibitory effect on roughage intake in this species. It would also add a radically new dimension to current thoughts on what limits roughage intake by ruminants. Infections of the abomasum with the parasite *Ostertagia circumcincta* are known to be accompanied by reductions in food intake (McLeay *et al.* 1973; Titchen & Anderson, 1977) and by hypergastrinaemia (Anderson *et al.* 1976; Titchen & Anderson, 1977). The possibility arises that hypergastrinaemia in infected sheep may cause hypophagia and rumen atony. This conclusion would seem to be supported by the finding that 9 μg pentagastrin/kg per h produced a substantial depression in intake and motility (Expts 1 and 2). McLeay (1971) as cited by McLeay *et al.* (1973) also found that pentagastrin suppressed food intake by sheep but the dosage used was not reported. The significance of gastrin-like activity in the reticulum and the rumen (Jury & McLeay, 1977) is not known. Whether this is the same as the gastrin found in vagal nervous tisse (Rehfeld *et al.* 1979) is not known. However with four times the gastrin-like activity in these organs relative to the pyloric antrum there is good reason to wonder what factors will release this

activity and if food intake will be affected. The only note of discord in the argument that gastrin may influence food intake is that food intake and circulating gastrin levels in the rat both increase during lactation (Lichtenberger & Thier, 1979). However chemicals with gastrin activity have not been shown to depress intake in this species with reasonable doses whether injections were made peripherally (Smith *et al.* 1974; Lorenz *et al.* 1979) or centrally (Manaker *et al.* 1979). Smith *et al.* (1974) reported that pentagastrin reduced intake but the dose required was eighty times that for maximal secretion of acid in this species so the response was not considered significant biologically.

Expt 3. *Secretin and food intake*

The threshold for the depression of food intake by secretin in sheep may, on the basis of these limited data, be less than 0·5 CU/kg per h. This observation contrasts with the lack of a response of food intake to secretin in rats (Glick *et al.* 1971; Gibbs *et al.* 1973). Infusion of 5 CU/kg per h into the portal vein of sheep tripled the flow of bile (Heath, 1970) and there was no indication that a plateau was being approached. The elevated flow due to the secretin was also similar to the maximum value arising from the infusion of taurocholate into the portal vein (Heath, 1970). If the stimulation of bile flow is a physiological function of secretin in sheep, it follows that limiting food intake may also be one of its functions since these effects were produced with similar rates of infusion of secretin. At present the receptor site for the food intake response is not known and the comparison of the bile secretion and food intake responses is somewhat hazardous since the infusion sites differed in the two experiments. However the response of bile secretion to secretin should be similar whether the infusion was made into the portal vein or the jugular vein because the liver is not known to degrade appreciable amounts of secretin (Lehnert *et al.* 1974; Rayford *et al.* 1976) and the secretion of fluid from the intrahepatic bile ducts and canaliculi can be stimulated only by secretin that has returned to the heart and been distributed to the liver via branches of the common hepatic artery (Konturek *et al.* 1977; Netter, 1957).

Mixed bile and pancreatic secretions of sheep were found by Horn & Huber (1975) to increase to 1·8 times that of control values when 6·88 units secretin (Calbiochem)/kg per h were infused into the jugular vein. The activity of this product is described in terms of Crick–Harper–Raper units (CHR) which have been found equivalent to 0·25 CU by radioimmunoassay (Boden *et al.* 1974) or to approximately 0·125 CU by biological assay (Stening *et al.* 1968). Thus the infusion of Horn & Huber (1975) was approximately 1 CU/kg per h and its effect on secretion was moderate relative to that noted by Heath (1970) who infused 5 CU/kg per h. These observations and the findings that 3·96 CU/kg per h was within the physiological range for the dog (Sugawara *et al.* 1969) and that 1–4 CU/kg per h stimulated maximal secretion of water and electrolyte by the pancreas and the liver of man and pigs (Johnson & Grossman, 1968; Wormsley, 1969; Vaysse *et al.* 1974; Häcki *et al.* 1977; Schaffalitzky *et al.* 1977) indicate that the suppression of food intake in sheep was achieved with a reasonable rate of administration of secretin.

Expt 4. *Ceruletide, octapeptide, CCK and food intake*

The depressions in food intake with 810 ng ceruletide and 3 μg octapeptide/kg per h failed to reach significance but they were similar to that produced by CCK. In other experiments (unpublished observations), the effects were statistically significant. Suppressions of intake by hungry sheep have also been found by Symons (1978) in response to intravenous injections of octapeptide. Doses of 810 ng ceruletide and 1·5 μg octapeptide/kg per h had quite marked inhibitory effects on the frequency of contractions of the reticulum (Table 1) but if the threshold for ceruletide on intake is approximately 90 ng or 66 pmol/kg per h this affect on motility is probably inconsequential. One wonders, however, why the

threshold for ceruletide on intake appears to be so low relative to that for CCK (5 to 15 IDU or 425 to 1276 pM/kg per h) and octapeptide (0·75 to 1·5 μg or 656 to 1312 pM/kg per h). The lower limits on threshold for CCK and octapeptide would have had some depressing effects on motility. From this point of view, the odds that satiety can be signalled by normal peptides having CCK activity would be greater if the effective doses were lower. The sensitivity of the animals to the drugs may be increased with another feeding regimen and this would make CCK more attractive as a signal of satiety in sheep. In anaesthetized dogs, infusions of ceruletide and octapeptide at rates of 1·4 μg/kg per h increased the secretion of pancreatic amylase by 2·4 and 5 times respectively (Nakajima, 1973). With this information as a reference point, the doses used in sheep were not unreasonably high. The amounts of ceruletide used to suppress intake in other species have varied from 200 ng/kg in the rabbit (Houpt & Anika, 1977) to 400 and 2000 ng/kg in the rat (Gibbs *et al.* 1973; Stern *et al.* 1976; Anika *et al.* 1977). For octapeptide, 0·91–3·64 μg/kg have been required in the rat and the monkey (Gibbs *et al.* 1973; Gibbs *et al.* 1976; Anika *et al.* 1977; Nemeroff *et al.* 1978). Another report indicated that 40 IDU CCK activity/kg given as octapeptide decreased the intake of solid and liquid food but not water by weanling rats (Bernstein *et al.* 1976). The gall-bladder of sheep as outlined on X-rays was reduced in size by 50% after only 10 ng ceruletide/kg was injected intravenously (Beretta *et al.* 1973) and the transit time of barium sulphate through the small intestine of man was decreased from 130 to 19 min after a single intravenous injection of 20 ng octapeptide/kg (Levant *et al.* 1974). It is clear that extremely small doses of ceruletide and octapeptide have marked effects on the gut and thus one must be cautious in interpreting their effects on food intake.

CCK depressed food intake in the sheep and the threshold appeared to between 5 and 15 IDU/kg per h. The rate of 1·66 IDU/kg per h had a negligible effect on reticular motility but 5 and 15 IDU/kg per h decreased the frequencies of contractions 21 and and 63% from normal respectively without affecting their amplitude. These effects on frequency of contractions were substantial but since motility was not abolished by the infusions, the question of the biological significance of CCK in depressing intake by sheep is still pertinent. The numerous reports mentioned in the introduction that CCK depressed food intake were not produced with the continuous-infusion technique so comparisons of the dosage required for effect in sheep and other animals are difficult to make at this time. However after deprivation periods ranging from 5–21 h, 5 IDU CCK/kg significantly depressed food intake by the rat (Gibbs *et al.* 1973), rabbit (Houpt & Anika, 1977) and the monkey (Gibbs *et al.* 1976) for periods ranging from 15 min to 1 h. If the threshold for effect in sheep is close to 5 IDU/kg per h one might venture that the sheep were perhaps as sensitive as the other animals to the apparent satiating influence of CCK. However a single injection of 20 IDU/kg was required to depress significantly intake in lambs deprived of food for 3·5 h (Grovum & Baile, unpublished results). The intravenous infusion of 6·88 CHR units CCK/kg per h in sheep, a dose which is equivalent to 1·7 IDU/kg per h (Jorpes & Mutt, 1973), caused a 60% increase in mixed pancreatic and bile flow, a 3·8-fold increase in bicarbonate secretion and a transient but small increase in protein secretion by the pancreas (Horn & Huber, 1975). The dose of CCK required for maximal stimulation of protein secretion by the pancreas was 7·5 CHR or 1·9 IDU/kg per h in human beings (Malagelada *et al.* 1973) and it ranged from 2 (Vaysse *et al.* 1974) to 6 (Debas & Grossman, 1973) IDU/kg per h in the dog (Wormsley, 1969). These reports indicate that the amount of CCK required in sheep to suppress intake is not unreasonably high but it exceeds the amounts required to stimulate maximally enzyme secretion by the pancreas in other species.

Whether the suppression of intake by CCK is a direct effect or an indirect one due to general malaise is still open to question and it may be difficult to resolve because reductions in intake may be a more sensitive indicator of malaise or nausea than any known test of

conditioned taste aversion (Deutsch & Gonzalez, 1978). A dose of 1–4 IDU CCK/kg per h produced mild to moderately severe abdominal cramps in man (Gutiérrez *et al.* 1974; Sturdevant & Goetz, 1976) and 3 IDU/kg per h produced loose stools in three out of ten human subjects but they increased rather than decreased their food intakes during the infusion (Sturdevant & Goetz, 1976). An intravenous injection of 50 IDU over 1 min in human beings markedly accelerated the passage of barium through the small intestine with transit times being reduced from 227 to 8 min (Parker & Beneventano, 1970). Mild abdominal cramps and nausea were commonly reported for up to 10 min after the injections. CCK at the rate of 8 IDU/kg per h has been reported to produce retching in dogs (Stening *et al.* 1969) but adverse reactions have not been noticed in sheep. However the outputs and dry-matter contents of faeces have not been monitored during these experiments. In the rat, a dose of CCK that reduced intake was shown to decrease markedly motility of the duodenum (Deutsch *et al.* 1978). The role of CCK in normal satiety in human beings and animals is still not known as a non-specific effect is difficult to rule out when complaints of nausea and abdominal cramps are made by human subjects injected with less CCK/kg body-weight than that required in animals to reduce meal size (Glick, 1979). However there appear to be wide species differences in the effect of CCK on the gut and generalities about the side-effects of a given rate of CCK infusion obviously cannot be made safely. The fact that CCK does not depress water intake by thirsty animals indicates the response is specific for food (Gibbs *et al.* 1973; Mueller & Hsiao, 1977; Kraly *et al.* 1978). The demonstration that CCK elicited the behavioural sequence associated with normal satiety in the rat (Antin *et al.* 1975) is a good argument for its involvement in the control of food intake in that species.

Although the physiological significance of CCK in satiety in sheep is certainly open to question, it appears that CCK may still be important in animal production because sheep infected with *Trichostrongylus colubriformis* markedly decreased their food intakes concomitant with rises in activity of CCK in blood (Symons, 1978). Following treatment with an anthelmintic the CCK activity and food intakes returned to normal. It is also possible that the retardation of growth due to the presence of trypsin inhibitors in the diet may be mediated in part through an effect of CCK on intake. Liener (1979) indicated that the growth of the pancreas in these instances is probably due to elevated concentrations of CCK because the trypsin inhibitors interfere with the normal negative feedback mechanism of trypsin on the cells which produce CCK.

Expt 5. Interaction between reticular distension and pentagastrin

The effect of 800 ml reticular distension was substantial in that it depressed the 0–30 min intake by 45% which agrees with results reported previously (Grovum, 1978) but there was little additional effect of pentagastrin except perhaps for a weak effect at 9 μg/kg per h (Fig. 1). The results are really not conclusive and the Graeco Latin Square is perhaps not the best tool to use in assessing whether two factors combine influences to affect food intake. The main weakness of the design is that each treatment combination is examined in only one animal.

General discussion of the role of gastrointestinal hormones in the brain

Little is known about the mechanism of action of gastrointestinal hormones on food intake but Dafny *et al.* (1975) and Schanzer *et al.* (1978) have shown that intraperitoneal injections of CCK, pentagastrin and secretin modified neuronal activity in areas of the brain involved in feeding behaviour. Whether this is a direct or indirect effect is not known but the possibility of it being direct cannot be discounted because pentagastrin has been shown to act on central structures to depress motility of the reticulo-rumen (Chapman *et al.* 1979),

to induce gastric secretion in rats (Tepperman & Evered, 1979) and to induce acetylcholinesterase activity in the cerebral cortex but not in the cerebellum of rats (Nandi Majumdar & Nakhla, 1978). Also the net stimulatory effect of cerulein on intestinal motility in the dog was shown to consist of a direct stimulatory effect on the intestine and to an inhibitory effect mediated through a sympathetic area in the brain cranial to the superior colliculus (Neya *et al.* 1973). The activity of cortical neurons but not hypothalamic neurons was shown to be modified by the direct application of CCK (Ishibashi *et al.* 1979) but there is still no evidence that peripheral CCK can function in this manner. Various peptides with gastrin and CCK activities (including the COOH-terminal tetrapeptide amide common to both hormones) and have been found in cerebrospinal fluid in human beings (Rehfeld & Kruse-Larsen, 1978) and octapeptides with CCK activity have been isolated from the brains of sheep (Dockray *et al.* 1978) and turkeys (Dockray, 1979). If the distribution of activity in the brain were similar to that in cattle, pigs and the rat the activity would have been greatest in the cerebral cortex but otherwise widely distributed except for small amounts in the epithalamus, the cerebellum and the pituitary (Rehfeld, 1978). There is thus good reason for suspecting that CCK activity in the cerebral cortex may modify CNS activity but whether this will alter feeding behaviour is not known. Gastrin activity by contrast has been found restricted to the pituitary gland (Rehfeld, 1978) and bombesin activities were highest in the hypothalamus (Walsh *et al.* 1979). Bombesin, as Walsh *et al.* (1979) comment, is also concentrated in the fundus of the stomach. Additional examples of hormone and peptide activity which have been found in the gut and the brain are insulin, vasoactive intestinal polypeptide (VIP), neurotensin, substance P and somatostatin (Havrankova *et al.* 1979; Pearse & Takor Takor, 1979), gastric inhibitory polypeptide (Guillemin, 1978), motilin (Grossman, 1979) and secretin (Mutt *et al.* 1979). The functional significance of the gastrointestinal hormones in the brain is only starting to emerge but evidence has been found that obese mice contained significantly less CCK activity in the cerebral cortex than normal animals (Straus & Yalow, 1979*a*) and gastrin injections into the third ventricle but not intravenously in rats suppressed plasma TSH, LH and prolactin levels and increased the concentrations of growth hormone (Vijayan *et al.* 1978). As well, VIP activity was nineteen times higher in portal hypophyseal blood than in systemic arterial blood making it possible that this chemical may also influence the function of the pituitary gland (Said & Porter, 1979). Injections of CCK into the lateral cerebral venticle depressed food-rewarded lever pressing in hungry rats (Maddison, 1977) and injections of pentagastrin (Grovum *et al.* 1974) and octapeptide of CCK (Della Fera & Baile, 1979) but not secretin (Grovum *et al.* 1974) into the lateral cerebral ventricles depressed food intake by hungry sheep. Tritiated cerulein was selectively bound to tissue in the ventromedial hypothalamus and micro-injections of cerulein into the ventromedial but not the lateral hypothalamus depressed food intake in rats (Stern *et al.* 1976). However it is unlikely that only the ventromedial hypothalamus is involved in these anorectic responses since the intake of food by hungry rats with lesions in the ventromedial hypothalamus was also depressed by the intraperitoneal injections of the octapeptide of CCK (Kulkosky *et al.* 1976). This might be expected if the brain has a number of tiers of control over ingestive behaviour as was sugested by Mogenson & Huang (1973). In summary it appears that pentagastrin and cerulein can act directly on the brain to alter body function (Neya *et al.* 1973; Chapman *et al.* 1979; Tepperman & Evered, 1979) and that gastrointestinal hormones in the brain may be acting as neurotransmitters (Guillemin, 1978; Pinget *et al.* 1979; Rehfeld *et al.* 1979; Straus & Yalow, 1979*b*), as true hormones involving the pituitary gland (gastrin and VIP), or in the instance of secretin, VIP and glucagon as modulators of the rate of turnover of norepinephrine in hypothalamic areas involved in the control of food intake (Fuxe *et al.* 1979). The latter observation is extremely interesting because it relates to the recently-proposed scheme of Anderson (1979)

that energy intake regulation is influenced by brain catecholamine activity whereas protein intake is governed largely by brain serotonin levels. The means by which gastrointestinal hormones may gain access to the brain and the humoral factors and types of afferent activity which will release gastrointestinal hormone activity in the brain clearly needs to be investigated in future studies on food intake. The peripheral administration of brain opiates was not effective in producing analgesia but yet the other actions of the chemicals such as changes in behaviour were still being observed (Kastin *et al.* 1979). Numerous mechanisms for these observations were suggested (Kastin *et al.* 1979) but the mode of action is still not known.

There appears to be reasonably good evidence that CCK and gastrin are involved in the modulation of food intake in ruminants at least when certain gastrointestinal parasites are resident in the gut but whether these hormones limit intake in healthy animals is uncertain. Secretin has not been demonstrated to have anorectic properties in other species and its normal role in satiety in sheep is also not known. Although these experiments have focused on the effects of gastrin, cholecystokinin and secretin one must bear in mind that hepatic-portal injections of serotonin (Rezek & Novin, 1975) and glucagon (Martin & Novin, 1977; Martin *et al.* 1978; Vanderweele *et al.* 1979) have suppressed feeding in rats and rabbits, that pancreatic polypeptide has been shown to reduce food intake in mice (Malaisse-Lagae *et al.* 1977), that bombesin decreased intake in rats (Gibbs *et al.* 1979) and that insulin which is released by volatile fatty acids in the rumen or the blood (Bhattacharya & Alulu, 1975; Weekes, 1975) has also been implicated in the control of food intake (Bhattacharya & Alulu, 1975; Panksepp *et al.* 1975; Rezek, 1976). The depressing effects of gastrointestinal hormones on food intake by ruminants in health and disease needs to be investigated further. Radioimmunoassays applicable to ruminants need to be developed to ascertain whether the concentrations of hormone which suppress intake during infusion studies are within the normal range or within the range found in animals infected with parasites.

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