Concurrent studies on the flow of digesta in the duodenum and of exocrine pancreatic secretion of calves

The collection of the exocrine pancreatic secretion from a duodenal cannula

By J. H. TERNOUTH* and H. L. BUTTLE
National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

(*Received* 12 *June* 1972 – *Accepted* 23 *October* 1972)

1. Two surgical techniques were developed in the calf to study the role of pancreatic secretion in digestion. The secretion was collected through a cannula placed in a small sac of duodenum into which the pancreatic duct drained. The continuity of the duodenum was re-established in the first technique by duodenal re-entrant cannulas, and in the second technique by end-to-end anastomosis of the duodenum with a cannula placed cranial to the anastomosis to return the pancreatic secretion. The accessory pancreatic duct was ligated.

2. The flows of digesta through the duodenum of milk-fed calves were 5505, 6369 and 7709 ml/12 h at 7, 24 and 63 d of age respectively, similar to values reported previously in the literature. In a 12 h collection period 297, 441 and 602 ml pancreatic fluid were secreted by calves of 7, 24 and 63 d of age respectively. The secretion from the mucosa of the duodenal sac was 40 ml/12 h in two other calves.

3. The rate of secretion from the pancreas varied markedly in milk-fed calves, being lowest 2–3 h and highest 6–10 h after feeding. Changes in the concentration of chloride and bicarbonate with pancreatic secretion rate were indicative of a secretin stimulus to secretion.

4. The rates of inactivation of pancreatic enzymes collected from the duodenal sac were measured at 4°, 20° and 39°.

Few long-term studies of the exocrine secretion of the bovine pancreas have been undertaken. Wass (1965*b*), Gorrill, Thomas, Stewart & Morrill (1967), McCormick & Stewart (1967) and Aust & Cook (1968) inserted tubes into the main pancreatic duct and measured the volume of secretion at various times after feeding; however, autolysis of pancreas duct tissues limited the survival of animals cannulated in this manner (McCormick & Stewart, 1967).

In this paper, two surgical techniques are described with which it has been possible to study the exocrine secretion of the pancreas in calves for several months. Essentially, these techniques consisted of the preparation of a small isolated segment of the duodenum into which the main pancreatic duct opened: the continuity of the intestine was maintained either by re-entrant cannulas, or by an anastomosis and a simple cannula placed cranial to the anastomosis for the return of pancreatic secretions.

These procedures are similar to those of Dragstedt, Montgomery & Ellis (1930–1) and Herrera, Kemp, Tsukamoto, Woodward & Dragstedt (1968) for dogs and to those of Sineshchekov (1965) and Aliev (1966).

The anatomical relationships of the pancreatic ducts, bile duct and duodenum vary between different species (Hallenbeck, 1967). In adult cattle, the main pancreatic duct

* Present address: Department of Animal Husbandry, Faculty of Veterinary Science, University of Queensland, St Lucia, Queensland 4067, Australia.

enters the duodenum about 300 mm caudal to the sphincter of Oddi (Sisson & Grossman, 1956), and Wass (1965*a*) has demonstrated that an accessory pancreatic duct joins the bile duct in most cattle.

MATERIALS AND METHODS

Animals

Newborn Friesian and Ayrshire bull calves that had not suckled were purchased and checked for absence of absorbed colostrum by a zinc sulphate turbidity test (Aschaffenburg, 1949) on a sample of plasma. Over the next 48 h each calf was fed four times with a total of 7 l of pooled colostrum collected from cows during the first 24 h after parturition. This regimen was modified when surgery was performed within 48 h of birth so that the calf did not have a full abomasum.

Surgical techniques

Anaesthesia was induced with a mixture of cyclopropane and oxygen given through an open mask; an endotracheal tube was inserted and anaesthesia maintained with cyclopropane–oxygen in a closed circuit apparatus.

A 150–200 mm incision was made, beginning over the last rib on the right side, which extended ventrally over the costro-chondral junctions on to the flank. The periosteum was reflected and a segment of the rib removed, and the incision deepened into the abdominal cavity. The anatomical relationships between the duodenum, liver, bile duct and pancreas were explored by blunt dissection and the juxtaposition of the cranial lobe of the pancreas with the bile duct was located. Ligatures were then passed around the pancreatic tissue as close to the bile duct as possible in an attempt to occlude the accessory bile duct.

The pancreatic secretion was collected from a cannula placed in a 30–40 mm segment of the duodenum into which the main pancreatic duct drained. The duodenal segment was formed by dividing the duodenum between clamps at sites 20–30 mm cranial and caudal to the pancreatic duct opening. One end of the segment was closed by oversewing the clamp (Markowitz, Archibald & Downie, 1964); a Perspex 'T piece' cannula (Fig. 1) was inserted through an incision in the anti-mesenteric border, secured with a purse-string suture, and then the other end of the segment was clamped and oversewn. The continuity of the intestine was restored either by joining the ends through re-entry type cannulas (technique 1) or by an end-to-end anastomosis (technique 2).

Technique 1. The two ends of duodenum that remained were closed by oversewing the clamps. A Perspex 'T piece' cannula was inserted through the anti-mesenteric border of the duodenum near each of the closed ends and secured by a purse-string suture. All three cannulas were exteriorized through stab wounds in the right side, a polyethylene disc and aluminium clip were used to stabilize each cannula, and all three were connected externally with polyvinyl chloride (PVC) tubing.

Technique 2. The two ends of duodenum that remained after the cannulated segment had been formed were anastomosed by an end-to-end anastomosis (Markowitz *et al.*

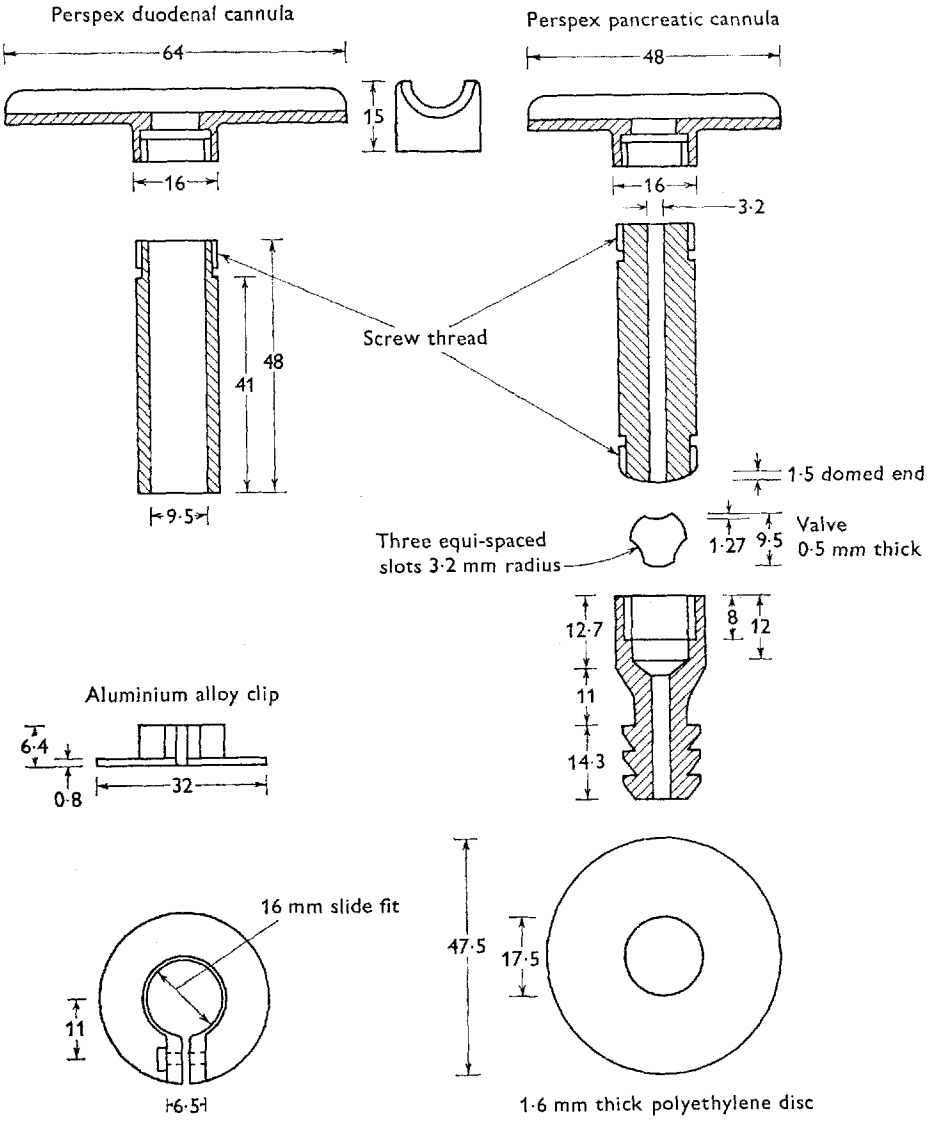

Fig. 1. Drawings of cannulas. (All measurements in mm.)

1964). A single 'T piece' Perspex cannula for return of the pancreatic secretion was inserted into the duodenum anterior to the anastomosis and both cannulas were exteriorized through stab wounds and connected externally. The first three calves in which this technique was used died within 48 h from blockage at the anastomosis. Subsequently, interrupted sutures only were used for the end-to-end anastomosis (some short lengths of continuous suture had been used in the first three) and the patency of the anastomosis was maintained by inserting 100 mm PVC tubing of external diameter 8 mm through the anastomosis. The tubing was held in place by threads which passed through the duodenal cannula and was withdrawn, through the cannula, 3 d after surgery.

On two calves a further modification of technique 2 was used, in order to separate pancreatic secretions from the secretion from the mucosa of the duodenal sac. PVC tubing was inserted through the duodenal orifice of the pancreatic duct and ligated into position, this tube was brought out through the cannula draining the duodenal sac, and was removed 7 d after surgery.

Technique 1 was used on eleven calves; on seven of these the surgery was done within 48 h of birth and on the other four when they were 3–5 d old. Technique 2 was used on fifteen calves 3–36 d old.

Management of calves

Feeding with colostrum or milk was reinstated as soon as possible after recovery from surgery. After healing, the external parts of the cannulas and the surrounding skin were washed frequently. Impaction of ingesta in the external tubing which connected the re-entrant cannulas rarely occurred except when hay diets were offered to older calves. Latterly a non-return valve (Pekas, 1965) was incorporated into the cannula which drained the duodenal sac to prevent back-flow of ingesta.

Sampling techniques

The calves were fed at 09.00 and 21.00 hours. Collections of duodenal digesta and of the secretion from the pancreas with the secretion from the mucosa of the duodenal sac (sac effluent) were made from 08.30 till 21.00 hours. The duodenal digesta were collected in a polyethylene bottle attached to the calf by the technique of Tagari & Roy (1969). The bottle was emptied as soon as 200 ml were collected and also at the conclusion of each 1 h period. The sac effluent was collected in a second smaller bottle attached to the calf. The duodenal digesta were homogenized and a one-tenth sample was taken; a one-fifth sample was taken of the sac effluent. The remainder of both effluents were mixed, warmed, and returned to the calf at a rate similar to the outflow of digesta from the duodenum.

Analytical techniques

Sodium and potassium in the pancreatic secretion were measured with an EEL flame photometer. The bicarbonate content of 5 ml of pancreatic secretion was estimated by the method of Langerlof (Varley, 1967) and Cooke, Nahrwold & Grossman (1967). Nitrogen was determined by the Kjeldahl method and protein content estimated on the assumption that 1 g nitrogen is derived from 6·25 g protein. Amylase, lipase, ribonuclease, trypsin and total protease were assayed by the methods described by Ternouth (1971) and Ternouth, Siddons & Toothill (1971). Estimates of the rates of inactivation of these enzymes in sac effluent were obtained by incubating samples of sac effluent at 4°, 20° and 39° and the activities of these enzymes were measured on subsamples taken at half-hourly or hourly intervals. The time required for one-half of the activity of these enzymes to disappear ($t\frac{1}{2}$) at each temperature was calculated by least square regression analysis.

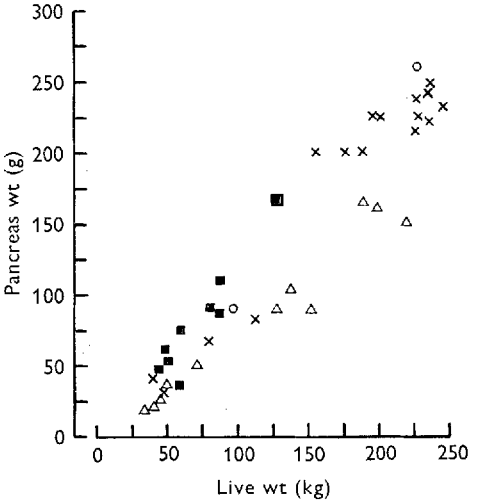

Fig. 2. Weight of the pancreas of cannulated (■) and non-cannulated (×) calves. △, ○, values for male and female calves taken from Schingoethe, Gorrill, Thomas & Yang (1970).

Autopsy

At post-mortem examination, the pancreas of each calf was weighed and examined macroscopically for the presence of a patent or occluded accessory duct. Selected blocks of the pancreatic tissue were examined histologically.

RESULTS

With technique 1, ten of the eleven calves recovered from surgery and were slaughtered at 39–173 d of age. With technique 2, eight of the fifteen calves recovered from surgery and were slaughtered at 62–207 d. Of the losses, one occurred because of a fractured cannula, three from intestinal blockage at the anastomosis and three from salmonellosis. Experiments on digestibility and nutrient balance were conducted on calves before and after surgery when technique 2 was used; after surgery there was a significant depression in the digestibility, and in the nitrogen and phosphorus retention from the diet given to the calves (Ternouth, 1971).

Pancreatic morphology

Macroscopically, the pancreas from each of the calves appeared normal. Gross pancreatic weights in relation to body-weight are shown in Fig. 2. The weights of the pancreases from cannulated calves were slightly heavier than values obtained for non-cannulated calves in other experiments at this Institute. A patent accessory duct was identified in one calf (1/3) in which technique 1 was used. On histological examination, the pancreases from cannulated calves appeared normal in that neither enlargement of the acini nor fibrosis of the ductules were seen; however, an occasional complete acini had degenerated and had been infiltrated by lymphocytes.

Table 1. *Mean milk intake, volume of duodenal digesta and of pancreatic secretion of calves at mean ages of 7, 24 and 63 d, during 12 h duplicate collection periods*

Mean age (d)	Calf no.	Live wt (kg)	Milk intake (ml)	Duodenal volume (ml)	Apparent secretion* (ml)	Pancreatic secretion†	
						ml	ml/kg live wt. h
7	1/1	36·0	3216	5500	2284	302	0·70
	1/2	36·0	3534	5120	1586	291	0·67
	1/3	34·0‡	2932	4810	1878	137	0·34
	1/4	44·0	3228	5670	2443	483	0·91
	1/5	37·0	3727	5895	2168	335	0·75
	1/6	34·0	3724	6050	2327	360	0·88
	Mean	36·8	3392	5505	2115	297	0·71
24	1/1	41·0‡	4941	7670	2729	365	0·74
	1/2	42·0	2875	4485	1611	328	0·65
	1/3	38·0‡	3182	5580	2398	258	0·56
	1/4	51·0	5183	7080	1893	890	1·45
	1/5	48·0	4183	6235	2053	375	0·65
	1/6	42·0	4472	7185	2714	429	0·85
	Mean	43·7	4140	6369	2233	441	0·85
63	1/3	51·0	4933	7415	2482	225	0·37
	1/4	67·0	4206	7035	2830	885	1·10
	1/5	68·0	4683	8255	3572	623	0·76
	1/6	59·0	5899	8130	2232	674	0·95
	Mean	61·3	4930	7709	2779	602	0·80

* Duodenal volume minus milk intake.
† 'Sac effluent' in text.
‡ No duplicate measurement.

Composition and volume of pancreatic secretion

The total volumes of pancreatic secretion plus the secretion from the mucosa of the duodenal sac (sac effluent) secreted by each of six calves at 7 and 24 d of age, and by each of four calves at 63 d of age (technique 1) are given in Table 1, which also shows the volumes of duodenal effluent and of milk intake. The average total volume of sac effluent increased with age, but the secretion rate remained constant in proportion to the live weight. The changes in flow of sac effluent and of duodenal effluent from these calves with time after feeding are given in Fig. 3, which shows that at 24 and 63 d of age marked changes occurred in flow of sac effluent with time after feeding, with least flow at 2–3 h and greatest flow at 6–10 h after feeding, whereas at 7 d of age this pattern of flow change was barely discernible. The volume of duodenal effluent collected over the same period decreased steadily with time after feeding.

The volume of secretion from the mucosa of the duodenal sac was measured in two calves (3/2 and 3/3) in which the pancreatic secretion had been separated from the secretions of the duodenal sac. The volume of secretion from the mucosa was $80 \pm 0{\cdot}9$ ml/d ($n = 13$), which represented about 7% of the total sac effluent from calves of similar age. The concentrations of electrolytes and the total nitrogen content were measured in sixteen samples of pancreatic secretion collected at the same time as the secretion from the mucosa of the duodenal sac (Table 2). The samples analysed

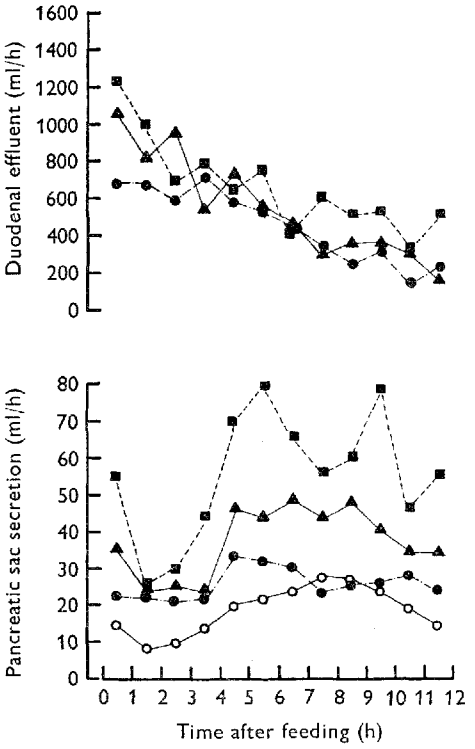

Fig. 3. Hourly changes in the mean volumes of duodenal effluent and of pancreatic sac secretions from six calves at 7 (●) and at 24 (▲) d of age, and from four calves at 63 (■) d of age. ○, values taken from McCormick & Stewart (1967).

Table 2. *Composition of the secretion of the pancreas and duodenal segment of a calf*

(Mean values with their standard errors)

	Pancreatic secretion	Duodenal segment secretion
No. of samples ...	16	6
Na^+ (mmol/l)	148·9±0·9	145·4±3·1
K^+ (mmol/l)	5·04±0·12	4·75±0·11
Cl^- (mmol/l)	89·4±5·2	142·3±3·8
HCO_3^- (mmol/l)	61·9±4·3	—
N (mg/l)	650±50	1480±60

were chosen to represent a wide range of pancreatic secretion rates, and the relationship between chloride, bicarbonate and protein concentrations and secretion rate are shown in Fig. 4.

The concentrations of amylase, trypsin and total proteases in the sac effluent collected from the calves at the three ages examined, both before feeding and 10–12 h after feeding (i.e. before the next feed), are given in Table 3; significant differences were not found between the results obtained at the two times of sampling.

The contents of the bottle used to collect the sac effluent were maintained at 20°.

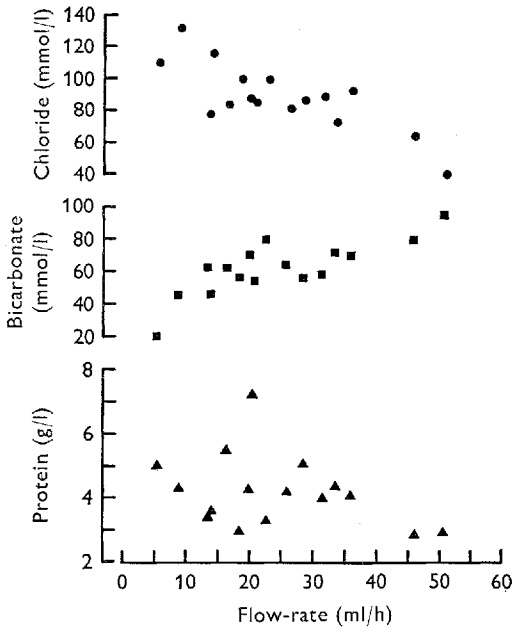

Fig. 4. Relationships between the flow-rate and the concentration of chloride (●), bicarbonate (■) and protein (▲) in the pancreatic juice of calf no. 3/3.

Table 3. *Comparison of the concentrations of enzymes (μg/ml) in pancreatic secretion of calves immediately before feeding and* 10–12 *h after feeding*

Enzyme	Before feeding	10–12 h after feeding	Mean	SE
Amylase	113	109	111	19
Trypsin	554	444	499	42
Proteases	5123	4933	5028	79

Table 4. *Mean times (h), with their standard errors, for one-half of the activities of enzymes of calves to disappear ($t_{\frac{1}{2}}$) from the combined secretions of the pancreas and duodenal segment after maintenance at* 4°, 20° *and* 39°

Enzyme	At 4°	At 20°	At 39°
Amylase	— (3)	4·70 ± 0·49 (3)	0·83 ± 0·06 (3)
Trypsin	— (5)	6·06 ± 0·58 (6)	1·79 ± 0·17 (6)
Total protease	20·63 ± 4·93 (6)	6·73 ± 0·85 (6)	3·33 ± 0·28 (6)
Lipase	— (3)	6·06 ± 1·24 (3)	0·64 ± 0·04 (3)
Ribonuclease	2·35 ± 0·47 (4)	1·06 ± 0·09 (4)	0·65 ± 0·07 (4)

Figures in parentheses are the numbers of experiments.

The times required for one-half of the activity of enzymes to disappear ($t_{\frac{1}{2}}$) from sac effluent maintained at 4°, 20° and 39° are given in Table 4. After storage at 20° ribonuclease was most rapidly inactivated ($t_{\frac{1}{2}}$ = 1·06 h), whereas amylase, lipase, trypsin and total protease were more stable ($t_{\frac{1}{2}}$ = 4·70, 6·06, 6·06 and 6·73 h respectively). At 4° only ribonuclease and total protease activity were lost ($t_{\frac{1}{2}}$ = 2·35

Table 5. *Mean daily secretion of exocrine pancreatic fluid by ruminant animals*

Reference	Daily pancreatic secretion (ml/kg live weight)	Age
Ruminating sheep		
Cited by Dukes (1955)	12·0	Adult
Magee (1961)	10·3	Adult*
Harrison & Hill (1962)	11·0	Adult
Taylor (1962)	8·6–10·3	Adult
Aust & Cook (1968)	10·2	?
Clary, Mitchell, Little & Bradley (1969)	4·3	Yearling wethers*
Ruminating cattle		
Cited by Dukes (1955)	14·0	Adult
Greene, Hirs & Palade (1963)	11·0	Adult†
Zherebtsov & Serykh (1962)	6·2‡	1–6 month
Wass (1965*b*)	7·2	Adult§
Aust & Cook (1968)	23·8	Calf
Milk-fed calves		
Zherebtsov & Serykh (1962)	5·9‡	1 month
Gorrill *et al.* (1967)	3·8	4 d
	7·0	21 d
McCormick & Stewart (1967)	9·5	3–56 d‖
Present experiments		
Calf 1/3	8·2	8 d
	13·4	24 d
	8·9	58 d
Calves 1/1, 2, 4, 5 and 6	19·4	8 d
	17·5	24 d
Calves 1/4, 5 and 6	22·6	58 d

* Assumed live weight of 50 kg.
† Assumed live weight of 550 kg.
‡ Recorded values were for 18 h experimental period.
§ Mean secretion of 100 l in 25 d with a live weight of 550 kg.
‖ Dry food available.

and 20·63 h respectively). The activities of all enzymes examined were lost rapidly after storage at 39°. The addition of the duodenal activator, enterokinase, to the sac effluent did not increase the proteolytic activities.

DISCUSSION

Both the surgical procedures used were considered satisfactory as methods of collecting and returning the pancreatic secretions of calves over a long period. Survival of the calves after surgery was good and no pathological changes in the pancreatic tissues were found.

The sac effluent collected was a mixture that contained pancreatic secretion and secretion from the short length of duodenum which formed the sac. The secretion from the sac influenced the pancreatic secretion in terms of volume, relative composition (15% of the nitrogen content was of sac origin) and by activation of the proteolytic enzymes. The daily volume per kg live weight of sac effluent produced by these calves varied between animals but the mean was approximately twice that found

for calves by other workers (Table 5). Part of the discrepancy may be explained by the presence of the duodenal sac, which contributed about 7% of the volume of the sac effluent, but most of the discrepancy would appear to be due to the failure, by other workers, to occlude the accessory pancreatic duct; one of our calves (1/3) which had a patent accessory duct, produced about half the volume of sac effluent of the others.

In calves 7 d old, the secretion of sac effluent varied little after feeding, but in older calves there was a distinct pattern in flow-rate, lowest at 2–4 h and highest 6–10 h after feeding, which was not proportionally related to duodenal flow. These changes, and the changes in the concentration of chloride and bicarbonate with pancreatic secretion rate seen in calf 3/3, agree with those reported by McCormick & Stewart (1967) and are entirely consistent with the known actions and pattern of secretion of secretin as has been found for many species (Bayliss & Starling, 1902; Grossman, 1958).

Failure to return pancreatic secretions resulted in changes in volume and composition of pancreatic secretion in dogs and sheep respectively (Annis & Hallenbeck, 1951; Taylor, 1962). In the present experiments, 80% of the pancreatic secretions were returned and differences in both volume and composition at the beginning and end of collections were insignificant.

Loss of enzyme activity occurred in the sac effluent with time, especially at room temperature and above, probably by autolysis, especially as there was an absence of alternative (dietary) proteins for the enzymes to degrade (Pelot & Grossman, 1962; Khayat & Christophe, 1969).

It was unlikely that pancreatic innervation was severed (Dragstedt *et al.* 1930–1; Thomas, 1950) since the more caudal position of the pancreatic duct in calves, in relation to other species, places the operative site remote from the vagus. The duodenal sac had its own intrinsic motility which was not related to the contractions of the duodenum immediately cranial to the sac (visual observations). The nervous plexuses controlling intestinal motility had been severed and probably resulted in the asynchrony of contractions, and the consequent pooling of the secretions within the sac is likely to render this technique unsuitable for short-term studies on pancreatic secretion.

The authors wish to thank Mr S. C. Watson for his assistance with the anaesthesia and Drs R. C. Siddons and Joyce Toothill for their assistance with enzyme assay techniques. Dr J. H. Ternouth wishes to thank the Australian Dairy Produce Board for their financial support.

REFERENCES

Aliev, A. A. (1966). Modern methods of operating on entire animals in studying the digestive and intermediate exchange substances of ruminants. Thesis. Vses. Nauch.-Issled. Inst. Fiz. Biokhim. Sel'.-khoz. Zhiv., Moscow.

Annis, D. & Hallenbeck, G. A. (1951). *Proc. Soc. exp. Biol. Med.* **77**, 383.

Aschaffenburg, R. (1949). *Br. J. Nutr.* **3**, 200.

Aust, S. D. & Cook, R. M. (1968). *J. Anim. Sci.* **27**, 1160.

Bayliss, W. M. & Starling, E. H. (1902). *J. Physiol., Lond.* **28**, 325.

Clary, J. J., Mitchell, G. E. Jr, Little, C. O. & Bradley, N. W. (1969). *Can. J. Physiol. Pharmac.* **47**, 161.

Cooke, A. R., Nahrwold, D. L. & Grossman, M. I. (1967). *Am. J. Physiol.* **213**, 637.

Dukes, H. H. (1955). *The Physiology of Domestic Animals*, 7th ed. London: Baillière, Tindall and Cox.
Dragstedt, L. R., Montgomery, M. L. & Ellis, J. C. (1930–1). *Proc. Soc. exp. Biol. Med.* **28**, 109.
Gorrill, A. D. L., Thomas, J. W., Stewart, W. E. & Morrill, J. L. (1967). *J. Nutr.* **92**, 86.
Greene, L. J., Hirs, C. H. W. & Palade, G. E. (1963). *J. biol. Chem.* **238**, 2054.
Grossman, M. I. (1958). *Vitams. Horm.* **16**, 179.
Hallenbeck, G. A. (1967). In *Handbook of Physiology*. Sect. 6: *Alimentary Canal*, p. 1007 [C. F. Code, editor]. Washington, DC: American Physiological Society.
Harrison, F. A. & Hill, K. J. (1962). *J. Physiol., Lond.* **162**, 225.
Herrera, F., Kemp, D. R., Tsukamoto, M., Woodward, E. R. & Dragstedt, L. R. (1968). *J. appl. Physiol.* **25**, 207.
Khayat, M. H. & Christophe, J. (1969). *Am. J. Physiol.* **217**, 923.
McCormick, R. J. & Stewart, W. E. (1967). *J. Dairy Sci.* **50**, 568.
Magee, D. F. (1961). *J. Physiol., Lond.* **158**, 132.
Markowitz, J., Archibald, J. & Downie, H. G. (1964). *Experimental Surgery*, 5th ed. Baltimore: Williams & Wilkins.
Pekas, J. C. (1965). *J. appl. Physiol.* **20**, 1082.
Pelot, D. & Grossman, M. I. (1962). *Am. J. Physiol.* **202**, 285.
Schingoethe, D. J., Gorrill, A. D. L., Thomas, J. W. & Yang, M. G. (1970). *Can. J. Physiol. Pharmac.* **48**, 43.
Sineshchekov, A. D. (1965). In *Physiology of Digestion in the Ruminant*, p. 254 [R. W. Dougherty, editor]. London: Butterworths.
Sisson, S. & Grossman, J. D. (1956). *The Anatomy of Domestic Animals*, 4th ed. Philadelphia: Saunders.
Tagari, H. & Roy, J. H. B. (1969). *Br. J. Nutr.* **23**, 763.
Taylor, R. B. (1962). *Res. vet. Sci.* **3**, 63.
Ternouth, J. H. (1971). Studies of the role of the abomasum and pancreas in digestion in the young calf. PhD Thesis, University of Reading.
Ternouth, J. H., Siddons, R. C. & Toothill, J. (1971). *Proc. Nutr. Soc.* **30**, 89A.
Thomas, J. E. (1950). *The External Secretion of the Pancreas*. Illinois: C. C. Thomas.
Varley, H. (1967). *Practical Clinical Biochemistry*. London: Heinemann.
Wass, W. M. (1965*a*). *Am. J. vet. Res.* **26**, 267.
Wass, W. M. (1965*b*). *Am. J. vet. Res.* **26**, 1103.
Zherebtsov, P. I. & Serykh, M. M. (1962). *Izv. timiryazev. sel'.-khoz. Akad.* no. 4, p. 214.

Printed in Great Britain