PANCREATIC EXOCRINE SECRETION AND DIGESTION IN THE SHEEP

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[Manuscript received 8 September 1971]

Abstract

The effects of pancreatic exocrine secretion on digestive processes in the alimentary tract of the sheep have been studied. Animals in which the pancreatic duct was ligated were compared with pair-fed controls in which the duct was intact. Lack of pancreatic exocrine secretion led to a reduction of 65% in the rate of wool growth and a 22% reduction in body weight over a 6-week period.

Significantly more nitrogen was excreted in the faeces of sheep lacking pancreatic exocrine secretion. No difference was found between the duct-ligated animals and controls in the amount of fat excreted in the faeces.

An important function of pancreatic exocrine secretion in the sheep appears to be associated with protein degradation in the intestine and any deficiency could lead to impaired protein metabolism and wool growth.

I. Introduction

Intestinal fistulae and cannulation of the common bile duct have been used to study pancreatic secretion in the alimentary tract of the sheep (Hill and Taylor 1957; Magee 1961). A role for pancreatic juice in the optimum absorption of fat has been reported (Heath and Morris 1964; Leat and Harrison 1969). However, although a relatively large amount of microbial protein passes from the foregut to the small intestine of a sheep each day, little information is available on the importance of pancreatic exocrine secretion on protein digestion in this species.

In the studies presented here a lack of pancreatic exocrine secretion was established in young and adult sheep by ligating the pancreatic duct, leaving the bile duct intact. Changes in body weight, wool growth, and various parameters of protein and fat metabolism were measured and compared with a similar group of animals which were sham-operated, leaving the pancreatic duct intact.

II. Methods

Three groups of animals were used. The first two groups were studied together and comprised eight 6-month-old and four 3-year-old Merino wethers. The third group, comprised ten 4-year-old wethers.

The animals were surgically prepared using halothane–oxygen anaesthesia with a closed-circuit apparatus. The pancreas was exposed and dissected from the edge of the bile duct and duodenum in all animals. In half of each group the pancreatic duct was ligated and severed near its junction with the bile duct, ensuring that the bile duct remained intact.

Each duct-ligated animal was then paired with a sham-operated control. The animals were fed daily a mixture of 750 g wheaten hay chaff and 250 g lucerne chaff. When the food intake of a duct-ligated animal declined its control paired-animal was offered this same amount of food the following day.

The animals were weighed at intervals and blood samples were taken for determination of serum amylase (Van Loon, Likins, and Seger 1952), plasma non-esterified fatty acids (Trout, Estes,

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and Friedberg 1960), and blood levels of glucose (Cramp 1967), urea nitrogen (Marsh, Fingerhut, and Miller 1965), and total protein, albumin, and globulin fractions (Varley 1962, pp. 184–6). Wool growth was determined by clipping from a tattooed area 10 by 15 cm on the side of each animal and determining the weight of clean scoured wool grown between two times of clipping.

Both before and at intervals after operation the animals were placed in metabolism cages for a period of 10 days during which time measurements were made on the daily nitrogen intake from the fodder and the nitrogen output in faeces and urine. The daily excretion in the faeces of total fat (ether-extractable fraction after acid hydrolysis of the dried faeces), free fatty acids (titratable acids from a non-hydrolysed fraction), neutral fat (ether-extractable fraction from non-hydrolysed faeces minus the free fatty acid fraction), and combined fatty acids were determined (Varley 1962, pp. 347–8).

III. Results

A statistical analysis of the data from the three series of paired animals showed all groups to be homogeneous with respect to within-group variations and treatment effect. A combined analysis of all groups for the various parameters was therefore made.

Although differences were apparent between the controls and the duct-ligated animals soon after operation, the data obtained 6 weeks after operation was used to assess, by an analysis of covariance with the pre-operation data as co-variate, the statistical significance between the animals.

At the end of the 6-week period there was a mean reduction of 22% of body weight (from 31·9 to 24·9 kg) in the ligated group, whereas the mean body weight of the paired sham-operated control group on the same food intake fell from 31·0 to 29·1 kg, a loss of only 3% (S.E. of mean for both groups before and post-operation 0·63 and 0·62 respectively). The net effect of ligation of the pancreatic duct was shown by a mean loss of body weight of $4 \cdot 2 \pm 0 \cdot 7$ kg ($P < 0 \cdot 001$) over the 6-week period. There were 11 animals in each group.

Similarly, for the same groups of animals, the loss of pancreatic secretion led to a daily mean reduction in wool growth over an area of 150 sq. cm of 65% (from 96·5 to 32·9 mg) whereas only a 6% reduction (from 100 to 94·4 mg) occurred in the controls (S.E. of the mean for both groups before and post-operation 6·2 and 4·1 respectively). The net effect of ligation of the pancreatic duct on wool growth over the above area at 6 weeks after operation was shown by a reduction in the daily rate of growth of $61 \cdot 5 \pm 5 \cdot 8$ mg ($P < 0 \cdot 001$).

Again, relatively more nitrogen was excreted in the faeces of the ligated group than in the control group on the same nitrogen intake. Thus for both groups of 11 animals on a mean nitrogen intake of 11·74 g/day over a 10-day period the control group at 6 weeks after operation was excreting a mean output of 11·35 g/day of which 3·20 g/day was excreted in the faeces. The ligated group was excreting a mean output of 11·95 g/day of which 5·42 g/day was excreted in the faeces. This difference in faecal nitrogen output was significant at $P < 0 \cdot 001$ (S.E. for both groups 0·139). There was also a significant difference ($P < 0 \cdot 01$) in the urinary nitrogen excretion. The mean value for the control group was 8·15 g/day and for the ligated group 6·53 g/day (S.E. for both groups 0·291). The total nitrogen excretion, however, was not significantly different between the two groups (S.E. 0·322).

The mean daily excretion of fat in the faeces was determined for the five control sheep and five duct-ligated animals of the later series during two collections of 10-day

periods at 2 and 6 weeks after operation. Total daily intake of fat varied, depending on food intake, between 17 and 22 g/day. The results are shown in the following tabulation and give values (all expressed as g/day) for total fat, free fatty acids, neutral fat, and combined fatty acids:

	Control	Ligated	S.E.
Total fat	8·17	8·58	0·44, n.s.
Free fatty acids	0·495	0·576	0·075, n.s.
Neutral fat	2·31	2·47	0·31, n.s.
Combined fatty acids	5·37	5·60	0·28, n.s.

No significant difference was found between the control group and the duct-ligated animals for any of these fractions.

Twenty-eight determinations of the concentration of plasma proteins were made on the 10 adult sheep of the later series (five animals in each group) over a period of 8–10 weeks after operation. Total protein was significantly lower ($P < 0 \cdot 01$) in the ligated group ($5 \cdot 35 \pm 0 \cdot 065$ g/100 ml) than in the control group ($5 \cdot 82 \pm 0 \cdot 065$ g/100 ml) and when assessed as an albumin or globulin fraction (as g/100 ml) the albumin was lower in the ligated group:

	Control	Ligated	S.E.
Albumin fraction	3·17	2·52	0·053, $P < 0 \cdot 01$
Globulin fraction	2·65	2·83	0·077, $P < 0 \cdot 05$

Of the other blood parameters measured there was a marked increase in serum amylase for 3–4 days after ligation of the duct. Values of 400 units/100 ml were obtained, thus indicating the adequacy of the ligation. No change from normal values (70 units/100 ml) occurred in any of the sham-operated group. The concentration of urea nitrogen in the blood ranged from 16–20 mg/100 ml indicating no significant change from normal levels in either the control or ligated group. The concentration of glucose in the blood of the duct-ligated group showed a tendency to be higher than the control group measured in the later series of 10 animals over a 10-week period of observation. The mean difference between the two groups was $14 \cdot 7 \pm 5 \cdot 6$ mg/100 ml ($P < 0 \cdot 05$) determined on a total of 32 observations.

Radio-immunoassay of insulin in plasma samples taken at various times after operation showed a wide variation (sham-operated 9–28 and duct-ligated 9–26 micro-units/ml) with no obvious trend between the groups of animals, nor with time after operation. At autopsy the pancreas of each duct-ligated animal was found to be reduced to a small mass of fibrous tissue. Histological examination showed no viable acinar tissue with pancreatic islet tissue staining normally amongst fibrotic remnants of acinar tissue. The majority of the animals in which the pancreatic duct had been ligated had deteriorated in food intake and body weight by 8–10 weeks after operation to warrant termination of the experiment. However, two of the animals survived for 16 weeks with less severe signs of deterioration.

IV. Discussion

Hill (1960) has reported a mean daily flow of pancreatic juice in the sheep of 360 ml with a protein content of about 4%. A large proportion of this protein is made up of proteolytic enzymes. Amylase and lipase are present, although the lipolytic

activity of ruminant pancreatic juice may not be very significant (Keller, Cohen, and Neurath 1958). Heath and Morris (1964) considered that both bile and pancreatic juice secreted into the duodenum of the sheep via the common bile duct are required for optimum absorption of fat. Changes in the proportion of non-esterified fatty acids and phospholipids in the small intestine of sheep deprived of both bile and pancreatic juice have been reported by Leat and Harrison (1969). They suggested that an enzyme in pancreatic juice may be responsible for the conversion of lecithin in bile to lysolecithin and thus aid in micelle formation and the absorption of fat into lymph. This current study on pancreatic-duct-ligated sheep and sham-operated, pair-fed controls did not specifically examine any change in the pattern of fatty acids and phospholipids in the small intestine. However, the data obtained on the excretion of fat in the faeces in the two groups of sheep indicate that loss of pancreatic juice had no significant effect on the excretion of total fat. The proportion present as combined fatty acids was also the same for both groups. The methods used in assessing fat digestion in this study measured rather broad parameters and would not distinguish between the relatively large amount of lipid material in the faeces derived from bacteria in the caecum and any lipid reaching the large intestine from more proximal parts of the alimentary tract. Under the conditions in which food intake was carefully balanced in duct-ligated animals and their pair-fed, sham-operated controls, when between 17 and 22 g of fat per day were available from the fodder, it would appear that no major abnormality was apparent in gross fat digestion due to lack of pancreatic juice. This is in contrast to the severe steatorrhaea associated with pancreatic insufficiency in some monogastric species. Further circumstantial evidence of no serious defect in fat metabolism may be adduced from the normal values of non-esterified fatty acids in the plasma of both groups. However, no challenge to the animals' capacity for fat digestion and absorption by specifically increasing the fat intake with a high-fat diet was attempted.

When the parameters of protein digestion and metabolism were examined it was apparent that severe restrictions on normal metabolism were imposed following loss of pancreatic juice. The increased excretion of nitrogen in the faeces of the duct-ligated sheep probably indicate a deficiency in the degradation or absorption of protein products in the alimentary tract. Hoogenraad and Hird (1970) have reported that trypsin can be responsible for an appreciable release of amino acids from cell walls of rumen bacteria. In view of the relatively large amount of microbial protein passing through the alimentary tract of the sheep any deficiency in proteolytic degradation of this material could affect the supply of amino acids available to the animal. This view would be consistent with the severe reduction of wool growth in those animals lacking pancreatic exocrine secretion.

From the nitrogen balance studies it is apparent that despite the greater excretion of nitrogen in the faeces of the duct-ligated group, there was a lower urinary nitrogen excretion compared with the controls. The excretion of total nitrogen was similar in both groups. A probable explanation is that the amino acids absorbed in the normal animal are converted into urea nitrogen and that in the duct-ligated animal such amino acids are not absorbed, thus leading to an increased excretion of faecal nitrogen and a decreased excretion of urinary nitrogen. This would be consistent with both the observed body weight changes and the observations on the nitrogen balance

studies. An abnormality in protein metabolism was also reflected in the lower levels of circulating plasma proteins with an albumin/globulin ratio of 1·2 in the control group compared with 0·9 in the duct-ligated group.

Idezuki, Goetz, and Lillehei (1969) have suggested that in dogs severe fibrotic degeneration of the exocrine tissue of the pancreas following occlusion of the duct may lead to secondary endocrine dysfunction. The data reported in this study on sheep showed an elevation in blood glucose in the duct-ligated group compared with the control group. This was more noticeable in those animals which survived for a longer period and may indicate some long-term impairment of insulin secretion associated with altered blood supply to the fibrotic remnant of pancreas.

However, plasma insulin levels were too variable to indicate any significant trend during the time of survival of the duct-ligated animals. In view of the severe reduction in wool growth, food intake, body weight, and changes in the pattern of nitrogen excretion, we suggest a serious effect of depletion of pancreatic juice appears to be associated with protein digestion.

V. Acknowledgment

The authors gratefully acknowledge the help of Mr. W. B. Hall, Division of Mathematical Statistics, CSIRO, for the statistical analysis of the data, of Miss J. M. Robinson for technical assistance, and of Dr. J. Bassett, Division of Animal Physiology, CSIRO, for the plasma insulin assays.

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