Proteins of the Succus Entericus from the Jejunum of the Sheep

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1. Suitable methods for studying the proteins passing into the small intestine are discussed. 2. The proteins passing into temporarily isolated jejunal loops between double re-entrant fistulae in four sheep were studied. 3. Loops about 60–70 cm. long secreted protein at a rate of 1–5 g./24 hr. The effect of slight stimulation of secretion by air pressure on the output of protein in 24 hr. was not regular. The total protein in the fluid part of the succus entericus is about 2½ times the serum albumin content of the fluid. 4. The additional protein contained in the cellular debris amounts to about 60% of the protein in the fluid part of the succus entericus. 5. Comparison of the proteins in succus entericus with those in serum by immunoelectrophoretic and other electrophoretic methods showed eight components in the fluid part of the succus entericus that appeared to be the same as those in serum and two components that appeared not to be present in serum. 6. Thin-layer gel chromatography in Sephadex G-200 and sedimentation analysis showed that the succus entericus contained two proteins not present in serum, one with sedimentation coefficient (uncorrected) 10s and one sedimenting slower than albumin: they move with the macroglobulin and slower than albumin respectively on gel chromatography. 7. These proteins could be secreted by the glandular epithelium of the small intestine or liberated from desquamated epithelial cells.

The small intestine is an important site of protein catabolism: this has been established by early experiments on isolated intestinal loops of dogs (Mosenthal, 1911) and by investigations into idiopathic hypoproteinaemia in the human (Cope & Goadby, 1935; Steinfeld, Davidson & Gordon, 1957) and on the physiology of the normal human subject (Holman, Nickel & Sleisenger, 1959). Proteins pass into the small intestine, where they are digested on mixing with ingesta and enzymes from higher up the digestive tract.

The proteins passing into the intestine have been shown by Steinfeld *et al.* (1957) and Holman *et al.* (1959) to include both serum albumin and γ -globulin. Barundun, Nusslé, Witschi & Büser (1962) have shown in addition that transferrin and α_2 -M-globulin are present in the intestinal juice. These workers used either immunochemical methods or methods depending on the use of proteins labelled with ^{131}I . Recent work on the passage of proteins into the small intestine with serum albumin labelled in this way, in both normal and pathological conditions, has been presented in two symposia (Schwartz & Vesin, 1962; Peeters, 1964).

Samples of the intestinal secretion, succus entericus, can be obtained for study *post mortem* or from acute experiments or by means of per-

manent fistulae. Samples obtained *post mortem* may not be normal, as Badawy, Campbell, Cuthbertson & Fell (1957) showed that there was a shedding of the intestinal epithelium at death. Those obtained from acute experiments may also be abnormal as Birke, Liljedahl, Plantin & Wetterfors (1960) found that increased protein losses arose from surgical procedures. Permanent fistulae would appear to avoid these drawbacks.

As the proteins passing into the intestine are subject to digestion and the breakdown products may be reabsorbed, it is difficult to examine the intestinal contents obtained from a single fistula for the proteins that have passed into the lumen or to assess them quantitatively. Digestion of protein may be stopped by collecting into saline containing trypsin inhibitor (Anderson, Glenert & Wallevik, 1964), or reabsorption of breakdown products can be avoided by the use of ion-exchange resins in the diet as used by Jeejeebhoy & Coghill (1961). These procedures do not, however, provide satisfactory samples for protein studies. These are best obtained by collecting the pure juice by means of two permanent double re-entrant fistulae in the jejunum below the point of entry of the common pancreatic and bile duct. These fistulae enable a Thirty-Vella type of loop to be formed for the

duration of the experiment by by-passing the flow of ingesta through the loop. The loop can then be washed out with physiological saline and pure intestinal juice obtained. The proteins present in samples obtained in this way appear to be stable, as Wright, Jennings, Florey & Lium (1940), in discussing the enzymes present in the secretion, report no proteases in the fluid, and Campbell *et al.* (1961), using ^{131}I -labelled albumin, found little evidence for protein breakdown in samples obtained in this way.

The succus entericus can be separated on centrifuging into two fractions, a straw-coloured fluid and a deposit consisting mainly of cellular debris arising from the intestinal epithelium. The rate of secretion can be markedly increased by distension of the loop and seems to be unaffected by the flow of ingesta through adjacent parts of the intestine (Scott, 1965). Samples obtained after washing out the loop may therefore at first represent a stimulated and possibly altered secretion but those obtained later will be more representative of the basal secretion.

Samples obtained from loops isolated by this type of fistulae have been used by Hogan (1957), Campbell *et al.* (1961) and Cloete (1964) to study the passage of protein into the small intestine of the sheep. The results obtained apply to the length of loop isolated in a particular part of the small intestine. There are uncertainties attached to extending them to the whole of the small intestine. It has to be assumed that the latter behaves uniformly along its length and a value for the relative lengths of the loop and small intestine has to be determined or estimated. In this connexion it has to be remembered that the length of the live small intestine may be only one-third of its length when dead, as observed for calves by Espe & Cannon (1940). If the results of different workers are expressed as the amount of protein passing into the small intestine in 24 hr. then Hogan's (1957) data give 50 g., Cloete's (1964) data give 40 g. and Campbell *et al.* (1961), using ^{131}I -labelled albumin, obtained 8 g. for the amount of albumin. The last-named workers did not identify other forms of protein passing into the intestine. Batty & Bullen (1961), however, have shown that these include antibodies from the serum of the sheep.

The present investigations are concerned with the nature of the proteins passing into the jejunum of the sheep: they also provide quantitative data on the protein passing in the fluid part of the succus entericus into an isolated loop, on the distribution of the protein between the fluid part and the cellular debris and on the effect of air pressure in the loop on the protein secreted in the fluid part.

The proportion of protein in the fluid part of the succus entericus due to serum albumin was estimated from electrophoresis in polyacrylamide gel.

The major proteins present in the succus entericus were compared with those in the serum of the sheep. Immunoelectrophoresis and other electrophoretic methods favouring the identification of specific proteins were used. Sedimentation analysis and gel chromatography were used to indicate the upper and lower size classes of proteins present. The electrophoretic properties, the size classes of the proteins and their hexose contents were correlated by preparative electrophoresis experiments.

MATERIALS

Samples of succus entericus were obtained from four sheep provided with intestinal loops isolated by re-entrant fistulae. The fistulae were fitted with two pairs of cannulae of the type developed by Ash (1962): the cannulae were numbered (1)–(4) in the direction of normal flow of digesta through the intestine. The sheep were either Blackface or Greyface breed, their average weight was 45 kg. and they were fed on dried grass. Sheep 166 (wether), 1378 and 395 (ewes) were the same animals as used by Scott (1965). The loops were 60–70 cm. long except in sheep 175 (wether), which had a 2 m. loop. In three animals, 166, 395 and 175, cannula (1) was at the iliac flexure of the duodenum, so that these loops were in the upper jejunum. In sheep 1378, however, the loop was situated about two-thirds of the way down the small intestine in the lower jejunum. Three of the sheep, 166, 395 and 175, eventually died or were shot and their loops examined: these appeared to be normal and free from ulceration or blockage.

When samples were required, the sheep was put in a stand and the flow of digesta made to by-pass the loop by connecting cannulae (1) and (4). The loop was then washed out with 0.15 M-NaCl at 38°, introduced through cannula (2) and allowed to drain to waste from cannula (3) for 1 hr. before collection was started. Unstimulated samples were obtained at hourly intervals for about 7 hr. Stimulated samples were obtained by applying a slight positive air pressure from a small pump to cannula (2) of the isolated loop via an adjustable leak to the atmosphere and a mercury manometer. The fluid was collected in a trap and the air escaped via a water-bubble flow indicator. A pressure of 10 mm. Hg was sufficient to disturb the sheep, whereas 6 mm. Hg was without this effect yet stimulated an increased secretion. The receivers for the samples were cooled in ice and contained a preservative, ethyl mercuri-thiosalicylate (Thiomersal; British Drug Houses Ltd., Poole, Dorset) to give a concentration of not less than 0.02 g./dl.; the samples were separated into fluid and cellular debris by centrifuging for 15 min. at 2000 g in an angle centrifuge. The cellular debris was dispersed in 0.15 M-NaCl by using an ultrasonic generator (Soniprobe type 1130A; Dawe Instruments Ltd., London, W. 3). Some of the ultrasonically treated material was centrifuged for 15 min. at 30000 g for the measurement of the soluble protein.

METHODS

Protein content was estimated either from measurements of refractive-index increment (McDougall, 1964) or by the biuret method (Kabat & Meyer, 1961).

Antisera to sheep succus entericus and serum were produced in rabbits by using Proom's method as given by Hirschfield (1960).

Immunoelectrophoresis was carried out as described by Wadsworth & Hanson (1960) but with a gel consisting of 0.6% agarose (Seravac Laboratories Ltd., Maidenhead, Berks.) in place of 1% agar. Electrophoresis in agarose was carried out as above, but without immunodiffusion. Proteins were stained with Light Green or Trifalgic acid and lipoproteins with Lipid Crimson (Smith, 1960).

Paper electrophoresis was carried out in horizontally suspended Whatman 3MM paper, in sodium veronal buffer, pH 8.6 and 10.05 (0.05M-sodium veronal-0.01N-diethylbarbituric acid), for 16hr. at 0.4ma/cm. width of paper. Proteins were stained with tetrabromophenolphthalein ethyl ester (Feigl & Anger, 1937) and glycoproteins were stained by the method of Laurell & Skoog (1956).

Starch-gel electrophoresis was carried out by the method of Smithies (1955) but with the phosphate buffer system (pH 7.8) of Ashton (1957). Polyacrylamide-gel electrophoresis was carried out as described by Cruft (1962) but with the above phosphate buffer. The starch and polyacrylamide gels were stained for proteins with Naphthalene Black 10B (Smith, 1960) and caeruloplasmin was detected in the gel by the oxidase stain with *p*-phenylenediamine (Uriel, 1958).

The stained polyacrylamide gel was scanned photometrically and an estimate made of the proportion of albumin in the total protein by weighing cut-out tracings of the photometric record.

Preparative electrophoresis was carried out in the apparatus of Hannig (1961) in tris-acetate buffer, pH 8.3 and 10.1 (0.1N-acetic acid-0.2M-tris). The concentration of protein was approx. 2g./dl. and the sample rate was 2-3ml./hr., throughput time 150min. The fractions obtained were concentrated by ultrafiltration through collodion thimbles.

Sedimentation-velocity analysis was carried out at a

protein concentration of 0.4-1.8g./dl. in potassium phosphate buffer, pH 7.5 and 10.2 (0.064M-K₂HPO₄-0.013M-KH₂PO₄), in the Spinco model E ultracentrifuge at 259700g.

Thin-layer gel chromatography was carried out by the method of Johansson & Rymo (1964) with Sephadex G-200 (superfine grade) [Pharmacia (G.B.) Ltd., London, W. 13]. The chromatograms were transferred to Whatman 3MM paper and stained in the same way as the paper electrophoretograms.

Hexose was estimated by the method of Schönenberger, Kellner, Südhof & Harper (1957).

RESULTS

Protein content. Table 1 shows the volume rate of production of succus entericus and the protein concentration and output in the fluid part of the succus, obtained in typical experiments from resting and stimulated loops in the upper and lower jejunum at successive collection periods. Table 2 shows the protein output in the fluid part in 24hr. from these loops and Table 3 the distribution of the protein between the fluid part and the cellular debris and between the total and soluble protein in the cellular debris.

Immunoelectrophoresis. Comparisons made by using rabbit antiserum to sheep serum showed that 13 components were present in serum of which eight appeared to be present in the fluid part of the succus and three in the cellular debris. Two lipoprotein lines present in serum were absent from succus. When anti-succus serum was used three regions were found containing components in succus that did not appear to be present in serum. These are seen at *a*, *b* and *c* in Fig. 1.

Electrophoretic components. Paper electrophoretograms (Fig. 2) show that the succus contains components comparable in mobility with the albumin, β - and γ -globulins of serum and in

Table 1. *Volume output of succus entericus and protein concentration and output in the fluid part of the succus entericus from resting and stimulated isolated jejunal loops*

Collection period: *a*, 1 hr.; *b*, 2½ hr.; *c*, 4 hr. Vertical arrow (↓), application of stimulus.

Collection period	Upper jejunal loop (sheep 166)						Lower jejunal loop (sheep 1378)		
	Resting			Stimulated			Stimulated		
	Vol. (ml./hr.)	Concn. (g./dl.)	Output (mg./hr.)	Vol. (ml./hr.)	Concn. (g./dl.)	Output (mg./hr.)	Vol. (ml./hr.)	Concn. (g./dl.)	Output (mg./hr.)
<i>a</i> 1	37.5	0.59	209	14.5	1.28	186	<i>b</i> 16.6	0.54	36
<i>a</i> 2	33.5	0.91	291	6.5	2.06	134	<i>b</i> 6.0	0.49	29
<i>a</i> 3	12.5	1.56	187	4.3	1.63	70	<i>b</i> 5.4	0.61	37
<i>a</i> 4	5.5	1.73	87	17.0	0.81	138	<i>c</i> 3.5	0.51	18
<i>a</i> 5	13.0	1.93	221	54.0	0.21	113	28	0.22	62
<i>a</i> 6	3.0	2.29	69	49.0	0.18	88	27	0.17	44
<i>a</i> 7	7.0			20.0	0.20	40	↓ 57	0.06	33

Table 2. Protein output in the fluid part of succus entericus from isolated jejunal loops

Sheep no.	Date	Collection period (hr.)	Protein output (g./24hr.)	Remarks	
166	1. 9.64	7	4.9	Resting	Upper jejunal loop
	3. 9.64	6	3.6	Resting	Upper jejunal loop
	9. 9.64	7½	3.7	Resting	Upper jejunal loop
	21.10.64	7	3.1	Resting	Upper jejunal loop
	23.10.64	3	3.8	Resting	Upper jejunal loop
		3	1.9	Stimulated	Upper jejunal loop
		3	2.3	Resting	Upper jejunal loop
	30.10.64	4	2.0	Stimulated	Upper jejunal loop
395	26. 2.63	4	17.5	Resting	Upper jejunal loop
	— 4.63*	24	2.9	Resting	Upper jejunal loop
	23. 5.63*	24	4.4	Resting	Upper jejunal loop
1378	13. 9.64	7½	0.8	Resting	Lower jejunal loop
		3	1.1	Stimulated	Lower jejunal loop

* This result is quoted from Cloete (1964) for comparison.

Table 3. Distribution of protein in succus entericus from an isolated loop in sheep 166 between fluid and cellular debris

Collection date	...	...	10.12.64	21.1.65	26.1.65	5.2.65
Volume of succus (ml.)			77	56	55	71
Period of collection (hr.)			7	7	6.5	6
Concn. in fluid (g./dl.)			1.05	1.46	1.23	0.85
Output in fluid (g./24hr.)			2.40	2.34	2.22	2.01
Output in cellular debris (g./24hr.):						
Total protein			1.52	2.22	1.24	0.59
Soluble protein			1.03	1.02	0.43	—

Fig. 1. Immunoelectrophoretic comparison of proteins in sheep succus entericus and in sheep serum by using rabbit antisera to succus entericus and serum proteins. su and se indicate the positions of wells for succus entericus and serum antigens, and A-su and A-se indicate the positions of troughs for antisera to succus entericus and serum proteins. a, b and c indicate the approximate positions of precipitin lines characteristic of succus entericus.

addition a component with a mobility intermediate between those of β - and γ -globulin. Staining for glycoproteins showed that these were present in the succus in the β - γ -globulin mobility region. Electrophoresis in agarose gel showed clearly that there were two γ -globulin zones present in serum and both were detectable in succus samples. Starch-gel electrophoresis (Fig. 3) shows that the succus contains traces of transferrins and a strong zone of lower mobility than these components that is not present in serum; this zone could also be seen in the cellular debris. Appropriate staining also showed that traces of caeruloplasmin were present. The slow α -globulin of serum was absent from the succus. Electrophoresis in polyacrylamide gel showed that albumin accounted for 38% of the protein present in the succus compared with 51% in the serum.

Sedimentation components. The sedimentation components of succus, cell debris and serum are illustrated in Fig. 4. In addition to the components with sedimentation coefficients 4s, 7s and 16s found in the serum, the succus contains appreciable amounts of a 10s component and there are indications of a component slower than 4s. All these

Fig. 2. Paper electrophoretograms of proteins of sheep succus entericus (su) compared with those of sheep serum (se). Veronal buffer, pH 8.6 and 10.05, was used. The current was 0.4 mA/cm. width of paper for 16 hr. Serum proteins: Alb, albumin; α, β and γ, globulins.

Fig. 4. Sedimentation diagrams of the proteins of sheep succus entericus compared with those of serum and the soluble proteins of the cellular debris from the succus entericus, in potassium phosphate buffer, pH 7.5 and 10.2. The arrow indicates the direction of sedimentation at 259 700g. (a) Serum proteins (1.3 g./dl.); (b) succus-entericus proteins (1.0 g./dl.); (c) cellular-debris proteins (0.4 g./dl.). The time in each case is after 32 min.

Fig. 3. Starch-gel electrophoretogram of proteins of sheep succus entericus (su) compared with those of sheep serum (se) and cellular debris (cd) in phosphate buffer, pH 7.8. Serum proteins: Alb, albumin; Tf, transferrin; Sα, slow α-globulin.

components were found in the cell debris. The composition of succus, cell debris and serum in terms of these components is given in Table 4.

Thin-layer gel chromatography. The succus was found to contain, in addition to components with the behaviour of macroglobulins, 7s globulins and albumin, a component moving slower than albumin. These components are considered further in the experiments below.

DISCUSSION

Reasons were given in the introduction for considering the samples obtained from double re-entrant cannulae as normal. When ingesta passes through the intestine, it will produce some mechanical stimulation. The samples obtained under air stimulation are therefore probably more representative of the normal secretion than those from the resting loop. However, although an increase in the volume output occurs on stimulation, the effect on the protein output in 24 hr. was small.

One of the results in Table 2 calls for comment. The protein secreted in the first experiment

Table 4. *Sedimentation analysis of proteins in the succus entericus fluid and ultrasonically treated cellular debris compared with those of the serum of sheep*

Expt. no.	Sheep no.	Concn. (g./dl.)	Sedimentation coefficient (s)					Percentage composition			
			Component ...	1	2	3	4	1*	2	3	4
Succus entericus fluid											
1	1378	1.8		4.0	6.5	9.9	15.2	72.3	20.2	4.6	2.9
2	166	1.3		4.0	6.8	10.5	13.9	49.7	18.2	24.2	8.0
3	166	0.8		4.0	6.3	10.9	18.1	59.6	11.0	19.8	9.5
4	166	0.7		4.3	6.8	10.4	18.1	50.0	26.5	17.9	5.6
5	175	1.0		3.9	6.4	11.1	17.1	51.0	22.3	24.0	2.6
Ultrasonically treated cellular debris											
6	166	—		4.1	6.8	9.4	—	64.1	20.6	15.3	—
7	166	1.2		4.2	6.3	10.5	16.4	56.7	16.8	23.7	2.8
Serum											
8	1378	1.4		3.8	6.4	—	15.3	71.1	26.2	—	2.8
9	166	1.4		3.7	5.9	—	15.8	67.8	28.1	—	3.7
10	175	1.3		3.9	6.2	—	16.0	49.2	47.8	—	3.0

* Component 1 in succus entericus samples includes some slower unresolved component.

Fig. 5. Preparative electrophoresis of proteins in sheep serum (a) and succus entericus (b) in tris-acetate buffer, pH 8.3 and 1.0. The current was 200 mA and throughput time 150 min. A-G, Fractions of serum and succus entericus that were concentrated and used to produce the data in Fig. 6 and Table 5.

reported on sheep 395 is far above average. The secretion obtained also differed in that the proteins in it more closely resembled those of serum. The explanation for this is not clear as the later collections gave lower values. Extrapolation of the results to give the total losses of protein raises the question of how uniform is the secretion rate throughout the small intestine. The results show that the loop in the lower jejunum gave lower values than those obtained from loops in the upper jejunum. Assuming a live length of 15 m. for the small intestine, this would give a minimum value of 20 g./24 hr. and a maximum of up to 100 g./24 hr., if the very high value is excluded. A better estimate requires additional data from different parts of the small intestine.

The relation between the albumin and the total protein passing into an intestinal loop can be estimated from the present results. The electrophoresis in polyacrylamide gel showed that the total protein in the fluid part of the succus was about 2½ times the albumin content. The average of the values given in Table 3 shows that the additional protein contained in the cellular debris in samples from the upper jejunum amounted to some 60% of the protein in the fluid part of the succus.

Not all the protein in the fluid part of the succus derives from the serum. The sedimentation analyses and thin-layer gel chromatography show that there was a larger 10S component and a protein smaller than albumin present in succus and absent from serum. The former appears to correspond with the strong precipitin line at c in Fig. 1, and in view of the hexose analyses on the fractions from the preparative electrophoresis experiment it may be a glycoprotein related to the faster-sedi-

Table 5. Hexose content of electrophoretic fractions of proteins of succus entericus and serum of sheep

Fraction...	...	Hexose content (%)						
		A	B	C	D	E + F	G	
Succus entericus		2.3	6.2	4.1	4.8	5.7	4.1	
Serum		1.2	—	3.7	—	1.5	1.3

Fig. 6. Thin-layer chromatography in Sephadex G-200 (superfine grade) gel of electrophoretic fractions A-G of proteins in sheep serum (a) and in succus entericus (b). R, Reference solution containing rabbit serum proteins and a horse serum macroglobulin preparation; S, starting position. Serum proteins: Alb, albumin; Gl, globulin; M, macroglobulin. Sodium veronal buffer, pH 8.6 and 10.05, was used. The gels show different flow rates in the two experiments.

menting components of the γ_{1A} -globulins described by Heremans, Heremans & Schultze (1959). The latter may correspond to the zone noted in succus

on paper and starch-gel electrophoresis. Table 4 shows that the 10s component may amount to about one-quarter of the protein in the fluid part of the succus. Hence more than one-quarter of the protein may not come from the serum. These proteins could be secreted by the glandular epithelium of the small intestine or liberated from desquamated epithelial cells or both.

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