SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

VII.* SECRETION OF SULPHUR AND NITROGEN IN SHEEP PANCREATIC AND BILE FLUIDS

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Abstract

[^{35}S]taurine or [^{35}S]Na_2SO_4 was given as a single intraduodenal infusion and the combined bile and pancreatic (CBD) secretions continuously collected, sampled, and returned. In two sheep 41–51% of ^{35}S from [^{35}S]taurine was recovered in CBD fluids, less than 1% in the faeces, and less than 4% in the urine, over 3 days. When the ^{35}S-labelled CBD fluids were infused an estimated 90–113% of the ^{35}S was recycled; in 6 days less than 2% was excreted in faeces and less than 4% in the urine. With other sheep 6–8% of ^{35}S from [^{35}S]Na_2SO_4 was recovered in the CBD fluids and 63–76% in the urine over 4–5 days. When the ^{35}S-labelled CBD fluids were infused 14–29% of the ^{35}S was recycled.

The composition of ^{35}S incorporated into the CBD fluids after infusion of [^{35}S]-taurine and [^{35}S]Na_2SO_4, respectively, was: soluble organic sulphur, 97–98% (68–77% in taurocholate, 3–7% in other tauroconjugates, less than 2% in taurine *per se*) and 18–19% (less than 11% in taurocholate); reducible sulphur, 0·1–0·2% and 80–81% (25–54% in inorganic sulphate, 29–47% in ester sulphates); protein sulphur, 1·8–3·4% and 0·5–0·7%.

The daily secretion of fluid, sulphur, and nitrogen in CBD secretions from four sheep was 366–871 g, 142–245 mg, and 1063–2039 mg, respectively. The mean nitrogen : sulphur ratio was 7·60, and there was a significant linear relationship between output of sulphur and nitrogen. The composition of the sulphur was: soluble organic sulphur 78%; protein sulphur 10%; ester and inorganic sulphate sulphur each 6%. Urea plus ammonia nitrogen comprised 17·5% of the total nitrogen.

The results show that taurine is efficiently conserved, thus contributing to the sulphur economy of the sheep.

I. Introduction

Large amounts of endogenous protein are secreted into the intestine of the monogastric animal (e.g. Nasset, Schwartz, and Weiss 1955; Twombly and Meyer 1961; Ochoa-Solano and Gitler 1968). Of the total secretion in man, pancreatic protein secretions have been variously estimated to be *c.* 8 g/day (Howard, James, and Evans 1951) and 12–30 g/day (Nasset 1965) and bile protein secretion *c.* 1 g/day (Russel, Fleck, and Burnett 1964) or 2–3 g/day (Nasset 1965). Other estimates of pancreatic and bile fluid total nitrogen, non-protein nitrogen, and protein secretion for man and other vertebrates may be roughly calculated from the data compiled by Altman and Dittmer (1968). Data for sheep are presented in the present paper.

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The sulphur in sheep faeces is largely organic and is excreted in substantial amounts (Bird 1971). The nitrogen : sulphur ratio may be considerably narrower than the corresponding ratio for the feed (Barrow and Lambourne 1962). These authors suggested that the excretion of bile taurocholic acid might account for this. The bile acids in sheep are predominantly tauroconjugated (Peric-Golia and Socic 1968). In monogastrics these bile salts participate in the enterohepatic circuit, with little loss from the system (e.g. see Gray, Nicholson, and Quincey 1968), except for germ-free animals (Gustafsson *et al.* 1957) or those treated with antibiotics (Linstedt and Norman 1957). Any bile salts not absorbed are mainly deconjugated and degraded by bacteria in the large intestine (Gustafsson *et al.* 1957). The sulphide so formed is absorbed and is excreted in the urine largely as inorganic sulphate (Schram and Crokaert 1957; Boquet and Fromageot 1965). Although little of the taurine sulphur is lost in the faeces of rats (Boquet and Fromageot 1965), there is no information on the fate of this compound in the sheep.

In the present experiments the incorporation of ^{35}S into the bile–pancreatic secretions, the composition of ^{35}S in the bile–pancreatic secretions, and the excretion of ^{35}S in urine and faeces was followed over 3–5 days after the duodenal infusion of [^{35}S]taurine or [^{35}S]Na_2SO_4. The efficiency of ^{35}S recycling from the radioactive bile–pancreatic fluids was similarly estimated. In addition, data are also presented on the secretion of total sulphur, and the distribution of that sulphur, in the bile–pancreatic fluids of the sheep.

II. Materials and Methods

(*a*) *Experimental Animals*

The common bile and pancreatic duct (CBD) in mature Merino sheep was permanently cannulated. Access to the CBD on the right-hand side of the sheep was gained by the removal of a 10-cm segment from the mid-section of the 10th or 11th rib. The CBD was severed about 2 cm from the sphincter of Oddi, i.e. several centimetres distal to the entry of the pancreatic ducts. A flexible open-ended catheter (int. diam. 2 mm) was inserted 3 cm into the CBD proximal to the point of transection. Sutures placed distal to a vinyl collar 2 cm from the end of the cannula were used to retain the cannula in the duct. Another soft catheter was inserted approximately 6 cm into the duodenum via the severed CBD (i.e. through the sphincter of Oddi) and both cannulae were exteriorized through stab wounds. The cannulae were patent for 3–5 weeks; they were eventually dislogded or rendered non-functional by blockage. Six sheep, weighing between 36 and 45 kg, were used in the experiments. Two of these, W2 and O6, did not regain appetite after the operation, despite treatment with antibiotics. No radioisotope infusions were given to sheep W2, but CBD secretions were collected from all sheep, including 1–2 days prior to isotope infusion (sheep W22 and B95) (Table 1).

(*b*) *Collection and Return of CBD Secretions*

The sheep were confined in metabolism cages and the CBD secretion collected continuously into a narrow closed polyethylene bag suspended from the sheep's harness. A peristaltic pump transferred the fluid to a closed polyethylene container in a refrigerator (0–5°C). From there the fluids were pumped back into the duodenum at the average rate of secretion. The delivery lines were periodically cleaned with NaOH solution and deionized water.

(*c*) *Infusion of Radioactive Solutions*

[^{35}S]taurine or [^{35}S]Na_2SO_4, supplied by the Radiochemical Centre, Amersham, England, was dissolved in sterile water, then frozen and stored until required for either intraduodenal or intravenous infusion.

(i) [^{35}S]*taurine Infusions*

Single doses containing 99–136 μCi of radioactivity, in 10·4–16·5 g solution, were given to sheep W22 intraduodenally and intravenously 3 weeks later, and to sheep B95 and sheep B53 intraduodenally. Sheep B95 accidentally dislodged its proximal cannula shortly after the infusion was made, therefore no isotope data were obtained from this animal.

The radioactive CBD secretions, urine, and faeces were collected over three separate but successive periods each of 3 days; within these 3-day periods a 5% aliquot of each individual daily collection was separately frozen and stored. The remaining CBD secretions were combined and returned to the duodenum of the respective sheep over the first 2 days of the following period. No secretions were infused on the third day of the second and third periods in order to increase the synthesis of bile acids (Bergstrom and Danielsson 1968) and thereby aid the clearance of ^{35}S from the system over each period. Non-radioactive CBD secretions, obtained several days prior to the experiments, were infused over the first period following [^{35}S]taurine infusion, in order to maintain a normal flow of bile (Harrison 1962).

(ii) [^{35}S]Na_2SO_4 *Infusions*

A single dose containing 146–173 μCi of radioactivity, in 25·0 to 30·2 g solution, was infused into the duodenum of sheep O6 on two occasions (A and B) with 3 weeks between doses, and into sheep R25.

The radioactive CBD secretions and urine of sheep R25 and O6(A) were collected over two separate but successive 4-day periods. After sampling, the radioactive CBD secretions collected in the first period were returned to the sheep over the first 3 days of the second period. No CBD secretions were infused on the final day of the second period, nor were infusions made during the first 2 days following [^{35}S]Na_2SO_4 infusion. However, non-radioactive secretions obtained prior to the experiment were returned during the last 2 days of this period. The second infusion of [^{35}S]Na_2SO_4 was given to sheep O6(B) when, as with sheep W22, the residual ^{35}S activity in the CBD secretions had fallen to near background values. This process was hastened by discarding the secretions collected in the final period. The radioactive secretions were continuously collected for 5 days and non-radioactive secretions from sheep R25 were infused during those days.

(*d*) *Diets*

Five of the sheep were offered 775 g of a ration comprising 57% oaten chaff, 29% of a proprietary concentrate (Wesfeeds sheep cubes), 13% lucerne chaff, and 1·3% minerals. The ration contained 2·1% N and 0·17% S. The other sheep (R25) was given a mixture of 46·5% oaten chaff, 46·5% lucerne chaff, 5% starch, and 2% minerals. This ration contained 1·8% N and 0·16% S.

(*e*) *Analytical*

(i) *Nitrogen*

Total nitrogen in CBD secretions and rations was determined by the Kjeldahl technique. The urea plus ammonia nitrogen content in CBD secretions was determined by the urease micro-diffusion method (Conway 1957).

(ii) *Sulphur*

The methods described by Bird and Fountain (1970) were used for the analysis of total sulphur and reducible sulphur. Protein in CBD secretions was precipitated by adding 30 ml of 95% ethanol to a 10-g sample in a tared 50-ml polyethylene tube, bringing this solution to the boil and centrifuging at 5000 *g* for 20 min. The supernatant (containing mainly taurocholate) was decanted, the precipitate washed with 30 ml water, then 10 ml 95% ethanol added and the suspension centrifuged as before. The supernatant (containing any taurine or sulphate residues) was decanted and the washing process repeated. Finally, sulphur analysis of the precipitate was made as described by Bird and Fountain (1970). Inorganic sulphate sulphur, neutral sulphur, and soluble organic sulphur values were obtained by difference (see Table 2).

(iii) *Chromatography*

Separation of sulphur compounds in CBD secretions was made by thin-layer chromatography (t.l.c.). The samples were not deproteinized. A 0·1-ml sample was distributed, in two applications, over a 3·5-cm section of the baseline on a Gelman silica-gel support sheet. Duplicate areas on each of two sheets were prepared for each sample. On each sheet authentic standards were applied, viz. taurine, cystine, methionine, taurocholic acid (TA), taurodeoxycholic acid (TDC), taurochenodeoxycholic acid (TCDC), indoxyl sulphate, [^{35}S]Na_2SO_4, and [^{35}S]taurine. The applied spots were dried in a warm air stream and developed by the ascending technique in either of two solvent systems.

One solvent system was the upper layer of isoamyl acetate–heptane–formic acid (90%)–water (85 : 15 : 70 : 30 v/v) (see Sjovall 1964). The best separation of TA from TDC and TCDC was obtained when the mixture was left for a day before isolating the upper layer. The other solvent system was a butanol–glacial acetic acid–water mixture (120 : 5·0 : 25 v/v). Vigorous shaking was required to form a single-phase solution. Good separation of the sulphur amino acids was achieved by using this solution immediately after preparation. In both systems the solvents were run for 15 cm above the base line. The chromatograms were then dried and the sections of the sheets containing the bile acid standards, *S*-amino acid standards, indoxyl sulphate, and the radioactive standards were separately detached. The sulphur amino acids were located with ninhydrin reagent and the bile acids by spraying with 60% sulphuric acid and heating to 250°C. Areas corresponding with those of the authentic compounds were marked off on the sample strips and on the radioactive standard strips and the segments were cut off and placed separately into vials.

The isoamyl acetate–heptane–formic acid–water (AA–H–F–W) solvent system did not differentiate TDC and TCDC (R_F 0·93). TA was clearly separated (R_F 0·65) from these bile acids and from taurine (R_F 0·37). However, indoxyl sulphate (representing the ester sulphates) occupied a diffuse area over and about the position of TDC and TCDC, while, in most instances, methionine (R_F 0·67) was not distinguished from TA. The butanol–acetic acid–water (But–HAC–W) solvent system clearly separated methionine (R_F 0·97), taurine (R_F 0·57), and cystine (R_F 0·37). The bile acids migrated to the top of the sheet and so were not distinguished from methionine. Methionine sulphone (MS, R_F 0·53) and taurine were not separable. In both solvent systems inorganic sulphate did not migrate from the origin. Protein would probably also remain at the origin.

The total ^{35}S activity in urine and in CBD secretions was determined from balance-point liquid scintillation counting of 1-ml samples in 10 ml of the scintillant described by Bird and Fountain (1970). Stable counting rates were obtained for the CBD fluids if the vials were stored in the dark for several days, in which time much of the coloration also disappeared. An internal standard technique was used to determine counting efficiency. The determination of reducible ^{35}S and protein ^{35}S in CBD secretion and total ^{35}S in faeces was as described by Bird and Fountain (1970). Radioactivity in the vials containing strips cut from chromatograms was counted after the addition of 16 ml of scintillant [a mixture of 1500 ml toluene, 500 ml methanol, 8 g 2,5-diphenyloxazole, and 0·2 g of *p*-phenylene-bis(4-methyl-5-phenyloxaz-2-ole)].

III. Results

(a) *Secretion and Composition of Sulphur and Nitrogen in CBD Fluids*

Intake and CBD secretion data are shown in Table 1. These data were drawn from all collection experiments. The mean daily secretion of CBD fluids ranged from 366 to 1559 g/day; excluding sheep O6 and W2 the range was from 366 to 871 g/day (for individual values see Fig. 2). Sheep O6 and W2 did not eat following cannulation and on those grounds were excluded from any group means or regression analyses given in this text. The mean daily secretion of total sulphur was 142–245

mg/day and of total nitrogen 1063–2039 mg/day (see Fig. 1). The N : S ratio for these values ranged from 6·48 to 8·58 (mean 7·60±0·44). For sheep W22, R25, B53,

TABLE 1

INTAKE AND BILE–PANCREATIC (CBD) SECRETION DATA

Values are means ± standard error

Sheep No.	No. of collection days	Mean daily intake (g)			Daily CBD secretion			
		Dry matter	Sulphur	Nitrogen	Fluid (g)	Sulphur (mg)	Nitrogen (mg)	N : S ratio
W22*	10	775±0	1·32±0	16·3±0	503±35	197±17	1690±145	8·58
W22†	10	748±18	1·28±0·03	15·8±0·4	366±39	145±15	1152±137	7·96
B53	9	398±46	0·68±0·08	8·4±1·0	807±47	157±17	1063±141	6·48
B95	2	775±0	1·32±0	16·3±0	579±4	245±3	2039±15	8·34
R25	8	524±27	0·86±0·03	9·3±0·4	871±52	183±16	1338±98	7·30
O6*	8	0	0	0	367±39	142±22	381±28	2·96
O6†	5	0	0	0	508±13	184±35	664±35	3·98
W2	2	0	0	0	1559±47	692±104	1357±15	1·96

* First collection period, after [^{35}S]taurine infusion (W22) or [^{35}S]Na_2SO_4 infusion (O6).

† Second collection period beginning 3 weeks later, after a second infusion of [^{35}S]taurine (W22) or [^{35}S]Na_2SO_4 (O6).

and B95 the regression of total sulphur output (y, mg/day) on total nitrogen output (x, mg/day) (Fig. 1) was:

$$y = 35\cdot6+0\cdot102x \quad [r = 0\cdot92;\ P < 0\cdot001;\ \text{S.D. (regression) } 21\cdot5]$$

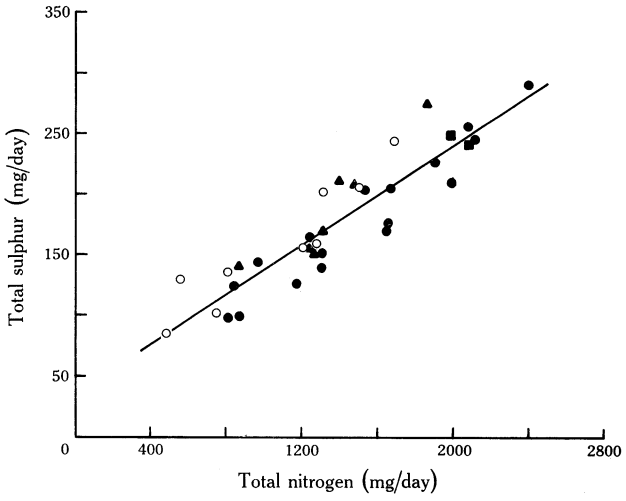

Fig. 1.—Relationship between the output of total sulphur and total nitrogen in the bile–pancreatic secretions (see text for regression equation).
● Sheep W22. ▲ Sheep R25.
○ Sheep B53. ■ Sheep B95.

Sheep W2 and O6 had exceptionally high outputs of sulphur compared with nitrogen; the N : S ratio ranged from 1·96 to 3·98. The regression of sulphur output on nitrogen output was not significant.

On those days when CBD secretions were not returned to the animal the amount of fluid, sulphur, or nitrogen secreted did not change significantly from those days on which they were returned (Table 2). There was no significant relationship between

TABLE 2

EFFECT OF METHOD OF COLLECTION ON THE SECRETION OF CBD FLUID, SULPHUR, AND NITROGEN

Values are daily means ± standard error

Sheep No.	No. of collection days	CBD secretion data		
		Fluid (g)	Sulphur (mg)	Nitrogen (mg)
		CBD fluids returned‡		
W22*	6	522±56	225±21	1849±210
W22†	6	361±49	143±19	1098±163
B53	5	853±71	153±24	1001±199
R25	5	897±83	198±24	1371±162
O6*	5	413±52	156±27	430±28
W2	1	1512	796	1342
		CBD fluids not returned		
W22*	4	475±33	156±11	1452±21
W22†	4	375±72	146±28	1219±254
B53	4	749±55	163±28	1141±223
R25	3	827±22	159±5	1283±15
O6*	3	291±41	109±34	259±111
W2	1	1605	588	1372

*,† See footnotes, Table 1. ‡ The previous day's collection, minus a 5% sample.

the concentrations of sulphur in the CBD fluid (y, μg/g) and the flow (x, g/day) of CBD fluids for individual sheep, however, when the data from the four sheep were combined there was a significant linear relationship (Fig. 2):

$$y = 532 - 0{\cdot}353x \quad [r = 0{\cdot}76;\ P < 0{\cdot}001;\ \text{S.D. (regression) } 73{\cdot}7]$$

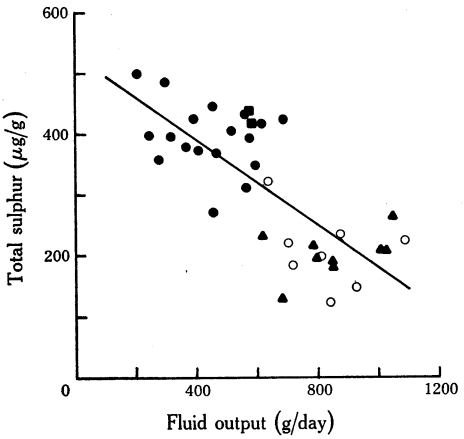

Fig. 2.—Relationship between the concentration of total sulphur in the bile–pancreatic fluids and the rate of bile–pancreatic fluid secretion (see text for regression equation).

● Sheep W22. ▲ Sheep R25.

○ Sheep B53. ■ Sheep B95.

The corresponding regression of total nitrogen concentration (y, mg/g) on flow (x, g/day) was:

$$y = 450-0\cdot324x \quad [r = 0\cdot73;\ P < 0\cdot001;\ \text{S.D. (regression) } 70\cdot5]$$

The regression of sulphur output (y, mg/day) on fluid flow for sheep W22 was:

$$y = 9+0\cdot371x \quad [r = 0\cdot91;\ P < 0\cdot001;\ \text{S.D. (regression) } 24\cdot2]$$

and

$$y = -40+0\cdot257x \quad [r = 0\cdot84;\ P < 0\cdot01;\ \text{S.D. (regression) } 26\cdot0]$$

for sheep R25. The regression for sheep B53 was not significant ($r = 0\cdot45$). The composition of the sulphur in the CBD secretions is shown in Table 3.

TABLE 3

SULPHUR CONTENT OF FRACTIONS OF CBD SECRETIONS

Values are means±standard error

Sheep No.	Sulphur content (as % of total sulphur)					
	Reducible	Ester sulphate	Protein	Neutral§	Soluble organic‖	Inorganic sulphate¶
W22*†	9·3±0·3	5·5±1·0	13·8±1·2	90·7	76·8	3·8
B53	12·6±1·0	8·1±1·6	8·1±3·2	87·4	79·3	4·5
B95	9·6±0·4	6·6±0·2	14·1±0·6	90·4	76·3	3·0
R25	17·2±0·9	4·4±0·4	3·6±0·3	82·8	79·3	12·8
O6*†	19·6±1·5	7·3±1·4	1·8±0·4	80·4	78·6	12·3
W2	9·1±1·3	5·2±0·8	2·8±0·6	90·9	88·1	3·8
Mean‡	12·2±1·8	6·2±0·8	9·9±2·5	87·8±1·8	77·9±2·6	6·0±4·5

*† See footnotes, Table 1. ‡ Excluding sheep O6 and W2.

§ Neutral sulphur = total sulphur minus reducible sulphur.

‖ Soluble organic sulphur = neutral sulphur minus protein sulphur.

¶ Inorganic sulphate sulphur = reducible sulphur minus ester sulphate sulphur.

The mean percentage of ester sulphate sulphur, inorganic sulphate sulphur, protein sulphur, and soluble organic sulphur of total sulphur was 6·16 (±0·79), 6·01 (±4·55), 9·90 (±2·52), and 77·93 (±2·59)%, respectively. The data for nitrogen content is shown in Table 4. Urea nitrogen plus ammonia nitrogen was 17·54 (±2·46)% of the total and the residual nitrogen was therefore 82·46% of the total. Both sheep O6 and W2 had from 41 to 42% of the nitrogen in the form of urea or ammonia or both but there was no corresponding increase in the inorganic sulphur concentration.

The regression of neutral sulphur output (y, mg/day) on residual nitrogen output (x, mg/day) was:

$$y = 74+0\cdot079x \quad [r = 0\cdot77;\ P < 0\cdot001;\ \text{S.D. (regression) } 30\cdot6]$$

TABLE 4

NITROGEN CONTENT OF FRACTIONS OF CBD SECRETIONS

Values are means±standard error

Sheep No.	Nitrogen content (as % of total nitrogen)	
	Urea plus ammonia	Residual§
W22*†	15·4±1·7	84·6
B53	24·7±3·4	75·3
B95	13·7±0·6	86·3
R25	16·3±1·1	83·6
O6*†	42·2±3·1	57·7
W2	41·3±1·6	58·7
Mean‡	17·5±2·5	82·5±2·5

*† See footnotes, Table 1.

‡ Excluding sheep O6 and W2.

§ Total nitrogen − (urea + ammonia) nitrogen.

(*b*) *Isotope Infusion Experiments*

(i) *Recovery of* ^{35}S *from* [^{35}S]*Taurine Infusions* (Table 5)

From 40·6 to 50·5% of the ^{35}S from an intraduodenal dose of [^{35}S]taurine was secreted in the CBD fluids over 3 days. An intraduodenal infusion to sheep W22 gave a recovery of 34·4%. Only 1·0%, or less, of the intraduodenal ^{35}S dose was excreted in the faeces and less than 4% of the dose was excreted in the urine in the 3-day period.

After intraduodenal infusions of ^{35}S-labelled CBD secretions the percentage of the ^{35}S dose apparently recovered in the CBD secretions was 90–111% in period B and 97–115% in period C. Since the amount of the ^{35}S dose collected on days 3, 6, and 9 was each a substantial portion of the total activity recovered, an adjusted estimate of dose recovery was made. In this calculation it was assumed that an amount of radioactivity equivalent to the amount recovered on the last day of each period would be secreted over the next period. In effect these amounts of ^{35}S would add to the ^{35}S-labelled CBD fluids infused in periods B and C. The adjusted estimate was calculated as either

$$^{35}\text{S recovery (\%)} = 100\times \frac{(^{35}\text{S recovered in period B}+^{35}\text{S recovered on day 6})}{(^{35}\text{S infused}+^{35}\text{S recovered on day 3})}$$

or

$$^{35}\text{S recovery (\%)} = 100\times \frac{(^{35}\text{S recovered in period C}+^{35}\text{S recovered on day 9})}{(^{35}\text{S infused}+^{35}\text{S recovered on day 6})}$$

The adjusted estimate of ^{35}S recycling from ^{35}S-labelled CBD fluids did not, however, differ greatly from the direct estimates. The values were 93–113% in period B and

TABLE 5

RECOVERY OF ^{35}S IN CBD SECRETIONS, URINE, AND FAECES OF SHEEP FOLLOWING THE INTRAVENOUS OR INTRADUODENAL INFUSION OF [^{35}S]TAURINE OR RE-INFUSION OF RADIOACTIVE CBD SECRETIONS INTO THE DUODENUM

Sheep No.	Route	Collection		^{35}S Recovery from:		
		Period	Day	CBD secretions‡	Urine§	Faeces§
W22*	Intraduodenal	A	1	25·1	4·6	0·6
			2	4·6		
			3	4·7		
				34·4		
	Re-infusion‖	B	4	47·4	1·1	0·9
			5	45·5		
			6	18·4		
				111·3 (113·4)¶		
	Re-infusion‖	C	7	110·6	1·0	0·7
			8			
			9	4·3		
				114·9 (101·6)¶		
Totals§					6·7	2·2
W22†	Intravenous	A	1	42·3	2·8	1·0
			2	4·8		
			3	3·5		
				50·5		
	Re-infusion‖	B	4	37·9	1·3	0·5
			5	53·9		
			6	5·0		
				96·8 (94·9)¶		
	Re-infusion‖	C	7	44·8	1·0	0·5
			8	39·6		
			9	13·0		
				97·4 (104·7)¶		
Totals§					5·1	2·0
B53	Intraduodenal	A	1	28·4	3·8	0·8
			2	8·5		
			3	3·7		
				40·6		
	Re-infusion‖	B	4	36·7	3·6	0·7
			5	41·5		
			6	12·0		
				90·2 (93·2)¶		
Totals§					7·4	1·5

*† See footnotes, Table 1.

‡ Values for period A expressed as % of ^{35}S from [^{35}S]taurine infused at zero time on day 1 and for periods B and C respectively as % of ^{35}S from CBD secretions collected during the previous period and infused over the first 2 days of the next particular period.

§ Values expressed as % of ^{35}S from [^{35}S]taurine infused at zero time on day 1.

‖ CBD secretions only.

¶ Alternative estimate—see Section III(*b*)(i).

102–105% in period C. A very small proportion of ^{35}S from the infused ^{35}S-labelled CBD fluids was excreted. Sheep W22 excreted 2·0 and 2·2% in the faeces over

9 days and sheep B53 excreted 1·5% of the dose in 6 days (both expressed as a percentage of the initial [^{35}S]taurine dose). Substracting the initial 3 days (period A) contribution from these totals, less than 1·6% of ^{35}S from ^{35}S-labelled CBD fluids was not absorbed and only 2·1–3·6% was excreted in the urine.

(ii) *Recovery of ^{35}S from [^{35}S]Na_2SO_4 Infusions* (Table 6)

From 6·4 to 8·3% of the [^{35}S]Na_2SO_4 dose was recovered in the CBD secretions and from 63·2 to 75·5% in the urine, over a 4- or 5-day collection period.

TABLE 6

RECOVERY OF ^{35}S IN CBD SECRETIONS AND URINE OF SHEEP FOLLOWING THE INTRADUODENAL INFUSION OF [^{35}S]SODIUM SULPHATE OR RE-INFUSION OF RADIOACTIVE CBD SECRETIONS INTO THE DUODENUM

Sheep No.	Route	Collection		^{35}S Recovery from:		
		Period	Day	CBD Secretions‡		Urine§
O6*	Intraduodenal	A	1	3·0	7·2	63·2
			2	1·9		
			3	1·2		
			4	1·1		
	Re-infusion‖	B	5	11·5	28·8 (25·6)¶	13·4
			6	7·5		
			7	8·6		
			8	1·2		
Totals§						76·6
R25	Intraduodenal	A	1	5·2	6·4	75·5
			2	0·8		
			3	0·2		
			4	0·2		
	Re-infusion‖	B	5	5·2	14·1 (14·2)¶	4·6
			6	7·0		
			7	1·3		
			8	0·6		
Totals§						80·1
O6†	Intraduodenal	A	1	4·7	8·3	—
			2	2·4		
			3	0·9		
			4	0·2		
	Re-infusion‖	B	5	0·1		

*† See footnotes, Table 1.

‡ Values for period A expressed as % of ^{35}S from [^{35}S]sodium sulphate infused at zero time on day 1 and for period B as % of ^{35}S from CBD secretions collected during period A and infused over days 5, 6, and 7.

§ Values expressed as % of ^{35}S from [^{35}S]sodium sulphate infused at zero time on day 1.

‖ CBD sections only.

¶ Alternative estimate—see Section III(*b*)(ii).

After intraduodenal infusions of this labelled CBD fluid 14·1–28·8% of the contained ^{35}S was apparently recovered in CBD fluid collected over period B. The adjusted estimate was calculated as:

$$^{35}S \text{ recovery } (\%) = 100 \times \frac{(^{35}S \text{ recovered in period B} + ^{35}S \text{ recovered on day 8})}{(^{35}S \text{ infused} + ^{35}S \text{ recovered on day 4})}$$

and ranged from 14·2 to 25·6%. Urinary excretion accounted for 76·6–80·1% of the initial $[^{35}S]Na_2SO_4$ dose over the two periods.

(iii) *Analysis of the ^{35}S-labelled Fractions in CBD Secretions* (Table 7)

With [^{35}S]taurine infusions 96·5–98·0% of the ^{35}S was in the soluble organic sulphur fraction, compared with 18·4–19·2% when $[^{35}S]Na_2SO_4$ was infused. Conversely, when the latter was infused 80·3–81·0% of the ^{35}S was in the reducible sulphur fraction, compared with 0·1–0·2% when [^{35}S]taurine was infused. The protein fraction contained 1·8–3·4% of the ^{35}S when [^{35}S]taurine was infused but 0·5–0·7% when $[^{35}S]Na_2SO_4$ was infused. The proportion of reducible ^{35}S in the CBD fluids collected after the infusion of either [^{35}S]taurine or of $[^{35}S]Na_2SO_4$ was 38–61% and 16–25% greater, respectively, than the proportion following the infusion of those respective radioactive secretions.

TABLE 7

^{35}S COMPOSITION DATA FOR CBD SECRETIONS COLLECTED FOLLOWING INTRADUODENAL OR INTRAVENOUS INFUSION OF [^{35}S]TAURINE‡ OR [^{35}S]SODIUM SULPHATE§

Values are mean percentages of total $^{35}S \pm$ standard error

^{35}S compound infused	Sheep No.	Sulphur fraction			
		Reducible	Protein	Neutral	Soluble organic
Taurine	W22†	0·1±0·03	3·4±0·93	99·9	96·5
	W22*	0·1±0·02	2·8±0·28	99·9	97·2
	B53	0·2±0·03	1·8±1·25	99·9	98·0
Sodium sulphate	O6*	80·3±3·95	0·5±0·03	19·7	19·2
	R25	81·0±9·10	0·6±0·33	19·0	18·5
	O6†	81·0±2·84	0·7±0·30	19·1	18·4

*† See footnotes, Table 1.

‡ Data obtained from pooled samples within each period.

§ Data obtained from daily collections within each period.

(iv) *Chromatographic Separation of ^{35}S Compounds in CBD Secretions* (Table 8)

Of the [^{35}S]taurine infused the activity incorporated in the CBD secretions was distributed as follows: 2·6–6·8% in TDC plus TCDC; 67·9–76·7% in TA, and 0·6–0·9% in inorganic sulphate plus the protein fraction. Recycled taurine, *per se*, may have accounted for 0·9–1·7%, and 1·0–2·2% incorporated into cyst(e)ine. The higher estimates for ^{35}S activity in taurine and cyst(e)ine with the AA : H : F : W system (5·5–8·3% and 4·6–7·8%, respectively) were due to unidentified sulphur compounds, with R_F values similar to those compounds in that system. Area (5) also

TABLE 8

CHROMATOGRAPHIC SEPARATION OF ^{35}S-LABELLED COMPOUNDS IN CBD SECRETIONS COLLECTED AFTER INFUSIONS OF [^{35}S]TAURINE, [^{35}S]SODIUM SULPHATE, OR ^{35}S-LABELLED CBD SECRETIONS

[^{35}S]taurine infusion data are means of day 1 and day 4 collections. [^{35}S]Na_2SO_4 infusion data are from day 1 collections. Values are mean percentages of total ^{35}S incorporated. Abbreviations used are defined in Section II(*e*)

Solvent system	Substance infused	Sheep	Area of ^{35}S activity corresponding to authentic compounds‡							
			(1) Ester sulphates	(2) TDC, TCDC	(3) Ester sulphates?	(4) TA, methionine	(5) ?	(6) Taurine	(7) Cystine	(8) Sulphate, protein?
AA–H–F–W	[^{35}S]taurine	W22*	2·6		2·7	67·9	91·5	8·3	6·2	0·8
		W22†	6·8		2·3	71·3	8·9	5·5	4·6	0·6
		B53	4·4		2·0	76·7	2·6	5·5	7·8	0·9
	[^{35}S]Na_2SO_4	O6*	20·5	9·1	6·4	9·1	2·4	5·9		46·6
		R25	27·7	11·4	7·7	10·9	3·2	5·3		33·8
		O6†	17·1	7·5	4·1	5·6	5·1	6·5		54·1
			TA, TDC, TCDC, methionine, ester sulphates				?	MS, taurine	Cystine	Sulphate, protein?
But.–HAC–W	[^{35}S]taurine	W22*	94·8				0·5	1·7	2·2	0·9
		W22†	95·1				0·8	0·9	2·1	1·1
		B53	95·9				1·1	1·3	1·0	0·7
	[^{35}S]Na_2SO_4	O6*	54·2				4·9	2·0	1·2	37·8
		R25	68·3				4·0	1·1	1·3	25·3
		O6†	**43·6**				**2·5**	**0**	**0**	54·0

*† See footnotes, Table 1.

‡ Areas (1)–(8) represent sections of chromatograms from solvent front (1) to the base line (8)—see Section II(*e*).

contained appreciable activity of unknown composition. The narrow, well-defined area (1) obtained with the But : HAC : W system contained activity equivalent to that spread over areas (1)–(5) of the AA : H : F : W system, plus the difference between areas (6) and (7) in the two systems. It is apparent from the results, in Table 7, that these unidentified compounds which contained about 17% of the total activity were organic compounds. The distribution of ^{35}S compounds in both sheep was similar in the day 1 and day 4 samples analysed. In particular, the mean percentage of ^{35}S in TA was 74·3 and 72·1% for day 1 and day 4 collections, respectively. The results were therefore combined for presentation in Table 8.

Of the [^{35}S]Na_2SO_4 infused, 25·3–54·1% of the activity incorporated in the CBD secretions was as inorganic sulphate only trace quantities were associated with protein (Table 7), and as 5·6–10·9% was probably as TA only traces would have been in TDC or TCDC; 28·7–46·8% was probably ester sulphate [areas (1)–(3)]. Very small quantities were possibly contributed by taurine (0–2%) and by cyst(e)ine (0–1·3%). Approximately 8% of the ^{35}S was not accounted for and was considered to be incorporated into other organic compounds. Only the samples obtained on the first day after infusion were analysed, since the specific radioactivity on subsequent days was very low.

IV. Discussion

The rate of secretion of CBD fluids in four sheep was found to be 366–871 g/day (mean 673). These values are similar to those reported by Leat and Harrison (1969) of 0·81–1·56 ml/kg secreted each hour over periods of 4–11 hr, or 500–1150 ml/day (mean 715). A daily flow of bile of 367–854 ml (Heath and Hill 1969) and pancreatic fluid of 200–400 ml (Taylor 1962) have been recorded in sheep.

The secretion of fluid, of sulphur, or of nitrogen compounds was not appreciably altered by not returning CBD secretions for 24 hr. This is contrary to the findings of Taylor (1962) and of Harrison (1962) for sheep and other animals (see Wheeler 1968) who all reported decreased secretion over short time periods. Deprivation clearly resulted in increased synthesis of tauroconjugated bile acids (Table 2) to maintain output over the 24-hr collection period. Bile acid synthesis is known to be homeostatically regulated by the rate of bile acid turnover (see Bergstrom and Danielsson 1968; Gray, Nicholson, and Quincey 1968); furthermore, bile fluid is considered to be directly associated with bile salt secretion in dogs (see Wheeler 1968) and in sheep (Heath, Caple, and Redding 1970), and therefore little change in the volume of fluid secreted would be expected. Although regressions of sulphur concentration on fluid flow rate were not significant for individual sheep, that for the pooled data over a wider range was negative and statistically significant. However, the regressions of total sulphur output on fluid flow were significant in two instances and as tauroconjugates represent some 78% of total sulphur, the secretion of these bile salts appear to be related to the fluid flow, despite changes in concentration. Since pancreatic and bile fluids were collected together, differences between these results and other data relating only to bile secretion may be due to the influence of pancreatic secretions. The osmotic effect of urea may partially account for the secretion of fluid in the CBD secretions.

The concentration of total nitrogen in the pancreatic fluids and in the bile fluids of man and dogs, as recorded by Altman and Dittmer (1968), was 1·0–9·36

and 0·65–1·05 mg/ml, respectively. The range of the mean concentrations of total nitrogen found in the CBD secretions in the present experiments was 1·32–3·52 mg/ml. The daily secretion of total nitrogen was 1–2 g. Of this 17·5% was in urea or ammonia or both. The remainder represents the maximum estimate for protein, i.e. 877–1681 mg nitrogen/day (or 4·3–8·7 g protein/day). However, as only 10% of the total sulphur was present as protein sulphur and assuming an N : S ratio for protein of 15, about 213–367 mg protein nitrogen only can be accounted for. The residual nitrogen fraction is therefore composed primarily of other organic nitrogen compounds, possibly alcohol-soluble protein, peptides, amino acids, and bile acid conjugates. Serum albumin is usually the major protein component of bile, while γ-globulin and the phospholipids also contribute nitrogen (see Schanker 1968; Wheeler 1968). Most, if not all, of the pancreatic juice proteins are enzyme proteins (Thomas 1950; Keller, Cohen, and Neurath 1958).

It is clear that, although total and non-protein nitrogen concentrations in the CBD fluids of the sheep are comparable with those of the pancreatic or bile fluids in other animals, the daily secretion of protein is substantially less than claimed for other animals. Thus, it has been estimated that 5–13 g protein is secreted daily in pancreatic plus bile fluids of man and dogs (Howard, James, and Evans 1951; Russel, Fleck, and Burnett 1964; Altman and Dittmer 1968); Nasset's (1965) estimate for man (14–33 g/day) is even greater. Such differences between, and within, species may be due to differing collection and analytical techniques, period of collection, the type of stimulation given (if any), and individual variation (see Thomas 1950).

The total sulphur secreted in the CBD fluids was 142–245 mg/day; ester sulphate and inorganic sulphate each accounted for 6%, protein was 10%, and soluble organic sulphur 78% of the total. There was a good correlation ($r = 0·92$) between total sulphur and total nitrogen secretion (Fig. 1), with the output of 9·8 mg nitrogen associated with 1 mg sulphur.

The major portion of the faecal sulphur is organic (Bird 1971) and is presumably derived from bacterial protein, since these residues are the main sources of protein in sheep faeces and may account for up to 78% of the faecal nitrogen excreted (Mason 1969). Since the N : S ratio of bacterial protein is about 11 (Walker and Nader 1968) other organic sulphur compounds must be excreted to give the narrower N : S ratio found for the faeces, e.g. 6·4–9·2 (Bird 1971). The mean N : S ratio in the CBD secretions was 7·60, suggesting that sulphur from this source could be excreted in the faeces and therefore affect the faecal N : S ratio. However, there was apparently an almost complete recovery of ^{35}S in the CBD secretions following infusions of CBD secretions previously labelled *in vivo* with [^{35}S]taurine (Table 5). The results obtained were consistent with the small fraction of the ^{35}S dose excreted in the faeces and urine (less than 2 and 4%, respectively, over 6 days). Since the ^{35}S incorporated in the CBD fluids was largely in the tauroconjugated bile acids (*c.* 80%) and present only to a small extent in protein ($\leqslant 3·4\%$), it is possible that bile or pancreatic protein could contribute relatively more sulphur to the faeces. Infusions of [^{35}S]-methionine or [^{35}S]cystine to label that protein (e.g. Hansson 1959) could clarify this situation. In the rat, pancreatic trypsin has a cystine content of 8·7% (Barnes, Kwong, and Fiala 1965) and a portion of this trypsin is not digested. However, in the present experiment only about 14–25 mg protein sulphur was secreted daily in the CBD fluids and assuming that 90% of this protein is digested and absorbed

(Twombly and Meyer 1961) insignificant amounts of this organic sulphur would have been excreted in the faeces. Apparently, therefore, other sources of organic sulphur are responsible for the narrow N : S ratio in the faeces.

The sheep absorbed 99%, or more, of the ^{35}S from the intraduodenal infusions of [^{35}S]taurine, consistent with results obtained from rats (Portman and Mann 1955). However, the rat excretes a greater proportion of the absorbed ^{35}S in the urine (up to 45%) than the sheep (< 4%) and, depending upon the intake of organic sulphur, there is a greater variation in the proportion either incorporated into bile or excreted (Portman and Mann 1965). This effect in the rat was presumably due to dilution of [^{35}S]taurine in the taurine pool resulting from increased taurine synthesis. Fluctuations in the supply of organic sulphur to the tissues of the sheep probably does not occur to the same extent. Irrespective of the diet fed most of the sulphur passing to the abomasum is contained in microbial protein (Bird and Hume 1971) which has a relatively stable amino acid composition (Purser and Buechler 1966). However, variation in the growth of microorganisms in the rumen is influenced by the amount of sulphur ingested (Hume and Bird 1970), depending upon the availability of other nutrients, including nitrogen and energy (Hume, Moir, and Somers 1970). Methionine is the most limiting amino acid in the tissues of the growing lamb (Nimrick *et al.* 1970); Reis (1967) and others had earlier indicated the scarcity of this free amino acid in the tissues, by the response to wool growth when abomasal infusions of methionine or cystine were given. In the present experiment, withholding secreted CBD fluids did not substantially reduce the amount of tauroconjugated bile acids synthesized over a 1-day period. Synthesis of taurine from cystine therefore appears to be an obligatory process and must reduce the availability of cystine for the synthesis of wool. When the diet is sulphur-deficient the rat can conjugate bile acid with glycine instead of taurine (Palmer and Hruban 1966), but glycine supplements did not alter the taurine : glycine ratio in man (Sjovall 1959) and there is no evidence that glycine can replace taurine in sheep. Peric-Golia and Socic (1968) found that the proportion of taurine : glycine conjugates decreased with age in lamb, but at 1–12 months the tauroconjugates comprised some 70% of the total. In view of the large requirement of cystine for wool synthesis the synthesis of taurine could be consistent with the minimal amount required for forming bile acid conjugates, and one might expect small urinary excretion of taurine compared with man (Soupart 1959) or rats (Boquet and Fromageot 1965). In the rat, and man, less than 4% of the absorbed bile salts are excreted in the urine (see Danielsson 1963); the sheep also efficiently transfers taurocholate from the blood to the bile (Heath, Caple, and Redding 1970). An efficient enterohepatic cycling of tauroconjugated bile acid in the sheep is indicated in the present experiment, since little ^{35}S was excreted in either the faeces or urine following the infusion of CBD fluids labelled *in vivo* with [^{35}S]taurine. In the rat, a substantial portion of the bile salts are deconjugated and taurine is degraded in the caecum, with subsequent absorption of H_2S and excretion in the urine as sulphate (Boquet and Fromageot 1965).

Only 6–8% of the ^{35}S from intraduodenal infusions of [^{35}S]Na_2SO_4 was recovered in the CBD fluids but the proportion recovered was 14–29% when the labelled CBD fluids were infused. About 20% of ^{35}S incorporated into the CBD fluids was organically bound; approximately half of this was accounted for in taurocholic acid but invariably less than 1% in either protein or taurine. The presence of the label in the

organic sulphur compounds probably resulted from the diffusion of $[^{35}S]SO_4^{2-}$ into the alimentary tract, with subsequent synthesis of *S*-amino acids by bacteria. If this occurred in the rumen then much of the synthesized organic ^{35}S compounds would subsequently have been absorbed. The tissue synthesis of *S*-amino acids from inorganic sulphur in most animals is negligible. Thus, Houvinen and Gustafsson (1967) found that the germ-free rat could not transfer ^{35}S from $[^{35}S]SO_4^{2-}$ into methionine, although a trace of ^{35}S was found in cysteine; conventional rats synthesized both methionine and cysteine in small amounts. Cats, however, can apparently use inorganic sulphate as the sole source of sulphur (Rambout and Miller 1965) and chickens can readily convert sulphate sulphur into taurine, but there the production of taurine and cysteine (but not methionine) may be independent of microbial intervention (e.g. see Mason, Hansen, and Weidner 1965).

Approximately equal amounts of ^{35}S were incorporated into inorganic sulphate and ester sulphate in the CBD fluids after $[^{35}S]Na_2SO_4$ infusion, and these compounds accounted for about 80% of the total ^{35}S incorporated. $[^{35}S]SO_4^{2-}$ is secreted in the pancreatic juice of mice in trace amounts (Hansson 1959), but in larger amounts in the bile of mice and rats (Everett and Simmons 1952; Cohen and Delassue 1959) in contrast with sheep where the chief route of $[^{35}S]SO_4^{2-}$ excretion is via the kidneys (Bray 1969). There are many ester sulphate compounds secreted in the bile, e.g. indoxyl sulphate (see Schanker 1968), bilirubin sulphate (Isselbacher and McCarthy 1959), cholesterol sulphate (e.g. Moser, Moser, and Orr 1966), and various steroid esters (e.g. Laatikanen and Vihkor 1970; Gustafsson, Gustafsson, and Sjovall 1968), all of which are found in the faeces.

Bray (1969) and others, have shown that, in the sheep, up to 20% of ^{35}S from an intravenous dose of $[^{35}S]Na_2SO_4$ was excreted in the faeces and it is probable that the pancreatic and bile fluids contribute to the loss of endogenous sulphur. Since the sheep can absorb up to 3·5 g sulphate sulphur daily from the small intestine (Bird and Moir 1971) little, if any, of the inorganic sulphate from the CBD secretions would be excreted in the faeces and therefore the ester sulphates are probably of greater significance.

Bird and Moir (1971) estimated, from the concentration of reducible sulphur in duodenal digesta, that about 126 mg/day of total sulphate sulphur flowed into the duodenum. Since the mean daily secretion of reducible sulphur in the CBD secretions in the present experiment was about 23 mg/day this contribution is about 20% of the total reducible sulphur in duodenal digesta.

The proportion of $[^{35}S]$taurocholate to total ^{35}S-labelled tauroconjugated bile acids was approximately 91–94% following $[^{35}S]$taurine infusions and there was little change in this proportion after infusing the ^{35}S-labelled CBD secretions, indicating that the synthesis of the secondary bile acid, taurodeoxycholate, was not significant. Perio-Golia and Socic (1968) reported that approximately 10 and 12% of the tauro-conjugated bile acids were present as taurodeoxycholate and taurochenodeoxy-cholate, respectively, values which are appreciably greater than indicated in the present experiment.

Although the sheep apparently has adequate synthetic capacity to replace the loss of tauroconjugates in the bile, and can incorporate some inorganic sulphur in these and other organic compounds, the reabsorption and recycling mechanisms

are extremely efficient, thus contributing appreciably to the sulphur economy of the sheep.

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VI. References

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