SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

I. THE ABSORPTION OF SULPHATE IN SHEEP AFTER INTRARUMINAL OR INTRADUODENAL INFUSIONS OF SODIUM SULPHATE

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Abstract

The mean apparent absorption of sulphur when sodium sulphate was continuously infused into the rumen or the duodenum of four sheep in amounts of 1·5, 3·0, and 6·0 g/day was 93·1, 95·3, 95·9% and 93·1, 92·8, 81·5% respectively. The net absorptive capacity of the entire intestine under these conditions was calculated to be up to 5 g of sulphate sulphur daily.

The rate of uptake of sulphur from duodenally infused sodium sulphate into the blood was estimated to be of the order 110–150 mg/hr and up to 3·5 g sulphate sulphur could have been absorbed daily from the small intestine.

A substantial flow of sulphate sulphur into the duodenum was evident 3–7 hr after commencing infusion of sodium sulphate into the rumen; peak concentrations of sulphate sulphur of up to 470 μg per millilitre of digesta were reached in 24–27 hr. These levels rapidly declined thereafter, coinciding with a marked increase in the concentration of sulphide in the rumen, indicating a rapid adaptation by the sulphate-reducing microflora. The concentration of reducible sulphur in samples of duodenal digesta obtained before, and subsequent to, the 14th day of infusion was $14\cdot1 \pm 1\cdot9$ μg/ml (mean $\pm$ S.E.). The flow of reducible sulphur to the duodenum was estimated to be about 126 mg/day.

Under normal physiological conditions the capacity of the sheep's intestine to absorb ingested sulphate sulphur is unlikely to be exceeded.

I. Introduction

Ruminants have potential for efficient absorption of ingested sulphate, due to the presence of microorganisms in the forestomach. Anderson (1956), Spais, Lazaridis, and Agiannidis (1968) and Bray (1969*a*, 1969*b*) have shown that inorganic sulphate is readily reduced by the microorganisms and rapidly absorbed as sulphide from the rumen, the absorption rate being apparently conditioned by ruminal pH. The sulphate ion *per se* is apparently not absorbed from the rumen (Bray 1969*b*) but the extent of uptake from the abomasum is unknown. Murphy, Dossetor, and Beck (1963) have, however, shown that sulphate is readily absorbed from the human stomach.

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The apparent digestibility of dietary sulphate in the sheep was first indicated by Warth and Krishnan (1935) to be of the order of 90%. More recently Moir, Somers, and Bray (1967) found the apparent digestibility of sodium sulphate, when added to a roughage diet, to be about 78% for the range of sulphur intake of 0·9–2·4 g/day.

The absorption of sulphate ion by the mammalian intestine has long been investigated but conclusions derived from these experiments are divergent. Thus Kun (1959) claimed that the ion was poorly absorbed, but Dziewiatkowski (1962) stated that the process was rapid. The consensus of opinion, however, appears to be that sulphate is but slightly absorbed, that different regions of the intestine differ in absorptive capacity, and that a number of physical and chemical factors may influence this process. A summary of the major contributions in this field is presented in Table 1.

TABLE 1

ABSORPTION OF SULPHATE FROM ISOLATED INTESTINAL LOOPS IN DOGS

Author	Loop Site	No. of Dogs	No. of Expts. per Dog	Compound Infused	Initial Osmolarity (m-osmoles)	Absorption Rate (mg sulphate sulphur per hour)
Cobet (1913)	Jejunum 50–85 cm	6	1	$MgSO_4$	218–1042	27–340
Andrews and Johnston (1933)		1	4	Na_2SO_4		6–11
Bucher *et al.* (1950)	30–40 cm	15	2	Na_2SO_4	303	7–12
Cobet (1913)	Ileum 50–85 cm	6	1	$MgSO_4$	218–1042	25–270
Nakashima (1924)	40–50 cm	2	2–4	Na_2SO_4	150–600	7–50
Ingraham and Visscher (1936)	Lower 18 in.	?	?	Half-isotonic Na_2SO_4	Isotonic*	0–27
Driver (1942)	Lower 12 in.	6	6	Half-isotonic Na_2SO_4	Isotonic*	42
Bucher *et al.* (1950)	25–40 cm	16	2	Na_2SO_4	303	0
Goldschmidt and Dayton (1919)	Colonic loops	4	1	Na_2SO_4	1622–105	8–13
		1	1	$MgSO_4$	592	4

* NaCl added.

The data has, for comparative purposes, been recalculated and expressed in terms of rate of absorption of sulphate sulphur per loop of intestine, since the common expression "percentage absorption" may be quantitatively misleading and indeed appears to account for much of the existing disparity of interpretation.

The jejunum is apparently capable of absorbing sulphate but absorption from the ileum is doubtful, according to the results of Bucher, Anderson, and Robinson (1950), who employed three techniques with similar outcomes. This group also had the advantage of previous work relating to factors influencing sulphate absorption (Verzar and McDougal 1933; Ingraham and Visscher 1936, 1938; Ingraham, Peters, and Visscher 1938; Driver 1942).

Bray (1969*a*) demonstrated qualitatively that $^{35}S^{2-}$ was absorbed from the duodenum of sheep, but that $^{35}SO_4^{2-}$ was poorly absorbed from the intestines.

The intervention of intestinal bacteria may result in the production and absorption of sulphide within the large intestine (Dziewiatkowski 1962). However, Dennis and Reed (1927) could not elicit a respiratory response by infusing H_2S gas into dog colons, although this occurred with infusions made into the duodenum. Using the same criterion for absorption it has been shown that H_2S is rapidly absorbed from the terminal ileum of sheep (Bird, unpublished data).

In the present experiment from 0 to 6 g sulphur per day, as sodium sulphate, was infused into the rumen or duodenum of sheep in order to estimate the absorptive capacity of the foregut and of the intestine. The forms of sulphur excreted are presented in the following paper.

II. Materials and Methods

(a) *Experimental Animals and Diets*

Four mature Merino wethers, fitted with rumen cannulae and duodenal cannulae (placed just distal to the common bile duct entry), were confined to metabolism pens for the duration of the experiment. One animal (sheep 2) died during the final period while receiving the ruminal infusion of water.

The sheep were fed 802 g dry matter daily at 9.00 a.m., comprising 645 g oaten chaff, 100 g corn starch, 30 g casein, 11 g urea, and 16 g sulphate-free mineral mix (Hume and Bird 1970). This ration supplied 0·7–1·1 g sulphur and 12·9–13·1 g nitrogen per day.

Deionized water was offered *ad libitum* and the daily intake recorded.

(b) *Experimental Design*

A 4×4 latin square design was used. In each period, 7 days stabilization on the basal diet preceded 6 days of continuous infusion of sodium sulphate per duodenum (of which the last 5 days were collection days) followed immediately by 15 days continuous infusion per rumen (the last 8 days being collection days).

(c) *Treatment Infusions*

The sodium sulphate was delivered in 800 ml of water over 24 hr to give 0, 1·5, 3·0, and 6·0 g sulphate sulphur per day (treatments A, B, C, and D respectively); 5·0 and 4·0 g sulphur per day only were infused for the highest level (treatment D) in the final two periods of ruminal infusion due to the occurrence of sulphide toxicity at higher levels.

(d) *Sampling*

Urine was collected daily into concentrated HCl and aliquots equal to 10% of the total volume were stored in bulk vessels with 1 ml of saturated, sulphur-free $CuCl_2$. Urine was also collected from the sheep at intervals on the day preceding and during the first day of intraduodenal sodium sulphate infusions. Blood samples were obtained from jugular cannulae over these 2 days. A 4·5% potassium oxalate solution was used as an anticoagulant. Faeces were collected daily, dried at 95°C, and aliquots equal to 10% of the total amount were stored for analyses. Rumen fluid samples were taken for sulphide determination 4 hr after feeding on the third and last day of the duodenal phase and the third, ninth, and fourteenth day of the rumen infusion phase. The material was strained through bolting silk and immediately analysed. Duodenal digesta were obtained by collecting digesta gushes from the cannula at intervals over the first, second, and fourteenth day of the ruminal infusion phase. After the first period no further duodenal samples could be obtained from sheep 4 due to a dislodged cannula.

(e) *Analytical Methods*

Sulphur analyses on rations, faeces, urine, whole blood, duodenal digesta, and rumen fluid were performed by the methods described by Bird and Fountain (1970).

(f) *Statistical Analyses*

The data was analysed by analysis of variance, or by Student's *t*-test methods where appropriate.

III. Results

The absorption of sulphate from the small intestine is demonstrated by the response of blood total sulphate sulphur concentrations and urinary total sulphate sulphur excretion to duodenal sodium sulphate infusion when compared with the previous day's basal values. Successive-day comparisons as shown in Figure 1 are

Fig. 1

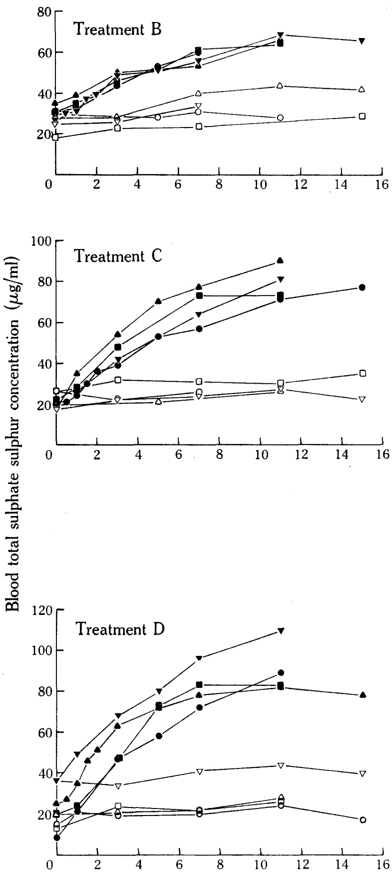

Fig. 2

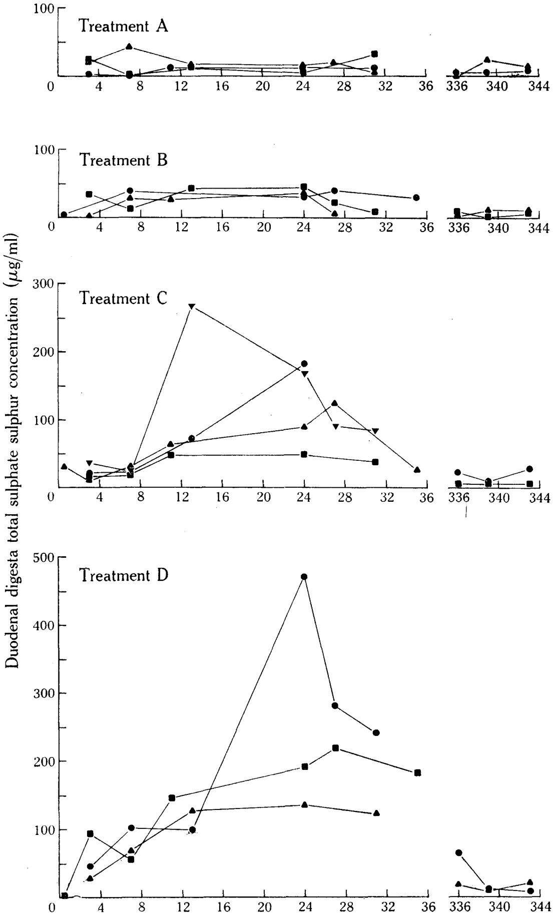

Fig. 1.—Effect of intraduodenal infusions (solid symbols) of sodium sulphate on the concentration of sulphate sulphur in blood compared with pre-infusion values (open symbols). ●, ○ Sheep 1. ■, □ Sheep 2. ▲, △ Sheep 3. ▼, ▽ Sheep 4.

Fig. 2.—Effect of intraruminal infusion of sodium sulphate on the concentration of sulphate sulphur in duodenal digesta. ● Sheep 1. ■ Sheep 2. ▲ Sheep 3. ▼ Sheep 4.

used, since there was some variation between periods in the sulphur content of the ration and its rate of intake, both of which affect blood sulphate concentrations. The results for each animal, at each infusion level, are listed from zero time (the commencement time of infusion or feed presentation or both) to 15 hr after commencement. The variation in basal blood total sulphate sulphur concentrations with time after feeding were small compared with the marked changes observed by Bray (1969) in experiments where the dietary sulphur intake was greater.

The approximate rate of sulphate sulphur absorption from the small intestine may be estimated by using Bray's (1969*c*) mean value for body sulphate space (27% of body weight) and the observed increment in blood sulphate concentrations after beginning the infusion. This calculation assumes equilibration of absorbed sulphate throughout the extracellular fluid space, and minimal excretion of absorbed sulphate during that period. The data in Table 2 indicate that urinary excretion of sulphate

TABLE 2

URINARY SULPHATE SULPHUR EXCRETION IN FOUR SHEEP IN RESPONSE TO THREE LEVELS OF SODIUM SULPHATE INFUSED PER DUODENUM

Levels B, C, D correspond to sulphate sulphur infusions of 1·5, 3·0, and 6·0 g/day. d_1, urinary excretion values after 24 hr infusion; d_0, previous day's basal excretion value

Time Interval (hr)	Urinary Sulphate Sulphur Excreted (mg): Level B		Level C		Level D		Level B		Level C		Level D	
	d_0	d_1	d_0	d_1	d_0	d_1	d_0	d_1	d_0	d_1	d_0	d_1
			Sheep 1						Sheep 2			
0–3	16	18	12	36	21	31	5	41	68	59	22	47
3–7	22	22	42	173	46	242	68	38	86	331	0	68
7–11	21	117	54	225	34	391	33	322	38	281	28	485
11–15			—	371	21	—	12	—	25	—		
11–24	64	530	49	1299	46	1794	60	316	70	1615	44	1820
			Sheep 3						Sheep 4			
0–3	38	78	29	22	31	152	4	64	23	21	42	264
3–7	60	34	27	164	23	410	95	113	10	39	109	736
7–11	38	153	28	303	26	577	40	121	28	335	57	260
11–15	20	—			—	849	—	98	7	—	20	—
11–24	54	938	44	1403	68	3618	56	495	13	860	68	2919

was not elevated substantially above basal up to 3 hr. The amount of sulphate sulphur infused into the duodenum in the respective treatments B, C, and D was 64, 125, and 250 mg/hr.

The sharp rise in blood sulphate sulphur concentration within 1 hr for sheep 3 and 4 (Fig. 1) indicates a rapid uptake of sulphate from the small intestine. However, at the same level of infusion (D) there was no clear-cut response for sheep 1 and 2 in this time. At that infusion level about 120 and 150 mg sulphate sulphur are estimated to have been absorbed by 1 hr in sheep 3 and 4 respectively, and 340 and 330 mg by 3 hr in sheep 1 and 2 respectively. In all sheep the estimated uptake of sulphate at treatment levels B and C approximated that infused into the duodenum. The absorptive capacity of the small intestine is therefore, from these estimates, of the order 110–150 mg sulphate sulphur per hour. The rise in blood sulphate sulphur levels is approximately linear in the initial stages, but the rate of increase declines as urinary excretion increases 3–5 hr after beginning the infusion. The blood sulphate sulphur levels tend to stabilize after 7–11 hr with, in general, the levels in treatment D higher than in C, and those in C higher than in B.

The effect of each level of intraruminal infusion of sodium sulphate on the concentration of sulphate in the duodenal digesta is shown in Figure 2. In each graph there is a discontinuity in the x-axis between the second and fourteenth day of sampling. The mean total sulphate sulphur levels for sheep 1, 2, and 3 on the basal treatment were 7, 15, and 20 μg/ml of digesta respectively. Within-day variations appeared to be random. There was a substantial initial flow of sulphate into the duodenum, with increased total sulphate sulphur concentrations in duodenal digesta being evident 3–7 hr after beginning the infusion; peak values were reached between 12 and 27 hr. A decline in these levels was seen thereafter, and at 14 days the collective mean for treatments B, C, and D of the total sulphate sulphur concentrations for sheep 1, 2, and 3 respectively were 24, 6, and 14 μg/ml of duodenal digesta. The concentration of sulphide in rumen fluid over this period is given in Table 3. By the third day of infusion

Table 3

SULPHIDE CONCENTRATION IN RUMEN FLUID IN RESPONSE TO FOUR LEVELS OF SODIUM SULPHATE INFUSION PER DUODENUM OR PER RUMEN

Values are for 4 hr after commencement of infusion on each day. Levels A, B, C, and D correspond to sulphate sulphur infusions of 0, 1·5, 3·0, and 6·0 g/day per duodenum and 0, 1·5, 3·0, and either 4·0 or 5·0 g/day per rumen (see text)

Infusion Site	Day of Infusion	Sulphide Concentration (μg/ml) for Levels:							
		A	B	C	D	A	B	C	D
		Sheep 1				Sheep 2			
Duodenum	3	0·47	0·14	0·23	0·40	0·34	0·94	0·64	—
Duodenum	7	0·47	0·17	0·25	0·40	0·37	0·34	0·47	0·22
Rumen	3	0·01	—	1·61	3·35	1·49	0·80	2·59	—
Rumen	9	0·01	3·22	5·56	1·34	—	2·01	2·61	9·25
Rumen	14	0·80	2·41	5·22	5·57	—	2·53	3·97	17·77
		Sheep 3				Sheep 4			
Duodenum	3	0·34	0·35	—	0·46	—	1·15	0·54	1·22
Duodenum	7	0·07	0·58	0·22	0·62	0·17	2·35	0·54	0·64
Rumen	3	0·40	5·76	—	5·95	—	5·33	2·01	11·06
Rumen	9	0·94	2·61	5·46	11·36	0·69	3·06	6·44	6·53
Rumen	14	0·23	2·13	8·05	9·54	—	2·79	11·32	5·92

the sulphide levels were substantially higher at 4 hr than those occurring during the duodenal infusion phase. These changes correspond with the decreased sulphate sulphur content of the duodenal digesta.

The flow of total sulphate sulphur to the duodenum of the sheep on the basal treatment and on the fourteenth day of intraruminal infusion of sodium sulphate may be estimated using the mean of 14·1 ($\pm$1·9) μg/ml (42 samples) and an assumed mean daily flow of 9 litres of duodenal digesta (Hogan 1965; Bruce *et al.* 1966). The estimated daily flow of about 126 mg total sulphate sulphur occurs regardless of intake of sulphur in the range used here, provided that the microflora of the rumen has adapted to sulphate loading.

The comparison between apparent absorption of sodium sulphate when infused per rumen or per duodenum may be seen in Table 4 and Figure 3. The increment above

TABLE 4

APPARENT PERCENTAGE ABSORPTION OF SULPHATE SULPHUR WHEN SODIUM SULPHATE AT THREE LEVELS WAS INFUSED PER RUMEN OR PER DUODENUM

Levels B, C, D as defined in Table 2. Mean values which are significantly different have similar superscripts: viz. level of significance for a, b, c is $P < 0 \cdot 10$; for d, $P < 0 \cdot 02$

Sheep No.	Level B		Level C		Level D	
	Rumen	Duodenum	Rumen	Duodenum	Rumen	Duodenum
1	91·88	92·52	93·73	88·19	95·74	75·48
2	—	90·24	—	94·81	—	84·38
3	94·73	94·16	97·61	91·70	94·67	75·10
4	92·57	95·53	94·60	96·44	97·15	90·96
Mean	93·06	93·11[a]	95·31	92·79[b]	95·85[d]	81·48[a,b,d]
(±S.E.M.)*	(±0·86)	(±1·14)	(±1·18)	(±1·82)	(±0·72)	(±3·82)

* Overall rumen mean 94·74 (±0·63)[c]; overall duodenum mean 89·12 (±2·10)[c].

basal in total faecal excretion of sulphur subtracted from the increment in sulphur intake above basal and expressed as a percentage of the increment in sulphur intake

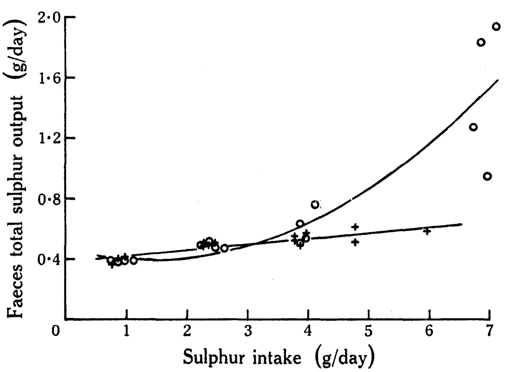

Fig. 3.—Effect of intraduodenal (○) or intraruminal (+) infusion of sodium sulphate upon total sulphur excretion in faeces. Regression equations are as follows:

Ruminal infusion:

$y = 382 + 38 \cdot 9x$
($r = 0 \cdot 88$, $P < 0 \cdot 001$).

Duodenal infusion:

$y = 464 - 97 \cdot 0x + 35 \cdot 6x^2$
($r = 0 \cdot 82$, $P < 0 \cdot 001$).

for each animal, at each treatment level, is used as the measure of apparent absorption of added sulphate sulphur:

$$\text{i.e. apparent absorption} = \frac{\text{increase in sulphur intake} - \text{increase in faecal sulphur}}{\text{increase in sulphur intake}} \times 100.$$

An estimate of the apparent absorption of infused sulphur may also be made from the increment in urinary sulphur excreted:

$$\text{i.e. apparent absorption} = \frac{\text{increase in urinary sulphur}}{\text{increase in sulphur intake}} \times 100.$$

Results based on this method of calculation are shown in Table 5.

There were no significant treatment differences in water intake or in urine volume due to amount of sodium sulphate infused or route of infusion (Table 5). There was therefore probably no between-treatment confounding effect of varying urine flow rates on excretion of the filtered inorganic sulphate load (Smith 1951; Walser and Mudge 1960).

TABLE 5

MEAN DAILY WATER INTAKE, URINE EXCRETION, AND APPARENT ABSORPTION OF INFUSED SULPHUR IN RESPONSE TO SODIUM SULPHATE INFUSION PER DUODENUM OR PER RUMEN

Infusion levels A, B, C, and D as defined in Table 3. Mean values which are significantly different have similar superscripts: viz. level of significance for a is $P < 0 \cdot 05$; for b, $P < 0 \cdot 01$

	Level A		Level B		Level C		Level D	
	Rumen	Duodenum	Rumen	Duodenum	Rumen	Duodenum	Rumen	Duodenum
Water intake (ml)	2936	3219	2789	3301	2841	3245	3301	3281
(±S.E.M.)	(±96)	(±258)	(±102)	(±170)	(±144)	(±305)	(±340)	(±324)
Urine volume (ml)	1724	1759	1649	2019	1862	1892	1976	1744
(±S.E.M.)	(±118)	(±450)	(±45)	(±182)	(±135)	(±231)	(±288)	(±295)
Sulphur absorption (%)*	—	—	108·1	97·2[b]	92·5	82·9	96·1[a]	71·1[a,b]
(±S.E.M.)			(±13·1)	(±3·8)	(±1·9)	(±4·7)	(±5·3)	(±2·9)

* See text for method of calculation.

IV. Discussion

The response of blood total sulphate sulphur levels to duodenal sodium sulphate infusions indicate that sulphate is readily absorbed from the small intestine. The estimated absorption rate of sulphate in the ovine small intestine (110–150 mg sulphur per hour), corresponds to the monogastric intestine (see Table 1). Although the sulphur ion is considered to be poorly absorbed from the intestine of humans and dogs it appears that the small intestine of sheep is capable of absorbing up to 3·5 g sulphate sulphur per day.

It is uncertain how rapidly the intraduodenal infusate travelled through the small intestine in this experiment, since there are irregular periods of from 15 to 20 min when no flow occurs at all (Phillipson 1952) but gushes of 20 g or more may be expelled in rapid succession over "active" periods of 5–10 min (Bruce *et al.* 1966). It is probable that within 3 hr the sodium sulphate "front" would still be within the small gut and therefore the calculations that have been made are a reasonable indication of the rate of sulphate uptake from the small intestine. The absorptive area is probably the jejunum and possibly also the ileum since:

(1) In two sheep there was no evidence of uptake in 30 min; but there may have been a rapid transit of the infusate to the jejunum in the other two animals which had elevated blood sulphate concentrations in that time.

(2) There is no evidence that sulphate is absorbed specifically from the duodenum, but in dogs the jejunum is clearly capable of absorbing sulphate (see Table 1).

(3) In dogs the ileum is relatively impermeable to sulphate (see Table 1), although in rats this area transfers sulphate more rapidly than other regions of the small intestine (Dziewiatkowski 1970).

(4) An active transport system for sulphate in rat and rabbit jejunum (Sanz, Espliguera, and Astudillo 1963), in hamster jejunum (Wilson 1962), and in rat ileum (Dziewiatkowski 1970) has been described.

It is possible that at least part of the sulphate was reduced by intestinal bacteria to sulphide, since under certain conditions the small intestine of many animals may harbour large numbers of anaerobic bacteria (see Donaldson 1968). The short period of duodenal infusion employed in this experiment was intended to minimize the likelihood of a large increase in the dissimilatory sulphate-reducing bacterial population in the intestinal tract. It is not clear in what forms and to what regions sulphate may diffuse from the blood into the intestinal tract and the influence this may have on the bacterial sulphate-reducing population.

By comparing the apparent absorption of sodium sulphate when infused per duodenum and per rumen (see Table 4), it is evident that the capacity of the sheep intestine to absorb sulphate is high. The difference at the highest level of infusion is 14·4%. Since sulphate may be absorbed then excreted via the gastrointestinal tract (Bray 1969*a*) in the form of a mucin complex (Dziewiatkowski 1962), as inorganic sulphate, or in other organic compounds, the amount of sulphate actually absorbed is slightly underestimated here. The apparent absorptive capacity of the intestine is, from these data, up to 5 g sulphate sulphur per day, with about 3·5 g of this being absorbed in the small intestine.

The estimated daily flow to the duodenum of 126 mg total sulphate sulphur in the abomasal digesta, bile, and pancreatic secretions, indicates that sheep which have ruminal microorganisms adapted to sulphate loading allow little dietary sulphate to pass from the foregut, so that the absorptive capacity of the intestine is unlikely to be exceeded.

IV. Acknowledgments

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