SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

II.* ORGANIC AND INORGANIC SULPHUR EXCRETION BY SHEEP AFTER INTRARUMINAL OR INTRADUODENAL INFUSIONS OF SODIUM SULPHATE

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Abstract

A basal ration containing 0·86 g sulphur was fed daily to sheep receiving a continuous intraruminal or intraduodenal infusion supplying 0–6 g sulphur, as sodium sulphate, per day.

Infusion of sulphate by either route significantly increased the excretion of total sulphur in faeces and the excretion of total sulphur, ester sulphate, and inorganic sulphate in urine. Inorganic sulphate excretion in faeces was increased significantly only by intraduodenal infusions, and the excretion of neutral sulphur in faeces and urine only by intraruminal infusions.

When sodium sulphate was infused intraruminally 87–94% of the faecal sulphur was in the neutral sulphur fraction, 4·1–5·4% was ester sulphate sulphur, and 0·5–4·0% was inorganic sulphate sulphur. It is suggested that the intake of sulphur and the supply of digestible energy to the fermentative rumen and hindgut regions primarily determine the amount of organic sulphur excreted in the faeces by affecting the synthesis of bacterial sulphur.

On the basal treatment 15–20% of the urinary sulphur was inorganic sulphate sulphur (30–47 mg/day), but the proportion was 80–90% when sulphate was infused.

The mean urinary neutral sulphur outputs were increased from 70 up to 353 mg/day by intraruminal infusions of sodium sulphate, compared with from 51 up to 176 mg/day by the corresponding intraduodenal infusions. Similarly, the mean urinary ester sulphate sulphur outputs were increased from 134 up to 392 mg/day by the intraduodenal infusions, compared with 136–205 mg/day by the intraduodenal route. These results indicate that the ruminant's ability to metabolize ingested sulphate is substantially influenced by its ruminal microorganisms. Non-ruminants would therefore be expected to absorb and metabolize less sulphate than ruminant species.

I. Introduction

There is an obligatory requirement of sulphate for the synthesis of mucopolysaccharides of cartilage, bone, connective tissue, keratin, the mucous secretions of the alimentary, bronchial, and urinary tracts (e.g. Belanger 1954; Dziewiatkowski 1962; Varadi, Cifonelli, and Dorfman 1967), for inactivation and regulatory mechanisms (e.g. Kun 1961), and at least a facultative requirement of sulphate for detoxification purposes (e.g. Folin and Dennis 1915; Cornish and Ryan 1965). Warth (1932) and Warth and Krishnan (1935) reported that little free sulphate was excreted in the urine

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or faeces of straw-fed sheep or cattle. The addition of sulphate to the diet resulted in an increased excretion of ester sulphates in the urine. No inorganic sulphate was excreted in the urine of rats fed a sulphur-free diet (Wellers and Chevan 1959), so that its excretion might, therefore, be considered subordinate to the requirement for sulphation and the excretion of ester sulphates.

The excretion of endogenous nitrogen and of neutral sulphur in the urine both parallel basal energy metabolism, with body weight ($W^{0.74}$) being the reference base for each process (Brody 1945). Folin (1905) regarded the excretion of neutral sulphur as independent of diet, but it was later shown that changes in the intake of protein or sulphur or both affected the amount excreted (e.g. Amman 1933; Beach *et al.* 1942; Bray and Hemsley 1969).

Wellers, Boelle, and Chevan (1960) reported that neutral sulphur accounted for most of the sulphur in rat faeces, and the data of Warth and Krishnan (1935) suggested that ruminants do not differ in this regard. The increased output of total sulphur in the faeces, in the experiments of Moir, Somers, and Bray (1967) and of Bray and Hemsley (1969), where sulphate was added to the diet of sheep, was therefore probably due to an increased excretion of organic sulphur.

In the present experiment an examination was made of the effect of infusion of 0–6 g sulphate sulphur by way of the rumen or duodenum on the urinary and faecal excretion of neutral sulphur, ester sulphate, and inorganic sulphate in sheep. An assessment might then be made of the ruminal microorganisms' contribution towards the digestion and subsequent metabolism of ingested sulphate, and thus indicate likely differences between ruminant and non-ruminant species.

II. Materials and Methods

The treatments imposed were four levels of sodium sulphate, from 0 to 6 g sulphate sulphur per day, infused continuously either per rumen or per duodenum as described in the preceding paper (Bird and Moir 1971).

The liveweight of the sheep ranged between 36 and 49 kg.

Analytical methods for the determination of sulphur in its various fractions were those of Bird and Fountain (1970).

III. Results

There were increases in excretion of faecal total sulphur (FS) and faecal neutral sulphur (FNS) above basal when sodium sulphate was infused into the rumen ($P<0{\cdot}05$). Between-treatment comparisons show no significant increases for sulphate additions greater than 1·5 g sulphur/day (Table 1). The regression of FNS excretion (y, mg/day) on sulphur intake (x, g/day) was

$$y = 377+26{\cdot}3x \quad (r = 0{\cdot}67, P<0{\cdot}01).$$

The regressions of FS and FNS excretion on faecal dry matter output or of FNS output on faecal nitrogen (FN) excretion were not significant, but that of FNS concentration on sulphur intake was significant ($P<0{\cdot}001$); the quadratic equation (Fig. 1) gave a significantly better fit ($P<0{\cdot}025$) than the linear equation.

The output of FNS was greater on treatment B than on the basal treatment ($P<0{\cdot}01$) or treatments C or D ($P<0{\cdot}05$) with sodium sulphate infused into the

TABLE 1

EFFECT OF AMOUNT OF SODIUM SULPHATE INFUSED INTO THE RUMEN OR DUODENUM UPON THE EXCRETION OF SULPHUR FRACTIONS IN URINE AND FAECES

Values expressed as mg/day ± standard error of the mean. Each observation is the mean of four sheep, with the exception of treatment A and D, rumen infusion route, where the mean of three sheep is taken. Treatments A, B, C, and D correspond to infusion of 0, 1·5, 3·0, and 6·0 g sulphate sulphur per day. Significant differences between treatments (a, b, x, y), or between infusion routes for a given treatment (t, r) are indicated by similar superscripts: viz. x, x_1, x_2; y, y_1, y_2; t, t_1: $P<0·05$; a, a_1, a_2, a_3; b, b_1, b_2, b_3, b_4, b_5: $P<0·01$; r: $P<0·001$

	Treatment A		Treatment B		Treatment C		Treatment D	
	Rumen	Duodenum	Rumen	Duodenum	Rumen	Duodenum	Rumen†	Duodenum
Sulphur intake*	863	922	2338	2422	3838	3952	5163^{a}	6922^{a}
	(±61)	(±81)	(±50)	(±81)	(±50)	(±61)	(±403)	(±81)
Faeces								
Total sulphur	$393^{a,a_1,x}$	386^{b}	496^{x}	485^{b_1}	533^{a}	606^{b_2}	$571^{a_1,r}$	$1497^{b,b_1,b_2,r}$
	(±15)	(±2)	(±5)	(±9)	(±18)	(±58)	(±31)	(±234)
Neutral sulphur	$369^{x,x_1,x_2}$	355^{b}	459^{x}	$431^{b,y,y_1}$	$502^{x_1,t}$	$392^{x_2,t}$	498^{x_2}	374^{y_1}
	(±15)	(±3)	(±8)	(±8)	(±23)	(±17)	(±45)	(±27)
Ester sulphate sulphur	20	$20^{b,b_1}$	24	$32^{b,b_3}$	22	$34^{b_1,b_4}$	30	$20^{b_3,b_4}$
	(±4)	(±1)	(±4)	(±4)	(±6)	(±6)	(±5)	(±2)
Inorganic sulphate sulphur	2	$3^{b,y}$	4	11^{b_1}	8	$174^{b_2,y}$	22^{r}	$1058^{b,b_1,b_2,r}$
	(±1)	(±1)	(±2)	(±3)	(±7)	(±67)	(±4)	(±76)
Urine								
Total sulphur	$207^{a,a_1,a_2}$	$234^{b,b_1,b_2}$	$1742^{a,x,x_2}$	$1678^{b,b_3,b_5}$	$2996^{a_1,x,x_1}$	$2738^{b_1,b_3,b_4}$	$4370^{a_2,x_1,x_2}$	$4503^{b_2,b_4,b_5}$
	(±31)	(±20)	(±169)	(±81)	(±69)	(±119)	(±655)	(±211)
Neutral sulphur	70^{a}	$51^{y,y_1,y_2}$	147^{x}	171^{y}	168^{x_1}	135^{y_1}	$353^{a,x,x_1}$	176^{y_2}
	(±31)	(±9)	(±42)	(±29)	(±74)	(±15)	(±85)	(±52)
Ester sulphate sulphur	$134^{a,a_1,x}$	136^{y}	$221^{a_2,x}$	175	$299^{a,x_1,t_1}$	163^{t_1}	$392^{a_1,a_2,x_1,t}$	$205^{y,t}$
	(±20)	(±15)	(±18)	(±21)	(±16)	(±20)	(±26)	(±20)
Inorganic sulphate sulphur	$30^{a,a_1,a_2}$	$47^{b,b_1,b_2}$	$1394^{a,a_3,x}$	$1375^{b,b_3,b_5}$	$2578^{a_1,x,x_1}$	$2439^{b_1,b_3,b_4}$	$3625^{a_2,a_3,x}$	$4061^{b_2,b_4,b_5}$
	(±6)	(±21)	(±124)	(±34)	(±41)	(±96)	(±546)	(±221)

* Values are dietary total sulphur plus infused sulphate sulphur.

† The amounts of sulphate sulphur infused were 5·0, 4·0, and 4·0 g respectively for the three sheep in this phase and not 6·0 g.

TABLE 2

EFFECT OF AMOUNT OF SODIUM SULPHATE INFUSED INTO THE RUMEN OR DUODENUM UPON APPARENT DIGESTIBILITY OF THE RATION, THE EXCRETION OF NITROGEN, AND THE CONCENTRATION OF NEUTRAL SULPHUR IN THE FAECES

Values are means ± standard error of the mean, as for Table 1, and treatments A, B, C, and D are as defined in that table. Significant differences between treatments are indicated by similar superscripts: viz. x, t: $P<0\cdot05$; b, b_1, b_2: $P<0\cdot01$; c: $P<0\cdot001$

	Treatment A		Treatment B		Treatment C		Treatment D	
	Rumen	Duodenum	Rumen	Duodenum	Rumen	Duodenum	Rumen	Duodenum
Nitrogen intake (g/day)	13·03 (±0·05)	13·01 (±0·05)	13·06 (±0·04)	13·01 (±0·05)	13·06 (±0·04)	13·01 (±0·05)	13·08 (±0·05)	13·01 (±0·05)
Apparent digestibility of dry matter (%)	$59\cdot9^{x}$ (±0·63)	$63\cdot2^{b}$ (±1·45)	63·1 (±0·63)	$58\cdot1^{b,y}$ (±2·10)	64·7 (±1·33)	60·8 (±0·35)	$65\cdot0^{x}$ (±1·51)	$62\cdot1^{y}$ (±2·27)
Total nitrogen excreted in faeces (g/day)	3·39 (±0·06)	$3\cdot16^{b}$ (±0·03)	3·40 (±0·09)	$3\cdot84^{b,b_1,b_2}$ (±0·15)	3·29 (±0·09)	$3\cdot16^{b_1}$ (±0·09)	3·20 (±0·06)	$3\cdot25^{b_2}$ (±0·11)
Total nitrogen excreted in urine (g/day)	8·27 (±0·03)	7·69 (±0·37)	7·88 (±0·13)	8·02 (±0·06)	8·12 (±0·32)	7·71 (±0·17)	8·33 (±0·25)	8·05 (±0·47)
Faecal neutral sulphur (mg/g faecal dry matter)	$1\cdot152^{x,c}$ (±0·039)	1·242 (±0·065)	$1\cdot565^{x}$ (±0·051)	1·298 (±0·057)	$1\cdot739^{c}$ (±0·050)	1·257 (±0·050)	$1\cdot824^{x}$ (±0·115)	1·218 (±0·044)

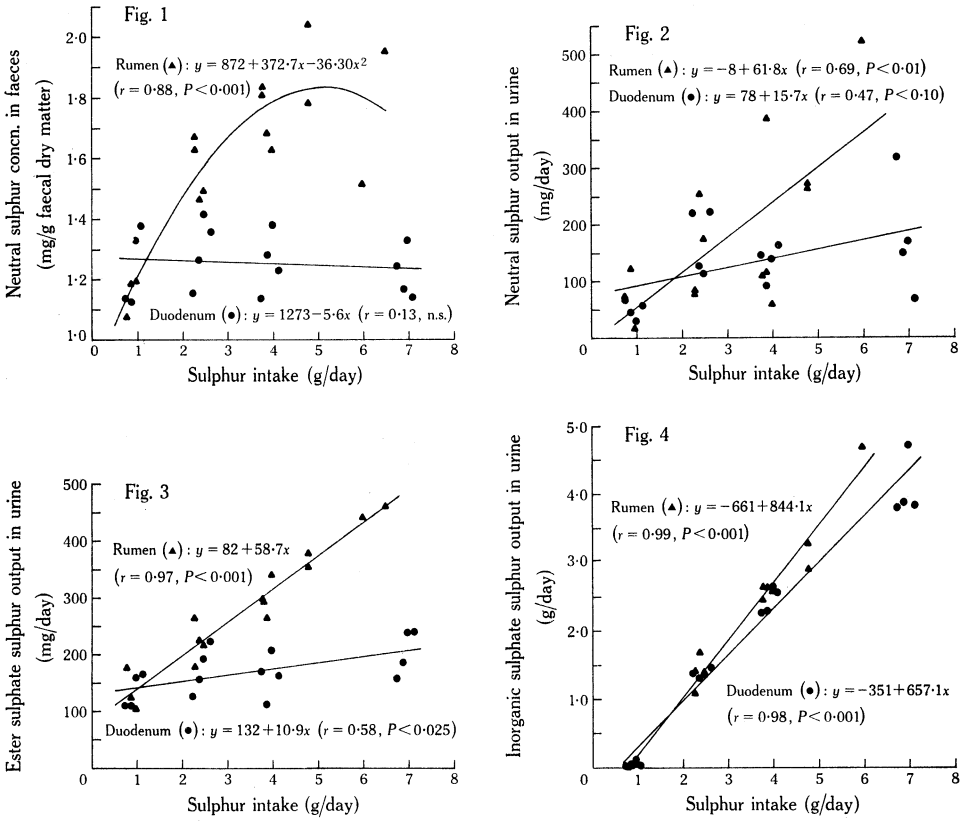
duodenum. This effect was associated with a higher apparent digestibility of the ration on treatment A ($P<0\cdot01$) or D ($P<0\cdot05$), as shown in Table 2. The regression of FNS output (y, mg/day) on faecal dry matter (x, g/day) was

$$y = 76+1\cdot0x \quad (r = 0\cdot71, P<0\cdot005),$$

and for FNS output (y, mg/day) on FN (x, g/day)

$$y = 109+83\cdot0x \quad (r = 0\cdot69, P<0\cdot005).$$

Sodium sulphate infused into the rumen did not influence the output of faecal ester sulphate (FES), but the duodenal infusion treatments A and D (Table 1) resulted in lower outputs than both treatments B and C ($P<0\cdot01$).



Figs. 1–4.—Effect of intraruminal (▲) or intraduodenal (●) infusion of sodium sulphate on the concentration of neutral sulphur in faeces (Fig. 1), and on the excretion of neutral sulphur (Fig. 2), ester sulphate sulphur (Fig. 3), and inorganic sulphate (Fig. 4) in the urine. Regression equations are given for each curve.

Intraduodenal infusions of sodium sulphate increased the excretion of inorganic sulphate in the faeces only, whereas both the urinary total sulphur (US) and inorganic sulphate outputs were increased by infusions of sodium sulphate by either route ($P<0\cdot001$) (Table 1). The relationship of urinary inorganic sulphate excretion and sulphur intake is shown in Figure 4.

Urinary neutral sulphur output (UNS) was increased ($P<0\cdot05$) by the infusion of sodium sulphate into the rumen (Table 1). The relationship of UNS output and sulphur intake for either route of infusion is shown in Figure 2. The apparently illogical variation in UNS output with different duodenal infusions led to the examination of other factors; UNS output (y, mg/day) was found to correlate with apparent digestibility of dry matter (x, %):

$$y = 790-10\cdot7x \quad (r = 0\cdot52, P<0\cdot05).$$

The relationship was not significant for the rumen infusion route ($r = 0\cdot24$).

Urinary ester sulphate excretion (UES) increased by sodium sulphate infusion into the rumen ($P<0\cdot005$), or into the duodenum ($P<0\cdot05$). The relationships between UES excretion, route of infusion, and sulphur intake are shown in Figure 3.

While urinary nitrogen excretion was unaffected by the infusions, faecal nitrogen excretion was increased only with duodenal infusions ($P<0\cdot01$) (Table 2).

Overall, the apparent digestibility of dry matter varied significantly with the duodenal infusions of sodium sulphate ($P<0\cdot025$), due mainly to an inexplicable decrease on treatment B, but did not do so with the ruminal infusions (Table 2).

IV. Discussion

Because of the capacity of the rumen microorganisms to metabolize and incorporate inorganic sulphate, some differences in the excretory pattern of sulphate infused into the rumen or into the duodenum—i.e. a ruminant *v.* a non-ruminant condition—may be anticipated.

The faecal excretion of total sulphur increased with sulphate intake, as previously recorded by Moir, Somers, and Bray (1967) and by Bray and Hemsley (1969). As 87–94% of this was in the neutral sulphur (i.e. organic, carbon-sulphur) fraction, the excretory pattern is similar to that in the rat (Wellers, Boelle, and Chevan 1960). The neutral sulphur concentration increased with ruminal infusions up to 3 g sulphate sulphur per day, but no consistent increase was evident with duodenal infusions (Fig. 1).

Under some circumstances dietary sulphate supplements have improved the digestibility of roughage (e.g. Bray and Hemsley 1969) and have also increased the production of ruminal protein (e.g. Hume and Bird 1970; Bird, unpublished data). In the present experiment increased FNS output was associated with those treatments in which increased digestibility of the ration occurred. An increased microbial protein synthesis may also be expected under these circumstances and an increase in the FNS output could result. Hogan and Weston (1968) have estimated that approximately three-quarters of the metabolic faecal nitrogen (MFN) comes from the foregut of sheep, due to the incomplete digestion of rumen bacteria. Assuming that the protein of MFN has a nitrogen/sulphur ratio of 10–15:1, up to 215 mg sulphur/day could then be derived from the rumen bacteria. The basal FNS excretion in the present experiment was 355–370 mg/day and the mean maximum increment in FNS output above basal due to sulphate infusion was 133 mg/day. This would demand a concomitant excretion of about 1·3 g FN/day if all the FNS was in protein. However, no increase in the FN output was observed, consequently substantial changes in the relationship between the faecal nitrogen and organic sulphur components must be suggested. The FN/FNS ratio in treatments A and D for the ruminal infusion of

sodium sulphate was 9·2 and 6·4 respectively, and 8·9 and 8·7 respectively for the duodenal infusion, therefore if all the FN was in protein form some organic sulphur other than in protein was excreted in the faeces. Although the sulphur-containing vitamins thiamin and biotin are produced by intestinal microorganisms and could thus contribute to the FNS fraction, only minor quantitative changes are possible due to this synthesis (Garrigus 1970).

Taurine conjugates of cholic, deoxycholic, and chenodeoxycholic acids are present in the sheep bile (Peric-Golia and Socic 1968). As inorganic sulphate supplementation may increase the production of ruminal protein (Hume and Bird 1970) increased absorption and metabolism of methionine and cystine could result in an increased synthesis of taurine and tauro-conjugated bile acids (Boquet and Fromageot 1967). Sulphate infused into the duodenum may also be reduced in the large intestine to hydrogen sulphide and absorbed. If not rapidly oxidized there arises the possibility of cystine synthesis from conjugation with serine in the liver (Brüggeman and Waldschmidt 1962; Waldschmidt 1962) again contributing to an elevation in the synthesis and excretion of taurine.

About 5% of bile acids are lost from the enterohepatic circuit in man (Gray, Nicholson, and Quincey 1968); however, due to microbial hydrolysis in the large intestine (Midtveldt and Norman 1967), conjugated bile acids are not usually excreted in the faeces (Weiner and Lack 1967), except from germ-free animals (Gustafsson *et al.* 1957) or animals treated with antibiotics (Lindstedt and Norman 1957). Presumably, therefore, tauro-conjugated bile acids do not normally contribute much sulphur to the FNS fraction in sheep. Taurine arising from the deconjugation of taurocholic acid by the intestinal flora is degraded in the gut, with the sulphur appearing in the urine mainly as inorganic sulphate (Boquet and Fromageot 1965, 1967); the sulphur requirement of bacteria in the large intestine might therefore normally be met from this source.

Those intraduodenal infusions of sulphate treatments resulting in a reduced digestibility of the basal ration produced an increased output of FNS and FN. It is possible that a reduced ruminal digestion ensured a greater supply of energy to the hindgut fermentation area, a condition which results in a substantial increase in FN output (Thornton *et al.* 1970) and in FNS excretion (Bird and Thornton, unpublished data). Within the narrow range of FN data obtained, the excretion of 1 g nitrogen is associated with about 83 mg FNS; the nitrogen/sulphur ratio being 12. This ratio is similar to that in bacteria (Walker and Nader 1968) so that variations in FNS output in response to the duodenal infusion of sodium sulphate may be accounted for in terms of variation in the amount of bacterial protein produced in the large intestine. Endogenous protein secretion probably contributes little to the FNS since the digestibility is very high (e.g. Snook and Meyer 1964) except in the germ-free animal (Loesche 1968), or in rats given antibiotics which result in an increased excretion of the cystine-rich trypsin (Barnes, Kwong, and Fiala 1965).

Faecal ester sulphates may arise from the sulphation of bilirubin (Isselbacher and McCarthy 1959), triiodothyronine (Roche *et al.* 1959), cholesterol (Drayer *et al.* 1964; Moser, Moser, and Orr 1966), steroid hormones (Gustafsson, Gustafsson, and Sjovall 1968*a*; Erickson, Gustafsson, and Sjovall 1969), plant and animal sterols (Gustafsson, Gustafsson, and Sjovall 1968*b*), and intestinal mucins (e.g. Pasternak and Kent 1958; Dziewiatkowski 1962). In this experiment the output of FES was

independent of the sulphur intake and, although forming the major portion of the faecal sulphates (58–91%), was only 4·1–5·4% of FS. There was a tendency for a greater FES output to be associated with an increased output of faecal dry matter, which would be expected if mucin excretion is proportioned to the FDM output.

Faecal inorganic sulphate sulphur was a very small fraction of the FS (0·5–4%) when sodium sulphate was infused ruminally and this may represent sulphate arising from mucin which is degraded by intestinal bacteria (Lindstedt, Lindstedt, and Gustafsson 1965), or sulphate secreted into the hindgut.

Urinary total sulphur (US) excretion was a linear function of sulphur intake. 75–85% of the sodium sulphate infused into the rumen and 65–69% of that infused via the duodenum were excreted in the urine. Inorganic sulphate sulphur contributed 80–90% of the total in both cases. The urinary output of inorganic sulphate sulphur on the basal treatments was only 15–20% of the total, that is 30–47 mg/day. No free sulphate was found in the urine of sheep fed 0·8 g sulphur/day by Warth and Krishnan (1935), while sulphur-deficient rats excrete little, if any, inorganic sulphate (Wellers and Chevan 1959). The proportion of inorganic sulphate in the urine of the group of children studied by Beach *et al.* (1942) was *c.* 84%. A mixed protein diet supplying about 1 g sulphur and 13 g nitrogen daily was fed. Together, these data affirm that inorganic sulphate excretion is extremely variable and responsive to dietary sulphur intake. Inorganic sulphate in the urine may arise from catabolism of tissue *S*-amino acids and sulphate esters, from the oxidation of sulphide absorbed from the gut, or from sulphate absorbed *per se*.

The UNS excretion increased from 70 to 353 mg/day when sodium sulphate was infused ruminally, but variability was large, particularly at the higher infusion levels. Bray and Hemsley (1969) reported an increased UNS output from 55 up to 140 mg/day, when the sulphur intake was increased from 0·37 to 2·55 g/day. By contrast, the data of Folin (1905) and Beach *et al.* (1942) show that humans excrete 52–84 mg UNS/day, while pigs and dogs excrete about 30–50 mg/day (Amann 1933). Brody's (1945) prediction equation for urinary neutral sulphur, which was based upon data from monogastric mammals ranging in size from rats to horses, was UNS (mg/day) $= 6{\cdot}85\ W^{0{\cdot}74}$ (kg). On this basis, the sheep in this experiment (liveweight 30–50 kg) should excrete 85–124 mg neutral sulphur/day. The discrepancy between these values indicate that the UNS excretion in sheep is very responsive to dietary manipulation and may exceed the value predicted by up to three times.

The UNS excretion was not directly influenced by the amount of sodium sulphate infused duodenally but was significantly associated with the digestibility of the ration. Amann (1933) observed that monogastric animals excreted more UNS when fed high-protein diets. A partial explanation for the increased UNS excretion in these sheep may be due to a greater synthesis of microbial protein and its subsequent metabolism.

In man, taurine is the major organic sulphur compound in urine (Soupart 1959) and protein diets or diets supplemented with cysteine, cysteic acid or taurine increase this excretion (Schmidt and Clark 1922; Evered 1956; Wellers 1962; King *et al.* 1968). Cysteine and several conjugates of cysteine occur in urine (Kodama 1968) as does thiamin (Teeri *et al.* 1953), methionine, thiocyanate, coenzyme-A (Mitchell 1962) and other organic sulphur compounds of metabolic origin such as isobuteine and felenine.

A linear increase of UES excretion resulted from increasing the amount of sodium sulphate infused either by way of the rumen or duodenum (Fig. 3). The basal UES output of 134 mg/day was increased up to 392 mg/day for ruminal infusion and from 130 up to 205 mg/day for the duodenal route. Warth and Krishnan (1935) found that sheep ingesting 0·81 g sulphur/day excreted about 150 mg UES/day, a value comparable with that found in this experiment for a similar intake of sulphur. Estimates of the ester sulphate output by humans are 39–68 mg sulphur/day for children (Beach *et al.* 1942) and 60–100 mg sulphur/day for adults consuming a mixed diet (Folin 1905). It therefore appears that ruminants of about the same size, and ingesting similar amounts of sulphur, excrete more ester sulphate in the urine, presumably as a consequence of greater microbial fermentation of the diet.

Urinary indican and skatoxyl sulphates arise from conjugation with the bacterial fermentation products of tryptophan, indole, and skatole (e.g. Bauman and Brieger 1879; Lewis and Emery 1962; Bostrom, Gustafsson, and Wengle 1963). In man an estimated 83($\pm$36) mg indican is excreted daily (Bryan 1965). The simple phenolic sulphates arise from the degradation of tyrosine (e.g. Bauman 1879; Bakke 1969*a*, 1969*b*) and the output of these conjugates is increased on high protein intakes (e.g. Folin and Dennis 1915; Bakke 1969*b*). A longer retention time of digesta in the gut also appears to increase the UES excretion in man (Beach *et al.* 1942), presumably as a result of increased fermentation. A wide range of steroids (Bostrom 1964; Pasqualini and Jayle 1961, 1962), mucopolysaccharides (Dziewiatkowski 1962; Varadi, Cifonelli, and Dorfman 1967), and other products of physiological body function such as adrenalin, noradrenalin, serotonin (Bostrom 1964), bilirubin (Isselbacher and McCarthy 1959*a*), and triiodothyronine (Roche *et al.* 1959) yield sulphated products. In the germ-free rat phenyl sulphate and indoxyl sulphates are not produced and the total phenol excretion is markedly reduced. Ester sulphates other than this group are not affected, with liver sulphurylating activity remains unchanged compared with the normal rat (Bostrom, Gustafsson, and Wengle 1963). Phenols may also be excreted as glucuronides or in the unconjugated state (e.g. Folin and Dennis 1915) depending upon the availability of sulphate for detoxification (Schoenfield, Bollman, and Huffman 1962; Cornish and Ryan 1965). Warth (1932) found that *p*-cresol formed the bulk of the UES from cattle ingesting a hay ration, but this contribution was relatively unchanged by increasing the intake of sulphur.

When sodium sulphate was infused into the rumen in the present experiment, the UES produced was almost twice that resulting from infusion of sodium sulphate into the duodenum, yet little difference occurred in the amount of infused sulphate sulphur absorbed. Sulphate infused into the rumen therefore appears to promote the synthesis of compounds which are presumably sulphated after absorption from the gut.

The results show that the forestomach markedly influences the ruminant's response to ingested inorganic sulphate. The metabolic transformation of this sulphur to ester sulphates and organically bound sulphur, as indicated from the urinary and faecal excretion, is substantially greater when ruminal intervention occurs. Non-ruminants would therefore be expected to transform proportionately less of the dietary sulphate than do ruminants.

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