Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 4, Number 1, January, 1935.

Sulphur Metabolism.

I. The Absorption and Excretion of Flowers of Sulphur.

By J. H. KELLERMANN, M.Sc.(Agric.), Ph.D., Animal Nutritionist, Biochemical Laboratory, Onderstepoort.

THE important rôle that sulphur plays in Nature, is evident from the fact, that it does not only occur in all plants and animals but in both these kingdoms it is absolutely essential for growth and reproduction. Plants get their sulphur in inorganic forms from the soil whereas all animals receive theirs from protein synthesis in certain organic forms, either directly or indirectly, from the plants upon which they feed. It has always been and still is the belief that animals cannot synthesise any of the organic sulphur compounds (cystine, cysteine or methionine) so essential for growth but have to take them preformed in their food. Consequently the metabolism of many organic sulphur compounds has been studied very extensively, whereas only a small amount of work has been done upon the metabolism of inorganic sulphur.

Bernheim and Bernheim (1932) found that colloidal sulphur is reduced to hydrogen sulphide by the liver which first accelerated and later inhibited the oxygen uptake of this organ. Evidently then the reduction of large amounts of sulphur may have deleterious effects upon the organism, and Steyn (1931, 1932), and Lewis and Lewis (1927) have found that sulphur, when fed at high levels to sheep and rats, respectively, was definitely toxic, probably due to the formation of hydrogen sulphide in the intestine.

Some investigators believe that sulphur has some influence on carbohydrate and fat metabolism, although Lewis and Lewis (1927) did not obtain any difference in the glycogen-content of the livers of rats to which sulphur had been fed, as compared with control animals. Arnoldi and Kucera (1931), on the other hand, found an increase in the glycogen and a decrease in the fat-content of the livers of rats fed a carbohydrate-rich diet, whereas on a protein- and fat-rich diet there was an increase in the fat and a decrease in the glycogen-content. Niccolini (1933) noticed that sulphur inhibited the formation of glycogen in the livers of guinea pigs but had little effect on that in the muscles. Bürgi and Gordonoff (1926) found that the livers of rabbits fed sulphur for a long period had a glycogen-content two or three times that of normal control animals. Foldes (1928) likewise observed an increase in the glycogen-content of the livers and hyperplasia of the suprarenals of rabbits into whose ears sulphur had been rubbed, and Kubo (1930) found that the fat and lipoid-content of rabbits decreased whereas in mice the fat-content of the whole body and some organs increased upon the administration of sulphur. In later publications (1932, 1932a), he also stated that when adrenalectomised rats were fed sulphur the body weight increased more rapidly than in the adrenalectomised controls and the decrease in the fat-content of the different organs was stopped. Steyn (1934) likewise noticed a " striking amount of subcutaneous, intramuscular and intra-abdominal fat " in sheep that received sulphur over prolonged periods. However, it is clear that the results obtained by the different investigators are still too much at variance to warrant any definite conclusions and more work is needed to elucidate this point.

Sulphur also seems to have definite therapeutic values. Montgomery (1932) and Burman (1932) have found that the ingestion of an adequate amount of sulphur served as a good prophylactic in malaria probably due to the aroma produced in the superficial tissues which prevented the mosquitoes from settling and sucking blood. Philips, Carr and Kennard (1921) stated that sulphur when added at the rate of 2 per cent. by weight to the basal ration offered interesting possibilities of success in raising chicks in confinement possibly due to the checking of intestinal putrefaction. Ockmann (1928) obtained excellent results with sulphur in the treatment of mange in horses, scab in goats, scaly leg in birds, and pocks in the udder of cattle. Moreover, Krüger (1928) by the parenteral injection of sulphur (Sufrogel) cured a case of coxitis in a horse, chronic arthritis in the shoulder joint of another, and gonitis in a bovine.

Sulphur may also be of importance in certain cases of poisoning. Menegheth (1928) found colloidal sulphur to be a good antidote for corrosive sublimate ($HgCl_2$) probably due to the formation of mercuric sulphide which is less poisonous. Steyn (1929) reported that favourable results in regard to sulphur as a preventive for the prussic acid form of " geilsiekte " had been obtained, and in a later publication (1931) he stated that sulphur is at present being recommended as an efficient preventive for the foregoing disease. He also found that sulphur-treated rabbits (1932) and sheep (1932) showed a fair degree of resistance to potassium cyanide. The reason why sulphur-treated rabbits and sheep should be more resistant to cyanide poisoning is, as mentioned by him, probably due to the formation of thiocyanates which are less toxic. Sandberg and Holly (1932) in discussing the fate of cyanides in the organism stated: " It is known that one of the mechanisms which the body uses in the detoxication of cyanides is transformation into thiocyanates by combination of the cyanide group with the labile sulphur ". Lang (1933) is in agreement with this view and assumes that thiocyanic acid is formed in the animal body from sulphur and prussic acid. He found that the synthesis of thiocyanates is performed by an enzyme which is present in practically all the tissues except muscle and blood, but is particularly abundant in the adrenal gland and the liver. Smith and Malcolm (1930) likewise believe that thiocynate formation is the principal method of cyanide detoxication in the animal body. Consequently, in cases of acute cyanide poisoning the administration of a soluble sulphide should be more efficacious.

From a nutritional standpoint elementary sulphur is of no apparent value. This seems to be especially true of omniverous animals because Geiling (1917) and Daniels and Rich (1918) have shown that elementary sulphur and sulphates, respectively, could not serve as substitutes for cystine in the diet of white mice and rats. It is furthermore reported from the Iowa Agricultural Experiment Station (1924) that no favourable or unfavourable effect resulted in rats from the feeding of less than 0·5 per cent. of sulphur. Likewise Evvard, *et. al.* (1925), (1925a), and Sheeby and Senior (1930) found no beneficial effect following the addition of inorganic sulphur to the pig's diet.

With regard to the effect of sulphur on ruminants (sheep) there prevails some difference of opinion. At the Iowa Agricultural Experiment Station (1924) it was observed that daily doses of 0·5 oz. of sulphur had no effect in pregnant ewes or their offspring, and it is reported in the Sci. Rep. Govt. Agric. Chem., Coimbatore (1932-33), that compared with controls, there was no material increase in the live weight of sheep receiving 0·5 gm. sulphur per head daily. Furthermore, Peirce (1933), Seddon and Chamberlin (1933) and du Toit, *et. al.* (1934) could not confirm the results of Steyn (1931), (1932), who found that the administration of sulphur to Merino sheep had striking beneficial effects on their weight and wool yield. In a later paper Steyn (1934) found that the administration of sulphur had a similar effect on the growth of bovines. However, his observations are not without support in the literature because Ramaiyya (1932) has also found that the feeding of sulphur to Indian (native) sheep resulted in a heavier fleece and a higher cystine content of the wool as compared with those of the control animals. If the observations of these two investigators are true and correct, it is evident that inorganic sulphur may still prove itself to be of great economical value to the various wool producing countries.

In order, therefore, to gain more information on the functions of elementary sulphur, a series of experiments have been started at this Institute to study its metabolism, and it was felt, that in order to make an extensive study of its metabolism, knowledge concerning its rate of absorption and excretion, to begin with, is important.

EXPERIMENTAL.

Rat Experiments.

Absorption of Sulphur.

Because it was impossible to obtain a colloidal solution of sulphur that contained no other toxic substances, and yet was of such a concentration that the methods of Cori (1925) or Johnston (1932) could be used in the study of its absorption, the only alternative was to use the method of Bergeim (1926). It should be pointed out, however, that by means of this method results are obtained that are only of relative value, yet they may be of such manifestation as to be of importance. This seems to have been the case with sulphur.

A group of ten albino male rats of an average weight of 195 gm. was fed a sulphur-containing, protein-free diet for five days before they were killed. This period was deemed necessary in order to accustom the physiological functions of the organism to this unnatural diet and to make sure that all the residues of the former diet had been swept from the intestines. The composition of the diet used is given in Table I as Ration I.

TABLE I.

Composition of Rations Used in Rat Experiments

	Ration I.	Ration II.	Ration III.	Ration IV.
Dextrinized starch	60·7	—	55	40
Powdered sucrose	15	25	15·5	13
Yellow Maize Meal	—	30	—	30
Whole Wheat Meal	—	25	—	—
Merck's casein	—	18	10	12
Merck's prepared lard	10	—	8	—
Merck's precipitated sulphur	1	—	—	—
Modified Steenbock Salts 40	5	—	4·5	4
$CaCO_3$	—	1	—	—
NACL	—	1	—	—
Cod liver oil	2	—	3	—
Brewer's yeast	—	—	2	—
Agar	6	—	2	—
Iron oxide (Fe_2O_3)	0·3	—	—	—
Vitamin B, yeast extract equivalent to	—	—	—	(5 gm yeast per 100 gm. ration)
TOTALS	100·0	100·0	100·0	100·0
Sulphur content (mg. per 100 gm. ration)	—	195	108	120

The inorganic salt mixture used was a modification of Steenbock and Nelson salts 40 (1923) in which the $Mg\ SO_4 7H_2O$ was replaced by an equivalent amount of $Mg\ CO_3$. The rats were killed by a blow on the head. The whole intestinal tract was excised after tying its different sections (stomach, small intestine, etc.), and the oesophagus and rectum. After the excised intestinal tract was cooled on ice, it was divided into its different sections and the contents of each squeezed into about 30 c.c. of a solution containing 5 per cent. each of NaOH and H_2O_2. Care was taken to keep the open end of the intestinal loop through which the contents were squeezed submerged in the solution to prevent the loss of any volatile sulphide. The contents were then thoroughly mixed with the solution and dried. The iron was determined as described by Bergeim (1926a) and the sulphur by the method of Feigl and Schorr (1923). Excellent results were obtained with the latter method whereas several of the recognized methods for sulphur determination, that were tried, gave results that were too low. These methods may give satisfactory results for organic and inorganic sulphur forms, but not for elementary sulphur. The sulphur content of a sample of ration to which elementary sulphur was added was found to be 0·423 per cent. with the Feigl-Schorr and 0·440 per cent. with the combustion method of Lant-Eckl (1921), and it is obvious, therefore, that the results obtained with the two methods are in perfect agreement. Moreover, when using 21·1 mg. to 22·3 mg. samples of Merck's sulphur (extra pure crystals) the recoveries of this element as determined by the Feigl-Schorr method, were consistently 97·57 to 99·51 per cent.

The results are given in Table II.

TABLE II.

Percentage Recovery of Sulphur from the Contents of Different Parts of the Digestive Tract as Determined by the Bergeim Method.*

GROUP I.

Contents.	Sulphur in dry material mg per gm.	Iron in dry material mg per gm.	Ratio S : Fe.	Percentage recovery of S in food.
Ration I	9·83	1·65	5·95	—
Stomach	11·11	1·86	5·97	100·3
Small intestine	19·36	3·32	5·83	97·9
Caecum	29·59	7·05	4·20	70·6
Large intestine	16·91	4·47	3·78	63·5
GROUP II.				
Ration II	9·54	1·89	5·04	—
Stomach	9·91	1·95	5·08	100·7
Small intestine	25·28	5·07	4·98	98·8
Caecum	31·00	9·62	3·22	63·9
Large intestine	21·42	6·94	3·08	61·1

* To a standard protein-free diet was added a definite proportion of iron oxide which served as a standard for comparison, and flowers of sulphur, the absorption of which it was desired to study. The ratios of sulphur to iron were determined for the calculation. For example, if the S:Fe ratio of the ration were 10:1 and of the intestinal contents 6:1 then the intestinal contents would contain 6÷10, which would be 0·6 or 60 per cent. of the original sulphur.

It is seen that by the time the intestinal contents reached the small intestine, caecum and large intestine, only 97·9, 70·6 and 63·5 per cent., respectively, of the original sulphur in the ration were recovered. Very similar results, given in the same table, were obtained with a second group of rats. It is evident, therefore, that by the time the contents reached the colon, more than 38 per cent. of the original sulphur in the ration had disappeared. Whether this fraction was absorbed or lost as volatile sulphide cannot be ascertained from these results although one would expect that both processes took place to a greater or lesser degree.

In order, therefore, to study the transformation of sulphur in and the probable form(s) in which sulphur is absorbed from the intestinal tract, an experiment was carried out in which the volatile and sulphate sulphur were determined in the intestinal contents of rats on rations with and without sulphur. There were 30 adult

TABLE III.

Sulphate and Free H_2S Sulphur in the Intestinal Contents of Rats on a Protein-free Diet With and Without 1 per cent. of Sulphur.

GROUP I.—(*Protein-free diet.*)

Contents.	Moisture.	Free H_2S Sulphur Sample.		Sulphur in dry matter.	Sulphate Sulphur Sample.		Sulphur in dry matter.
		Weight dry.	Sulphur.		Weight dry.	Sulphur.	
	Per cent.	gm.	mg.	mg per 100 gm.	gm.	mg.	mg per 100 gm.
Stomach	70·08	2·973	0·055	1·8	4·867	1·305	26.8
Small intestine	84·29	3·403	0·080	2·3	5·191	3·353	60·0
Caecum	82·41	4·764	0·510	10·7	6·161	12·130	196·8
Large intestine	69·31	2·271	0·470	20·7	8·320	17·270	207·6
Faeces	56·49	10·140	0·790	7·7	7·982	14·780	185·1
TOTALS	—	23·551	1·905	8·1	32·521	48·838	150·1

GROUP II.—(*Protein-free diet plus 1 per cent. of sulphur.*)

Contents.	Moisture.	Free H_2S Sulphur Sample.		Sulphur in dry matter.	Sulphate Sulphur Sample.		Sulphur in dry matter.
		Weight dry.	Sulphur.		Weight dry.	Sulphur.	
Stomach	75·11	2·691	0·52	19·3	2·192	1·168	53·3
Small intestine	85·35	3·991	1·64	41·1	6·069	7·984	131·5
Caecum	81·17	7·749	3·85	49·6	8·244	25·800	312·9
Large intestine	71·12	4·156	2·75	66·1	7·155	19·720	275·6
Faeces	25·45	9·757	1·66	17·0	8·017	22·520	280·9
TOTALS	—	28·344	10·42	36·8	31·677	77·192	243·6

albino rats to each group and the ration was the same as the foregoing except that the iron oxide was replaced by an equivalent amount of starch. After the animals had been on the rations for five days, they were killed and the contents of the different sections of the intestinal tract collected. The volatile and sulphate sulphur determinations were made on the fresh pooled material of each group. The volatile sulphide was determined by the gravimetric method as $BaSO_4$ as described by Heffter and Hausmann (1904). The hydrogen peroxide used was a 5 per cent. ammoniacal solution of Merck's perhydrol. For the determination of sulphate sulphur the method of Woodman and Evans (1933) was used.

The data presented in Table III show that not only did the free sulphide but also the sulphate sulphur of the intestinal contents of the sulphur-fed rats exceed by far those of the control group. It is seen that in both groups the highest concentrations of sulphate- and free sulphide-sulphur were present in the caecum and large intestine. The lower concentrations of free sulphide-sulphur in the faeces (collected on filter paper during the last 12 hours before the rats were killed, and only those that were uncontaminated with urine) of both groups as compared with those in the large intestines were partially due, no doubt, to the easy escape of this fraction. On the other hand, the parts of the intestinal tract in which the greatest increase in sulphate- and free sulphide-sulphur took place on the feeding of sulphur were the stomach and small intestine. However, the values of the small intestine were influenced, no doubt, to a large extent by those of the stomach where no, or very little, absorption took place, and whence the sulphide and sulphate were passed on to the small intestine. The average values for the sulphide- and sulphate-sulphur of the stomach and small intestinal contents of the sulphur-fed groups were, respectively, 15·4 and 2·4 times as much as the corresponding material from the control animals whereas the respective average values of the caecal and large intestinal contents were only 3·9 and 1·4 times as much as those from the control group. That the increase in sulphate-sulphur was not due to the oxidation of sulphide to sulphate in the course of the determination was ascertained by the fact that when water, saturated with hydrogen sulphide, was added to parts of the intestinal contents of another group of rats on the control diet, and the determinations further carried out as before, there was found to be no increase, whatsoever, in the sulphate contents. This might have been due to a more copius flow of intestinal secretions induced by hydrogen sulphide irritation, or to a greater contentration of sulphates in the secretions of the sulphur-fed rats, or to both.

Absorption and Excretion of Sulphur.

The results obtained so far indicated that elementary sulphur was transformed in the digestive tract into an absorbable form, and that by the time the contents reached the colon about 38 per cent. of the original sulphur in the ration had disappeared. The extent to which sulphur is absorbed and excreted by the organism, therefore, still remains to be determined. However, it should be pointed out that, under the experimental conditions, the absorption of sulphur could not be calculated from the difference between the intake of sulphur in the ration and the output in the faeces in view of the fact that sulphur was lost in the faeces in the form of hydrogen sulphide. The only alternative, therefore, was to calculate the absorption of sulphur from the increase in total sulphur in the urine during the sulphur period over and above that during the control period.

The experiment was run in duplicate form with male albino rats with an average weight of 165 gm. The metabolism cages were of the same type as described by Falcon-Lesses (1930). The basal ration is given in Table I as Ration II. Fresh distilled water was at all times available to the animals. Since it was found by Lewis and Lewis (1927) that sulphur, when incorporated at 0·5 per cent. and higher levels in the ration of rats was toxic, it was feared that the animals would suffer from anorexia and subsequent cachexia, and therefore deemed impracticable to attempt to equate the daily food intakes of the rats under the experimental conditions. Consequently weighed portions of the experimental diets were supplied in such quantities that the animals could consume the diets *ad libitum* while at the same time the food intake was accurately known for each period. However, it will be seen from Table IV that the average food consumption per animal per day did not vary very much for the three periods, being 8·5, 10·0 and 9·3 gm., respectively, for the pre-sulphur, sulphur and post-sulphur periods. After a preliminary period of five days commencement was made with the collection of the excreta of the pre-sulphur period. The transition periods between the presulphur and sulphur, and the sulphur and post-sulphur periods were each three days long during which time no excreta were collected. These three-day transition periods were adopted because it was found in another experiment (Table VI) that after the commencement on a sulphur diet, the total sulphur in the urine of rats increased until the third day when it reached a more or less constant level, and it might be assumed, therefore, that by this time the tissues were saturated with sulphur (H_2S and sulphates) and that the excretion of the latter in the urine was equal to its absorption. The urine and faeces were collected daily and the latter immediately dried in a steam oven. At the end of each period the dried faeces were freed from all hair, weighed and pulverised for analysis. The total sulphur in the faeces was determined as previously described. In collecting the urine, the metabolism cage, funnel, separator and bottles placed for collection of faeces and urine, respectively, were washed down daily with a brush and distilled water. It was preserved with toluol in a glass stoppered bottle kept in an ice-chest until the end of each period when it was made up to volume and filtered through filter paper. The total sulphur was determined by the Benedict method as modified by Denis (1910).

The results obtained with the 0·5 per cent. sulphur ration are summarised in Table IV.

It will be seen that the extent to which sulphur was " stored and lost " daily during the sulphur period was, on the average, 2·8 times as great as during the pre-sulphur one. The excretion of sulphur in the faeces for every 100 gm. of food eaten was on the

TABLE IV.

Record of Sulphur Balance of Rats on Rations Low and High in Sulphur Content.

	Ration II.		Ration II + 0·5% S.		Ration II.	
Period	Pre-Sulphur.		Sulphur.		Post-Sulphur.	
Rat Number	1	2	1	2	1	2
Duration (days)	6	6	8	8	19	19
Gain or loss in weight (gm)	– 1	– 2	– 3	– 9	0	–3
Food consumed (gm)	49·0	52·5	84·5	76·0	189·5	163·5
Total sulphur intake (gm)	0·0955	0·1024	0·5163	0·4643	0·3430	0·2959
Dried faeces (gm)	4·78	4·98	8·87	7·77	18·47	14·78
Sulphur output (gm)—						
(*a*) in faeces (dry)	0·0173	0·0170	0·2446	0·2226	0·0683	0·0585
(*b*) in urine	0·0574	0·0650	0·1728	0·1862	0·2612	0·2458
Total sulphur output (gm)	0·0747	0·0820	0·4174	0·4088	0·3295	0·3043
Part as % of total S output in urine	76·8	79·3	41·4	45·6	79·2	80·7
Part as % of total S intake in urine	60·1	63·4	33·4	40·11	76·14	83·08
Balance (stored and lost) per day (mg)	+ 3·5	+ 3·4	+12·3	+ 6·9	+ 0·71	– 0·44

average 33·7, 291·1 and 35·9 mg. for the pre-sulphur, sulphur and post-sulphur periods, respectively. It is clear, therefore, that for eveyr 100 gm. of food eaten the animals excreted 2·2 mg. more sulphur in the post- as compared with the pre-sulphur period. Furthermore, when it is remembered that the animals consumed on an average 0·8 gm. more food per rat per day during the post- as compared with the pre-sulphur period with a consequent shorter period of time to finish 100 gm. of food, it is evident that a transition period of three days was not long enough to clear the digestive tract of its sulphur of the previous (sulphur) ration. It will be observed further that the excretion of sulphur in the urine for every 100 gm. of food eaten was on an average 120·5, 223·6 and 143·6 mg. for the pre-sulphur, sulphur and post-sulphur periods. It is evident, therefore, that although the average loss of weight during the post-sulphur period was no more than that during the pre-sulphur one, the animals excreted for every 100 gm. of food consumed about 23 mg. more sulphur in their urine during the post-sulphur period as compared with the pre-sulphur one. This greater excretion of sulphur was partly due, no doubt, to the absorption and excretion of sulphur retained in the digestive tract and partly to the elimination of sulphur stored (unpublished data) in the body tissues.

The average parts, as percentage, of the total sulphur output in the urine were 78·05, 43·50 and 79·95 for the pre-sulphur, sulphur and post-sulphur periods respectively. This indicates that the extent to which the sulphur was excreted in the urine during the sulphur period was about 1·8 times less than during the pre- and post-sulphur periods, and suggests that the elementary sulphur was metabolised to a much smaller extent than the food-sulphur of this ration. However, all these values are slightly too high in view of the fact that some sulphur was lost in the faeces as hydrogen sulphide and a truer index will therefore be the percentage of the total sulphur intake, excreted in the urine. These values ran parallel with the foregoing ones and were 61·75, 36·75 and 79·61 for the respective periods.

From the the results given in Table IV the absorption of flowers of sulphur can roughly be calculated. In so doing it was assumed that the percentage of the "food-sulphur" intake, excreted in the urine during the sulphur period was equal to that during the pre-sulphur one and a correction made accordingly, and further that by the time of the third day on a sulphur ration the body tissues were saturated with this element and that, therefore, the excretion of sulphur in the urine was equal to its absorption. Furthermore, by disregarding the unequal catabolism of body tissues during the pre- and sulphur periods, an approximate value of 24·6 per cent. was obtained for the absorption of flowers of sulphur.

However, the high concentration of "food-sulphur" in the ration might have influenced the absorption of elementary sulphur or *vice versa*, and it was considered necessary, therefore, also to determine the absorption of elementary sulphur on a ration very low in "food-sulphur". For this purpose two different male rats were employed and the composition of the basal ration used is given in Table I as Ration III.

TABLE V.

Record of Sulphur Balance of Rats on Rations Low and High in Sulphur Content.

	Ration III.		Ration III + 1% S.		Ration III.	
Period	Pre-Sulphur.		Sulphur.		Post-Sulphur.	
Rat Number	3	4	3	4	3	4
Duration (days)	15	15	23	23	28	28
Gain or loss in weight (gm)	—2	—13	—3	—17	+4	—13
Food consumed (gm)	143	130·5	164	152	201·5	193·5
Total Sulphur intake (gm)	0·1544	0·1309	1·7991	1·6674	0·2176	0·2090
Dried faeces (gm)	8·48	8·86	10·75	12·95	13·38	16·41
Sulphur output (gm)—						
(*a*) in faeces (dry)	0·0763	0·0842	0·7502	0·8030	0·2051	0·1982
(*b*) in urine	0·0506	0·0701	0·7762	0·7620	0·0481	0·0625
Total sulphur output (gm)	0·1269	0·1543	1·5264	1·5650	0·2532	0·2607
Part as % of total S output in urine	39·9	45·4	50·8	48·7	19·0	27·4
Part as % of total S intake in urine	32·78	53·57	43·14	45·72	22·10	29·90
Balance (stored and lost) per day (mg)	+ 1·8	— 1·6	+11·8	+ 7·5	— 1·3	— 1·8

For a few exceptions the results, summarised in Table V, run parallel to those given in Table IV, and will therefore not be discussed in detail. The average food consumption per rat per day was 9·1, 6·9 and 7·0 gm. for the pre-sulphur, sulphur and post-sulphur periods, whereas the average loss in weight over the same interval of time was 0·5, 0·43 and 0·16 gm. for the respective periods. During the pre-sulphur and sulphur periods the sulphur balance showed a gain of 0·1 and 9·6 mg. respectively, per rat per day, whereas in the post-sulphur period there was a loss of 1·5 mg. per rat daily. The excretion of sulphur in the faeces for every 100 gm. of food eaten was on the average 58·6, 491·5 and 102·1 mg. per rat during the presulphur, sulphur and post-sulphur periods, respectively, again indicating that a transition period of three days was too short to clear the digestive tract of the sulphur of the previous ration. The excretion of sulphur in the urine for every 100 gm. of food consumed was on the average 44·1, 486·7 and 28·0 mg. for the respective periods. The reason why in this experiment the animals excreted less sulphur in the post- as compared with the pre-sulphur period is not quite clear. It should be remembered that the animals partook on the average 2·1 gm. less food during the post- as compared with the pre-sulphur period with a consequent longer interval of time to consume 100 gm. of food, and for that reason alone one would have expected even a greater excretion of sulphur in the urine during the post- as compared with the pre-sulphur period in view of the fact that the longer the time of observation the greater will be the elemination of endogenous sulphur.

The percentage of the total sulphur output, excreted in the urine, was on the average 42·6, 49·7 and 23·2, and those as percentage of the total sulphur intake were 43·2, 44·43 and 26·0 for the pre-sulphur, sulphur and post-sulphur periods respectively. These two sets of results run parallel and stress further the point that the extent to which sulphur was excreted during the post-sulphur period was much smaller than that during the pre-sulphur one, and one is therefore lead to the belief that the administration of sulphur to rats to the extent of 1 per cent. in their ration was, under the experimental conditions, injurious to their kidneys and interfered with the normal physiological function of the latter.

However, the extent to which sulphur was excreted in the urine, expressed as percentage of the total sulphur intake, of the pre- (and post-) sulphur period of Table V differed markedly from that in Table IV. In the latter table it is shown that the "food-sulphur" of Ration II was excreted in the urine to the extent (average) of 61·75 per cent. whereas that of Ration III (Table V) was excreted to the extent of only 43·17 per cent. With a difference of 18·6 per cent. one is bound to conclude that the "food-sulphur" of basal Ration II which contained casein- and cereal-S, was absorbed to a greater extent than that of basal Ration III which, except for the little sulphur present in the yeast added, only contained casein-S. However, basal Ration III also contained 8 per cent. of lard and it might be that the latter accounted to a certain extent for the smaller absorption of the "food-sulphur" of this ration.

The absorption of elementary sulphur, calculated from the results given in Table V (S-ration III) as previously described, was 44·6 per cent. This figure agrees fairly well with the value (± 38 per cent.) given in Table II but is 20 per cent. higher than that obtained on S-ration II. However, a comparison of the results given in Tables IV and V shows that when elementary sulphur is ingested with a basal ration that is rich in readily available " food-sulphur ", the absorption of elementary sulphur is low (Table IV), whereas, on the other hand, when the absorption of " food-sulphur " is low, the organism compensates by taking in more elementary sulphur with a consequent higher percentage absorption of the latter (Table V). This viewpoint seems to be supported by the fact that the percentage of the sulphur intake, excreted in the urine, on two rations that were quite different in their composition and sulphur content, were close to one another. From the results given in Tables IV and V, it will se seen that the average for these values were 36·75 and 44·43, or better, the difference between the percentage of the sulphur intake, excreted in the urine during the two different pre-sulphur periods, was 18·6 per cent., which was practically equal to the difference (20 per cent.) in the absorption of elementary sulphur on the two different sulphur-rations.

The level at which the feeding of elementary sulphur (hydrogen sulphide) turns to be toxic may, therefore, be controlled to a certain extent by the concentration of easily available " food-sulphur " in the ration, and the reason why Lewis and Lewis (1927) found the feeding of the same level of sulphur with the Osborne-Mendel low protein diet to be less toxic than with the Sherman-Merrill (1925) milk powder-starch diet might probably be explained by the fact that the former diet did not only contain a slightly higher protein content (food-sulphur) but also 4·5 per cent. of Osborne and Mendel salt mixture (1913) which contains sulphates. Furthermore, the cause of the low absorption (10 per cent.) of sulphur, as found by Denis and Reed (1927) with dogs, according to the same method, might partially be found in the omission of a few days sulphur-transition period during which time the percentage of the sulphur taken in, excreted in the urine, was undoubtedly below that of the succeeding days on the sulphur-ration, and partially in the high concentration of " food-sulphur " in their rations.

The Excretion of Sulphur.

In order to determine the forms in which the absorbed sulphur were excreted in the urine an experiment was performed in which the total nitrogen, total sulphur, total sulphate and inorganic sulphate were determined in every 24 hour urine samples.

The difficulty was of courses to acquate the daliy food consumption. This was considered absolutely essential if a clear insight were to be obtained as to what was happening to the absorbed sulphur in the animal organism when fed rations with and without the addition of sulphur. Preliminary experimentation with rats on Ration IV, Table I, showed that very soon after a 1 per cent. addition of sulphur to this diet, the animals began to suffer from anorexia and subsequent loss of appetite. However, it was eventually observed that the

TABLE VI.

Group I.

Distribution of Rat Urinary Sulphur on Rations Low and High in Sulphur Content.

Total gain or loss in weight per period. gm.	Day.	Total food consumed per day. gm.	Total N. mg.	Total S. mg.	Total Sulphate. S. mg.	Inorganic Sulphate. S. mg.	Ethereal Sulphate. S. mg.	Organic. S. mg.
				Ration IV.				
+ 15.........	1	28·5	244·0	7·10	6·04	5·49	0·55	1·06
	2	28·5	270·3	6·93	6·22	3·90	2·32	0·71
	3	28·5	268·5	7·17	5·87	5·31	0·56	1·30
	4	28·5	274·9	6·40	5·14	4·26	0·88	1·26
Average for 4 days...........			264·4	6·90	5·82	4·74	1·08	1·08
	(4)			Ration IV + 1% Sulphur.				
− 15.........	5	28·5	325·3	29·34	27·93	27·54	0·39	1·41
	6	28·5	347·8	44·46	42·47	40·81	1·66	1·99
	7	28·5	373·9	57·35	56·79	56·27	0·52	0·56
	8	28·5	324·0	57·20	55·43	54·88	0·55	1·77
	9	28·5	332·1	57·69	56·15	55·33	0·82	1·54
Average for last 3 days.......			343·3	57·41	56·12	55·49	0·63	1·29
	(9)			Ration IV.				
+ 13.........	10	28·5	351·9	42·57	40·47	39·91	0·56	2·10
	11	28·5	229·3	13·19	12·86	11·75	1·11	0·33
	12	28·5	339·0	13·52	12·30	10·75	1·55	1·22
	13	28·5	341·6	13·34	12·24	10·11	2·13	1·10
	14	28·5	289·8	11·35	10·58	9·47	1·11	0·77
	15	28·5	—	—	—	—	—	—
	16	28·5	354·0	10·31	8·80	7·87	0·93	1·51
	17	28·5	292·9	9·65	8·58	7·47	1·11	1·07
Average for last 3 determinations			312·2	10·44	9·32	8·27	1·05	1·12

addition of an alcoholic yeast extract (vitamin B_1) equivalent to 5 gm. yeast per 100 gm. ration and prepared according to the method of Guerrant and Dutcher (1932), the animals did not lose their appetite as readily and continued to eat about 9·5 gm. of the sulphur-ration per rat daily. Consequently Ration IV plus the yeast extract was used in the following experiment with and without the addition of 1 per cent. of sulphur, and the daily food consumption was kept constant throughout the entire experiment at 9·5 gm. food per rat daily.

The experiment was run in duplicate form with three adult albino male rats to a group kept together in a single metabolism cage. The urine was collected as previously described and immediately analysed for the constituents already mentioned. The sulphur fractions were determined by the method of Fiske (1921) and the total nitrogen by the direct Nesslerization method of Koch and McMeekin (1924).

The results obtained with Group I * are given in Table VI.

It is seen that the feeding of 1 per cent. of sulphur in the ration manifested its effects on the animal organism already on the first day of its administration when 60·9 mg. of nitrogen were excreted above the average for the pre-sulphur period. Furthermore, the effect of sulphur betrays itself right through the 8-day period since the sulphur was retracted from the diet and, curiously enough, even on the last day of observation when the animals had regained almost all of their weight lost during the sulphur period, the nitrogen excretion was still well above the average for the pre-sulphur one. Unfortunately it cannot very well be ascertained from the results as to whether the greater excretion of nitrogen during the sulphur and post-sulphur periods was due to a greater excretion of organic bases or to an increased excretion of endogenous nitrogen (creatinine) in view of the fact that only the total nitrogen in the urine was determined. However, judging from the excretion of organic sulphur in the urine, which in itself is an index of endogenous metabolism, it is clear that the increase in total nitrogen excretion during the sulphur and post-sulphur periods was in no way proportional to the increase in organic sulphur excretion and should, therefore, not be attributed to an increase in creatinine but to a greater elimination of some of the other nitrogenous compounds in the urine. This observation substantiates to a certain extent the observations of Cuthbertsen (1931) who found that the rise in the excretion of total nitrogen during conditions (human) of increased catabolism due to injury was mainly due to a proportionate increase in the amount of urea, whereas the creatinine (and neutral sulphur) showed little or no alteration in the absolute amount passed. Furthermore, when it is recalled that Lewis and Lewis (1927) observed a peripheral zonal necrosis in the livers of rats which died as a result of sulphur toxicosis, it is most likely that the rise in total nitrogen excretion in the urine of rats during the sulphur and post-sulphur periods of the present experiment was due to an enzymic solution of necrotic tissue, and a large part of its nitrogen was probably excreted

* The results obtained with Group II ran parellel with those of Group I and are therefore omitted for the sake of space.

as ammonium salts of the acids formed from the oxidation of hydrogen sulphide to sulphate in the animal body [Gamble, Ross, and Tindall (1923), Sherman and Gettler (1912), and Shingu (1932)].

With regard to the excretion of sulphur, it is evident that 24 hours after the rats were put on the sulphur ration, the total sulphur in the urine was already more than 4 times as high as compared with that on the basal diet, and that by the time of the third day on the sulphur ration, the total sulphur had reached a more or less constant level. Likewise the total and inorganic sulphate values run parallel to that of the total sulphur. As a matter of fact the increase in total sulphur and sulphate sulphur was practically due to the increase in inorganic sulphur alone in view of the fact that the ethereal sulphate and organic sulphur did not undergo any appreciable changes during the different periods.

The absorption of elementary sulphur, calculated as previously described and by taking the average total urinary sulphur excretion of only the last three days on the sulphur ration, was found to be 20·92 per cent. The reason for the rather low absorption of sulphur on this ration was propably again the presence of a large amount of easily available " food-sulphur " and, therefore, as will be expected, is in close agreement with that obtained on sulphur-ration II (Table I).

During the first two days after the retraction of sulphur in the ration, the inorganic sulphate (also the total- and sulphate-sulphur) dropped very markedly to a level still about 2·5 times as high as that during the pre-sulphur period. From here the inorganic sulphate returned very slowly to the normal level and 8 days after the discontinuation of sulphur feeding the inorganic sulphate was still 1·6 times as high as that during the pre-sulphur one. Evidently then the elimination of sulphur (sulphate) from the animal organism takes place at a very slow rate.

However, it was pointed out already in this article that a 3-day transition period was not long enough to clear the digestive tract of the sulphur of the previous ration, and it might be, therefore, that the slow urinary excretion of sulphur during the post-sulphur period was partially due to the absorption of sulphur still retained in the digestive tract. In order, therefore, to gain more information on this point, the distribution of sulphur in the faeces of rats on rations low and high in sulphur content was studied in greater detail.

Thirty adult rats were kept in large cages with screen bottoms and fed Ration IV (Table I), with and without 1 per cent. of sulphur, and distilled water *ad libitum*. The faeces were collected on filter paper and only those that were uncontaminated with urine were used for analysis. They were analysed for the following constituents: total S, sulphate S, combined H_2S-S, free H_2S-S and " rest "-S. The latter value was obtained from the difference between total sulphur and the sum of the other three constituents. The analyses were immediately carried out on the fresh material and the methods of determination were the same as described previously. The combined H_2S-S was determined in the same way as the free H_2S-S except that the faeces were diluted with 25 per cent. Hcl before distillation instead of with distilled water. The days on which the faeces were collected for analyses, were the 5th, 5th and 3rd of the pre-sulphur, (1) sulphur and post-sulphur (2) periods respectively.

The results are given in Table VII.

TABLE VII.

Distribution of Rat Faeces–Sulphur on Rations Low and High in Sulphur Content in terms of mg. Sulphur per 100 *gm. Dry Faeces.*

Moisture per cent.	Total S.	Sulphate S.	Combined H_2S–S.	Free H_2S–S.	" Rest " S.
		PRE-SULPHUR PERIOD.			
24·80	411·8	114·7	34·9	7·8	254·4
		SULPHUR PERIOD.			
13·16	6316·3	170·3	75·2	64·3	6006·5
		POST-SULPHUR PERIOD.			
17·71	822·1	127·8	36·2	8·1	650·0

It will be seen that all of the constituents in the faeces of the sulphur period were much higher than those of the pre-sulphur one. The " rest "-sulphur of the sulphur period was 23·6 times as much as compared with that of the pre-sulphur one and it is evident, therefore, that a tremendous amount of the elementary sulphur has passed through the alimentary canal without being affected by the intestinal flora. Furthermore, 3 days after the discontinuation of sulphur feeding (post-sulphur period) the total and " rest " sulphur values were still about twice as high as those of the pre-sulphur one, indicating that at that time a large amount of sulphur was still present in the digestive tract. Evidently then, the sulphur must have clung or been imbedded in the intestinal mucosa because it is well known that much less time is required by the rat to clear its digestive tract of the residues of the previous ration. As a matter of fact, Falcon-Lesses (1930) has found with rats that the beginning and end of different feeding periods, as determined by carmine, usually took from 18 to 24 hours for appearance, and it must be concluded, therefore, that the slow excretion of sulphur in the urine was partially due to the slow evacuation in the digestive tract.

(1) In this case no transition periods were allowed and by, for instance, the 3rd day of the post-sulphur period is meant the 3rd day after the discontinuation of sulphur-feeding.

(2) Because the sheep is a more suitable animal for a study of this nature, a more extensive account of the excretion of sulphur in the faeces is given in table XI.

Sheep Experiment.

Absorption and Excretion of Sulphur.

It was deemed necessary, for the sake of comparison, also to investigate the absorption and excretion of sulphur in sheep, in view of the fact that there are so many anatomical and physiological differences between the digestive systems of omniverous and herbiverous species.

Two 6-tooth Merino wethers, D.O.B. Nos. 32841 and 32871, were used in this experiment. They were put in metabolism cages and fitted with harnasses which, except for a few alterations, were constructed according to the descriptions of Ross, Bosman, *et al* (1927). The daily basal ration per sheep consisted of 200 gm. cut up lucerne hay, 400 gm. crushed yellow maize and 4 gm. common salt. This ration supplied, according to Armsby's (1922) figures, enough energy for maintenance. During the first transition and sulphur periods (Table VIII) each sheep also received daily 5 gm. of sulphur which was mixed with the yellow maize. The animals were only fed in the mornings and in all of the periods there was no refused feed at any time. Fresh distilled water was at all times available to the animals. At the beginning and end of the experiment sheep 32841 weighed 79·5 lb. and 79·5 lb., and sheep 32871 68·5 lb. and 73·5 lb. respectively. Before the experiment was started, about 150 lb. of lucerne hay was thoroughly mixed and put aside for this experiment. Likewise about 300 lb. of crushed yellow maize was mixed and stored. Approximately 500 gm. of each were ground up separately and kept in Mason jars for analysis.

The faeces and urine were collected daily but discarded for the first 12 days. The faeces were dried immediately in a hot air oven kept at approximately 70° C., mixed and weighed at the end of each period and about 50 gm. kept for analysis. The faeces were analysed for total sulphur and nitrogen. The sulphur was determined as previously described and the nitrogen by the Kjeldahl method. The urine was measured and analysed at once for total sulphur and its various fractions, as previously described, and for total nitrogen and creatinine. For the determination of total nitrogen 5 c.c. of urine were accurately measured out into 25 c.c. stoppered bottles and 10 c.c. of concentrated sulphuric acid added. The bottles were then stored at room temperature until the end of the experiment when their contents were emptied into 300 c.c. Kjeldahl flasks and the bottles rinsed out twice with 5 c.c. portions of strong H_2SO_4. The rest of the nitrogen determination was then carried out as described by Hawk and Bergeim (1931). The creatinine was determined according to the method of Folin (1914) with the use of a pure creatinine standard containing 1 mg. of creatinine per cubic centimeter.

The results [1] obtained with sheep 32841 are presented in Tables VIII, IX, and X.

---

[1] The data obtained with sheep 32871 ran parallel to those of sheep 32841 and were, therefore, omitted for the sake of space.

TABLE VIII.

Record of Nitrogen Balance of Sheep 32841 on Rations Low and High in Sulphur Content.

Period	Pre-Sulphur.	Transition.	Sulphur.	Transition.	Post-Sulphur.
Duration (days)	12	7	14	7	13
Total nitrogen intake (gm)	155·54	90·73	181·47	90·73	168·51
Nitrogen output (gm)—					
(*a*) in faeces	27·67	18·06	34·01	15·48	25·89
(*b*) in urine	107·16	64·64	124·52	63·58	124·39
Total nitrogen output (gm)	134·83	82·70	158·53	79·06	150·28
Part % of total N output in urine	79·48	78·16	78·54	80·42	82·77
Nitrogen balance per day (gm)	+ 1·73	+ 1·15	+ 1·64	+ 1·67	+ 1·40

TABLE IX.

Record of Sulphur Balance of Sheep 32841 on Rations Low and High in Sulphur Content.

Period	Pre-Sulphur.	Transition.	Sulphur.	Transition.	Post-Sulphur.
Duration (days)	12	7	14	7	13
Total sulphur intake (gm)	14·568	41·433	82·866	8·498	15·782
Dried faeces (gm)	1068·0	688·0	1348·0	632·0	1046·0
Volume of urine (L)	15 961	9·990	24·438	16·290	23·565
Sulphur output (gm)—					
(*a*) in faeces (dry)	4·592	18·232	47·719	9·290	5·491
(*b*) in urine	5·204	10·010	24·604	4·542	6·422
Total sulphur output (gm)	9·796	28·242	72·323	13·832	11·913
Part as % of total S output in urine	53·11	35·45	34·02	32·84	53·92
Part as % of total S intake in urine	35·72	24·16	29·69	53·44	40·69
Balance (stored and lost) per day (gm)	+ 0·3926	+ 1·8844	+ 0·7531	− 0·762	+ 0·2961

TABLE X.

Distribution of Sheep 32841 Urinary Sulphur on Rations Low and High in Sulphur Content.

Day.	Total N.	Creatinine.	Total S.	Total Sulphate S.	Inorganic Sulphate S.	Ethereal Sulphate S.	Organic S.
	gm.	gm.	mg.	mg.	mg.	mg.	mg.
			Period I. (Basal Ration.)				
1	9·98	2·095	465·8	456·0	184·4	271·6	9·8
2	9·33	2·195	413·7	395·2	169·8	225·4	18·5
3	8·87	1·979	450·7	416·5	191·1	225·4	34·2
6	8·62	1·943	465·4	427·9	205·1	222·8	37·5
7	8·87	1·918	388·4	380·9	179·3	201·6	7·5
9	9·04	2·680	458·6	404·8	178·0	226·8	53·8
10	9·23	2·244	427·5	404·2	190·5	213·7	23·3
Average for 7 determinations	9·14	2·150	438·6	412·2	185·4	226·8	26·4
12			Period II. (Basalt Ration + 5 gm. Sulphur daily.)				
13	9·71	2·064	843·1	774·7	546·9	227·8	68·4
14	8·20	1·714	1371·0	1334·0	1129·0	205·0	37·0
15	9·90	2·002	1472·0	1416·0	1234·0	182·0	56·0
16	10·86	1·831	1522·0	1482·0	1235·0	247·0	40·0
17	8·35	1·995	1242·0	1208·0	1037·0	171·0	34·0
21	9·86	1·780	1787·0	1634·0	1407·0	227·0	153·0
22	9·32	1·916	1797·0	1680·0	1509·0	171·0	117·0
28	9·48	2·051	1555·0	1490·0	1320·0	170·0	65·0
29	8·69	2·101	1596·0	1562·0	1353·0	209·0	34·0
31	9·37	2·278	1852·0	1793·0	1508·0	285·0	59·0
Average for last 4 determinations.	9·21	2·086	1700·0	1631·0	1422·5	208·5	69·0
33			Period III. (Basal Ration.)				
34	9·61	2·196	1237·0	1100·0	876·4	223·6	137·0
35	9·28	2·081	774·5	606·3	356·7	249·6	168·2
36	8·72	1·910	540·4	511·9	248·9	263·0	28·5
37	9·21	2·218	522·6	494·6	261·3	233·3	28·0
38	9·06	2·018	473·6	461·8	194·1	267·7	11·8
42	9·80	1·638	474·1	444·4	192·6	251·8	29·7
43	9·31	2·663	572·7	502·6	260·9	241·7	70·1
45	10·41	1·794	565·6	511·1	238·5	272·6	54·5
49	8·53	1·494	421·0	387·0	167·2	219·8	34·0
50	10·43	1·921	549·9	426·2	137·5	288·7	123·7
51	9·05	1·627	367·7	342·4	139·5	202·9	25·3
52	9·61	1·785	438·4	413·8	167·1	246·7	24·6
Average for last 4 days	9·40	1·707	444·2	392·3	152·8	239·5	51·9

It will be seen from Table VIII that the feeding of 5 gm. of sulphur per sheep daily had no effect on the daily nitrogen balance and the percentage of the total nitrogen output in the urine. The former varied from 1·15 mg. to 1·73 mg. with an average value of 1·52 mg. for the five periods, and the latter varied from 78·16 to 82·77 per cent. with an average value of 79·87 per cent. Evidently then the administration of 5 gm. of sulphur per sheep daily had, under the experimental conditions, no deleterious effect on the animals.

From Table IX it is clear that the greatest positive balance of sulphur (stored, and lost as H_2S) was during the first 7 days on the sulphur ration, probably due to the saturation of the tissues with hydrogen sulphide and sulphates whereas an actual negative balance was observed during the first 7 days after the retraction of sulphur from the ration due, of course, to the large excretion of sulphur stored in the tissues and retained in the digestive tract. This phenomenon is further stressed by the fact that the percentages of the total sulphur intake, excreted in the urine, were 24·16 for the transition period previous and 53·44 for the one after the sulphur period, being also the lowest and highest values for all of the five periods.

The " Stored " sulphur was excreted very slowly because the average daily excretion of sulphur in the faeces during the pre-sulphur and post-sulphur periods was 382·7 mg. and 422·4 mg., and the average daily excretion of total sulphur in the urine was 433·7 mg. and 494·0 mg. during the respective periods. Furthermore, the percentages of the total sulphur intake, excreted in the urine, was 35·72 and 40·69 during the pre- and post-sulphur periods, respectively, whereas, as would be expected, only 29·69 per cent. was excreted during the sulphur period.

The absorption of elementary sulphur during the sulphur period, calculated as previously described, was 28·13 per cent. which is somewhat higher than the values obtained with rats on mixed diets containing casein- as well as cereal-sulphur.

From the data presented in Table X it might be concluded that the daily administration of 5 gm. of sulphur per sheep had no effect on the total nitrogen, creatinine and ethereal sulphate excretion in the urine. Likewise, except for a few days of high organic sulphur excretion during periods II and III (which were not observed in the case of sheep 32871) the feeding of sulphur also seemed to have had no effect on the excretion of this fraction. It is clear, therefore, that, as with rats, the feeding of sulphur resulted chiefly in an increase in the inorganic sulphate-sulphur in the urine thus substantiating the observation of Denis and Reed (1927). It will be seen that the inorganic sulphate-sulphur fraction during the sulphur period (average of the last four determinations of period II) is 7·7 times as high as compared with that (average) of period I (basal ration) and, furthermore, that it took at least 11 days, after the discontinuation of sulphur feeding, for the inorganic sulphate-sulphur to return to its normal level.

TABLE XI.

Distribution of Sheep 32841 Faeces-sulphur on Rations Low and High in Sulphur Content.

Day.	Moisture per cent.	Faeces dry. gm.	Total S. mg.	Total S. mg. %	Sulphate S. mg.	Sulphate S. mg. %	Combined H_2S—S mg.	Combined H_2S—S mg. %	Free H_2S S. mg.	Free H_2S S. mg. %	"Rest" S. mg.	"Rest" S. mg. %
					PERIOD I. (Basal Ration.)							
1	28·87	89	349·7	392·9	30·9	34·7	44·8	50·3	1·7	1·9	272·3	305·9
2	31·23	80	334·3	417·8	33·3	41·6	35·6	44·5	3·9	4·9	261·5	326·8
3	31·71	83	343·4	413·7	28·6	34·5	37·2	44·8	4·8	5·8	272·8	328·6
TOTALS.	....	253	1027·4	407·7	92·8	36·8	117·6	46·7	10·4	4·1	806·6	320·1
4					PERIOD II. (Basal Ration + 5 gm. Sulphur daily.)							
10	44·54	90	3694·3	4105	50·9	56·5	65·1	72·4	51·1	56·8	3527·2	3919·3
11	35·85	93	3859·3	4150	46·8	50·3	55·7	59·9	42·6	45·8	3714·2	3994·0
13	34·30	98	4040·8	4124	48·4	49·4	78·9	80·5	58·1	59·3	3855·4	3934·8
15	38·37	94	3851·3	4098	57·6	61·3	54·0	57·5	54·6	58·1	3685·1	3921·1
TOTALS.	....	375	15445·7	4118·8	203·7	54·3	253·7	67·6	206·4	55·0	14781·9	3941·9
(15)					PERIOD III. (Basal Ration.)							
22	35·38	87	412·5	474·2	40·8	46·9	34·3	39·4	10·6	12·2	326·8	375·7
26	30·61	84	375·6	447·1	29·1	34·6	29·0	34·5	9·4	11·2	308·1	366·8
29	29·89	90	336·7	374·1	32·0	35·6	23·4	26·0	1·0	1·1	280·3	311·4
33	36·80	85	300·7	353·8	36·1	42·5	24·7	29·1	1·5	1·8	238·4	280·4
36	29·56	98	307·9	314·2	39·5	40·3	35·9	36·6	1·2	1·2	231·3	236·1
37	29·35	83	245·4	295·6	34·2	41·2	29·2	35·3	1·4	1·7	180·6	217·5
TOTALS.	....	527	1978·8	375·5	211·7	40·2	176·5	33·5	25·1	4·8	1565·5	297·0

In order to see to what extent the retention of sulphur in the digestive tract was responsible for the slow excretion of sulphur in the urine the experiment was repeated with sheep 32841 on the same ration and the faeces of only certain days collected. These were immediately analysed for total sulphur, sulphate sulphur, etc., as previously described. The results are given in Table XI.

It will be observed that all of the different sulphur fractions in the faeces during the sulphur period (period II) were much higher than those during the pre-sulphur one (period I). The average total and " rest " sulphur per 100 gm. dry faeces during the sulphur period were 10·1 and 12·3 times as great as compared with those during the pre-sulphur one, and it is evident, therefore, that a large amount of the sulphur must have passed through the digestive tract without being affected by the intestinal flora. Furthermore, as was found with the urine, it took most of the constituents about 11 to 12 days, after the discontinuation of sulphur feeding, to return to their normal level and it is clear, therefore, that the slow excretion of sulphur in the urine must have been partially due to the absorption of retained sulphur before all of it was swept out with the food residues.

With regard to the absorption of sulphates, Andrews and Johnston (1933) and Nakashima (1924) have shown that they are absorbed with difficulty, and the results presented in this article showed consistently that they are also excreted very slowly from the animal organism thus substantiating the observations of Dakin (1913), Denis and Hobson 1923), and Bialaszewicz (1931). Why this should be the case is not at all clear yet. Cope (1932) in his review of the literature with regard to the mechanism of inorganic sulphate excretion pointed out that the weight of opinion seems to be that sulphates are excreted mainly by the glomeruli and possibly also slighlty by the tubules of the kidney. However, whether this is the case and what bearing it has on the slow excretion of sulphates, are questions which can be answered only by further work.

DISCUSSION.

Evidence to date indicates that elementary sulphur can be transformed in the digestive tract into hydrogen sulphide in which form it can be absorbed. After having been absorbed it is subsequently oxidised to sulphate in the tissues and excreted as such in the urine.

Consequently it is difficult to see why the feeding of sulphur to sheep should be of any nutritional value in view of the fact that Henrici (1932), Askew and Bishop (1932) and Woodman and Evans (1933) have shown, respectively, that Karo bushes, New Zealand pastures, and feeding stuffs in general are fairly rich in sulphates and total sulphur. However, this does not seem to be universally the case. It was reported that the sulphur content of pastures in Coimbatore (1932-33), which ran parallel with that of calcium, was at all times of the year very low, and Aston and Lyons (1932) drew attention to the fact that the vegetation in certain mountainous regions in New Zealand is very low in sulphur and that sulphur-feeding might, therefore, be beneficial under such conditions. The form(s) in which inorganic sulphur may be beneficially utilised under those conditions remains to be seen. It may be that the inorganic sulphur is transformed through the symbiotic action of the intestinal bacteria, protozoa, etc., into organic sulphur compounds which in turn can be utilized by sheep in the synthesis of body tissues and wool-keratin as suggested by Rimington and Bekker (1932). However, if that is the case, one will expect that the soluble hydrogen sulphide (and sulphates), derived from elementary sulphur, will form better nutrients for the micro-organisms than the insoluble element. Furthermore, the observations of Sugata and Koch (1926) that hydrogen sulphide and sulphate sulphur can actually be converted into yeast protein and probably at least in part into cystine, and of Warth (1932) that part of the sulphates fed to cattle was transformed into some other form and not lost as H_2S, lend support in a way to the hypothesis of Rimington and Bekker.

On the other hand, it may be quite possible that elementary sulphur, under certain conditions at least, has a tonic effect on animals (sheep) and so indirectly influences their growth and well-being. Furthermore, when it is recalled that there is evidence to show that such unrelated substances to keratin structure as iodine (Veghelyi, 1932), iron (Cunningham, 1932, King, 1933), zinc (Todd and Elvehjem, 1934) and even lecithin (Rewald, 1933) have some influence on wool- and hair-growth, it may be that elementary sulphur also belongs to this apparent group of wool and hair growth-stimulating factors.

However, whatever the case may be, the effect of sulphur, if any, on growth and wool production of sheep remains still to be solved. This can only be done by careful and diligent experimentation, and it is rather unfortunate that several of the investigators who studied the effect of sulphur on sheep had planned and controlled their experiments so unsatisfactorily otherwise their results might have been less conflicting and more convincing. The experiment of Steyn (1931, 1932, 1934), for instance, "was planned to determine the toxic dose and not the nutritional value of sulphur", but on obtaining what appeared to be marked increases in body weight, Steyn was inclined to conclude that the comparative increases in the weights of the groups were significant whereas actually his results were only suggestive and not conclusive.

Larger groups genetically and nutritionally as homogenous as possible (efficiency quotient: Palmer and Kennedy, 1931; Morris Palmer and Kennedy, 1933) together with data relating to food consumption and the composition of the basal ration, apart from considering the advantages of individual feeding of the animals (Dunlop, 1933), are necessary to derive at more exact conclusions with regard to the effect of sulphur on body weight and wool growth of sheep.

SUMMARY AND CONCLUSIONS.

1. Experiments have been conducted with rats and sheep to determine the absorption and excretion of flowers of sulphur, and it was found that both processes took place at a relatively slow rate.

2. The steps in the metabolism of sulphur were its reduction in the digestive tract to hydrogen sulphide in which form it was absorbed. The sulphide absorbed as such was subsequently oxidised to sulphate and excreted in the urine as inorganic sulphates.

3. The excretion of the inorganic sulphates took place very slowly, because 8 days after the discontinuation of sulphur-feeding (Ration IV, Table I, containing 1 per cent. of elementary sulphur) the inorganic sulphate in the urine of rats was still 1·6 times as high as compared with that during the pre-sulphur period. In the case of sheep, it took 11 to 12 days, after the retraction of sulphur (5 gm. daily per animal) from the ration, for the inorganic sulphate to return to its normal level.

4. The slow excretion of sulphates in the urine was not only due to the storage of sulphur in the animal body but in part also to the long retention of sulphur in the digestive tract whence some was still absorbed before all of it was swept out of the digestive tract with the food residues.

5. The rise in the excretion of total sulphur in the urine during the sulphur period above that during the pre-sulphur one, with due allowance for a transition period, served as an index of the absorption of elementary sulphur, and when based on this assumption, the following absorption values were obtained:—24·6, 44·6 and 20·9 per cent. by rats on mixed Rations II, III and IV (Table I) containing 0·5 per cent., 1 per cent. and 1 per cent. of added flowers of sulphur, respectively. It will be seen that when the basal ration was high in easily available " food-sulphur " (Rations II and IV) the absorption of elementary sulphur was low, whereas when the absorption of " food-sulphur " was low (Ration III) that of the elementary sulphur was high.

6. Apparently the level at which the administration of elementary sulphur will commence to show toxic effects, will be controlled by the concentration of easily available " food-sulphur " in the basal ration. The larger the concentration of " food-sulphur " the higher will be the toxic level of elementary sulphur, and *vice versa*.

7. The absorption of elementary sulphur, as determined by the Bergeim method on a protein-free diet (Ration I, Table I) containing 1 per cent. of sulphur, was found to be about 38 per cent. This slightly lower value, as compared with that obtained on the low protein Ration III, containing 1 per cent. of elementary sulphur, might probably be explained on the grounds of the incompleteness of the protein-free ration from a nutritional standpoint, and the subsequent marked state of constitutional disorder.

8. The absorption of elementary sulphur by sheep when ingested with the basal ration (200 gm. cut up lucerne hay, 400 gm. crushed yellow maize and 4 gm. NaCl), to the extent of 5 gm. per animal per day, was found to be 28·13 per cent.

9. At this level the feeding of sulphur had no deleterious effect on sheep, whereas under the conditions of the rat experiments, the addition of 1 per cent. of sulphur to the basal rations showed definite toxic symptoms within a relatively short period of time.

10. The symptoms were anorexia and subsequent cachexia during the sulphur periods, and furthermore, an impairment of the normal physiological functions of the urinary excretory system.

11. The physiological principles underlying the metabolism of elementary sulphur would seem to be similar in rats and sheep.

BIBLIOGRAPHY.

ANDREWS, J. C., AND JOHNSTON, C. G. (1933). The Absorption of certain Sulphur Compounds from Intestinal Loops of Dogs. *J. Biol. Chem.*, Vol. 101, pp. 635-640.

ARMSBY, H. P. (1922). The Nutrition of Farm Animals, p. 713. New York: The Macmillan Company.

ARNOLDI, W., AND KUCERA, U. V. (1931). Vergleichende Untersuchungen über die Wirking einer Schwefel- einer Sulfat- und einer alkalischen Quelle auf den Glykogen- und Fettgehalt der Leber. *Zeitsch. f. ges. exp. Med.*, Vol. 79, pp. 311-318.

ASKEW, H. O., AND BISHOP, L. (1932). Influence of Season on the Inorganic and Organic Sulphur Contents of Perennial Rye-grass, White Clover, and Mixed Pasture. *New Zealand J. Sci. and Technol.*, Vol. 14, pp. 23-32.

ASTON, B. C., AND LYONS, J. (1932). Stock Licks and their Composition. *New Zealand J. Agric.*, Vol. 44, pp. 305-308.

BERGEIM, O. (1926). Carbohydrates and Calcium and Phosphorus Absorption. *J. Biol. Chem.*, Vol. 70, pp. 35-45.

BERGEIM, O. (1926a). A Method for the Study of Absorption in Different Parts of the Gastro-intestinal Tract. *J. Biol. Chem.*, Vol. 70, pp. 47-50.

BERNHEIM, F., AND BERNHEIM, M. L. C. (1932). The Action of Colloidal Sulphur on Liver Oxidations. *J. Biol. Chem.*, Vol. 96, pp. 331-339.

BIALASZEWICZ, K. (1931). *Arch. internat. de Physiol.*, Vol. 35, pp. 98-124. *Nutr. Abst. and Reviews* (1933), Vol. 2, p. 893.

BURGI, E., AND GORDONOFF, T. (1926). *Klin. Woch.*, Vol. 5, p. 466.

BURMAN, C. E. L. (1932). Impressions of and Personal Observations on the Recent Epidemic of Malaria in Maritzburg. *S.A. Med. Jour.*, Vol. 6, pp. 591-594.

(COIMBATORE) (1932-33). Seasonal Variations in the Sulphur Content of Pastures. *Sci. Rep. Govt. Agric. Chem., Coimbatore*, p. 25. *Nutr. Abst. and Reviews* (1934), p. 674.

(COIMBATORE) (1932-33). Sulphur Feeding to Sheep for Improvement in Wool-production. *Sci. Rep. Govt. Agric. Chem. Coimbatore*, p. 26. *Nutr. Abst. and Reviews* (1934), p. 866.

CORI, C. F. (1925). The Rate of Absorption of Hexoses and Pentoses from the Intestinal Tract. *J. Biol. Chem.*, Vol. 66, pp. 691-715.

COPE, C. L. (1932). Inorganic Sulphate Excretion by the Human Kidney. *J. Physiol.*, Vol. 76, pp. 329-338.

CUNNINGHAM, I. J. (1932). Influence of Dietary Iron on Hair and Wool Growth. *New Zealand Jour. Agric.*, Vol. 44, pp. 335-337.

CUTHBERTSON, D. P. (1931). The Distribution of Nitrogen and Sulphur in the Urine during Conditions of Increased Catabolism. *Biochem. J.*, Vol. 25, pp. 236-44.

DAKIN, H. D. (1913). Studies on the Intermediary Metabolism of Amino-acids. *J. Biol. Chem.*, Vol. 14, pp. 321-333.

DANIELS, A. L., AND RICH, J. K. (1918). The Role of Inorganic Sulphates in Nutrition. *J. Biol. Chem.*, Vol. 36, pp. 27-32.

DENIS, W. (1910). The Determination of Total Sulphur in Urine. *J. Biol. Chem.*, Vol. 8, pp. 401-403.

DENIS, W., AND HOBSON, S. (1923). A Study of the Inorganic Constituents of the Blood Serum in Nephritis. *J. Biol. Chem.*, Vol. 55, pp. 183-190.

DENIS, W., AND REED, L. (1927). Concerning the Effect Produced by the Administration of Sulphur on the Concentration of certain Sulphur Compounds in Blood and Urine. *J. Biol. Chem.*, Vol. 73, pp. 51-57.

DUNLOP, G. (1933). Methods of Experimentation in Animal Nutrition. *J. Agric. Sci.*, Vol. 23, pp. 580-614.

DU TOIT, P. J., MALAN, A. I., GROENEWALD, J. W., AND BOTHA, M. L. (1934). The Effect of Different Forms of Sulphur in the Diet upon the Growth and Wool Production of Sheep. This Journal.

EVVARD, J. M., *et al.* (1925). *Iowa Agric. Exp. Sta. Leaflet*, No. 2.

EVVARD, J. M., *et al.* (1925a). *Ibid.*, Leaflet No. 4.

FALCON-LESSES, M. (1930). The Cause of the Laxative Action of Bran. *J. Nutr.*, Vol. 2, pp. 295-310.

FEIGL, F., AND SCHORR, R. (1923). *Zeitsch. Anal. Chem.*, Vol. 63, pp. 10-29. *J. Chem. Soc.* (1923), Vol. 124 A. ii, p. 784.

FISKE, C. H. (1921). The Determination of Inorganic Sulphate, Total Sulphate, and Total Sulphur in Urine by the Bezidine Method. *J. Biol. Chem.*, Vol. 47, pp. 59-68.

FOLDES, E. (1928). *Zeitsch. ges. exptl. Med.*, Vol. 60, p. 571.

FOLIN, O. (1914). On the Determination of Creatinine and Creatine in Urine. *J. Biol. Chem.*, Vol. 17, pp. 469-473.

GAMBLE, J. L., ROSS, G. S., AND TISDALL, F. F. (1923). The Effect of Calcium Chloride Ingestion on the Acid-base Metabolism of Infants. *Amer. J. Dis. Child.*, Vol. 25, p. 455.

GEILING, E. M. K. (1917). The Nutritive Value of the Diamino-acids occurring in Proteins for the Maintenance of Adult Mice. *J. Biol. Chem.*, Vol. 31, pp. 173-199.

GUERRANT, N. B., AND DUTCHER, R. A. (1932). The Assay of Vitamins B and G as Influenced by Coprophagy. *J. Biol. Chem.*, Vol. 98, pp. 225-235.

HAWK, P. B., AND BERGEIM, O. (1931). Practical Physiological Chemistry, 10th. ed., pp. 814-15, Philadelphia: P. Blakiston's Son & Company.

HEFFTER, A., AND HAUSMANN, M. (1904). Über die Wirkung des Schwefels auf Eiweiszkörper *Beitr. Chem. Physiol. u. Path.*, Vol. 5, pp. 213-233.

HENRICI, M. (1932). Cystine and Sulphur Content of Bushes and Grasses in a Karroid Area (Fauresmith). *18th Rept. Dir. Vet. Ser. and Anim. Indust.*, Union of S.A., pp. 579-595.

Iowa Expt. Sta. Rept. (1924), pp. 38-39. *Expt. Sta. Rec.* (1925), Vol. 53, p. 768.

JOHNSTON, C. G. (1932). A Method for Making Quantitative Intestinal Studies. *Proc. Soc. Exp. Biol. and Med.*, Vol. 30, pp. 193-196.

KING, A. T. (1933). The Sulphur Economy of Animal Fibre Production. *Trans. Faraday Soc.*, Vol. 29, pp. 258-71.

KOCH, F. C., AND McMEEKIN, T. L. (1924). A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia. *J. Amer. Chem. Soc.*, Vol. 46, pp. 2066-2069.

KRÜGER, R. (1928). Ein Beitrag zur Behandlung chronischer Artritiden durch parenterale Verabreichung von Schwefel. *Tier. Rdschau.*, Vol 34, pp. 616-617.

KUBO, S. (1930). *Folia endocrinol. Japan*, Vol. 6, p. 65. *Chem. Abst.* (1932), Vol. 26, p. 3824.

KUBO, S. (1932). *Ibid.*, Vol. 7, p. 11. *Chem. Abstr.* (1932), Vol. 26, p. 5331.

KUBO, S. (1932a). *Ibid.*, Vol. 7, p. 189. *Chem. Abst.* (1933), Vol. 27, p. 5824.

LANG, K. (1933). Die Rhodanbildung im Tierkörper. *Biochem. Zeitsch.*, Vol. 259, pp. 243-56.

LANT-ECKL (1921). *Brennstoffchemie*, Vol. 2, p. 330.

LEWIS, G. T., AND LEWIS, H. B. (1927). The Effect of Elementary Sulphur on the Growth of the Young White Rat. *J. Biol. Chem.*, Vol. 74, pp. 515-523.

MENEGHETH, E. (1929). *Boll. soc. ital. biol. sper.*, Vol. 4, p. 133. *Chem. Abstr.* (1931), Vol. 23, p. 3746.

MONTGOMERY, W. M. (1932). Sulphur as a Prophylactic in Malaria. *S.A. Med. Jour.* Vol. 6, pp. 771-772.

MORRIS, H. P., PALMER, L. S., AND KENNEDY, C. (1933). An Experimental Study of Inheritance as a Factor Influencing Food Utilisation in the Rat. *Minn. Agric. Expt. Sta. Tech. Bull.*, No. 92, p. 56.

NAKAHSHIMA, K. (1924). Resorption of Sulphate from the Small Intestine. *J. Biochem. (Japan).*, Vol. 4, p. 277.

NICCOLINI, P. M. (1933). Ulteriori Indagini Sull' Azione Del Solfo Sul Ricambio Idrocarbonato, E Sul Meccanismo Della Medesima. *Arch. internat. de Pharmacolyn. et. de Therap.*, Vol. 45, pp. 54-88.

OCHMANN, D. (1928). Einige praktische Erfahrungen mit Sulfoliquid. *Berl. Tierarztl. Wschr.*, Vol. 4, p. 523.

OSBORNE, T. B., AND MENDEL, L. B. (1913). The Relation of Growth to the Chemical Constituents of the Diet. *J. Biol. Chem.*, Vol. 15, pp. 311-326.

PALMER, L. S., AND KENNEDY, C. (1931). The Influence of the Food Consumption and the Efficiency Quotient of the Animal. *J. Biol. Chem.*, Vol. 90, pp. 545-564.

PEIRCE, A. W. (1933). The Effect of the Body-weight and Wool Production of Merino Sheep of Adding Sulphur to the Diet. *J. Coun. Scientif. and Indust. Res.*, Vol. 6, pp. 294-298.

PHILIPS, A. G., CAR, R. H., AND KENNARD, D. C. (1921). Green Feed Versus Antiseptics as a Preventive of Intestinal Disorders of Growing Chicks. *J. Agr. Res.*, Vol. 20, pp. 869-873.

RAMAIYYA, P. V. (1932). Cystine Metabolism in Sheep. *Current Sc.*, Vol. 1, p. 12. *Biol. Abst.* (1933), Vol. 7, p. 1067.

REWALD, B. (1933). Lecithin a New Factor in Foodstuffs. *Food*, Vol. 2, pp. 149-150. *Nutr. Abst. and Reviews* (1933), Vol. 3, p. 185.

RIMINGTON, C., AND BEKKER, J. G. (1932). Is Preformed Cystine Essential to Sheep for Wool Production? *Nature*, Vol. 129, pp. 687-688.

ROSS, J. C., AND BOSMAN, A. M. (1927). Die Verteerbaarheid van Teffhooi vir Skape. *T.U.C. Bulletin*, No. 10, p. 25.

SANDBERG, M., AND HOLLY, O. M. (1932). On the Influence of Ethyl Isothiocyanate, Ethyl Thiocyanate, and Allyl Isothiocyanate on Sulphur Metabolism in Rabbits. *J. Biol. Chem.*, Vol. 97, pp. 31-35.

SEDDON, H. R., AND CHAMBERLIN, W. E. (1933). On the Effect of the Administration of Sulphur to Sheep. *Aust. Vet. J.*, Vol. 9, pp. 154-159.

SHEEBY AND SENIOR (1930). *J. Dept. Agric.* (*Irish Free State*), Vol. 30, p. 1.

SHERMAN, H. C., AND GETTLER, A. O. (1912). The Balance of Acid-forming and Base-forming Elements in Food, and its Relation to Ammonia Metabolism. *J. Biol. Chem.*, Vol. 11, pp. 323-338.

SHERMAN, H. C., AND MERRILL, A. T. (1925). Cystine in the Nutrition of the Growing Rat. *J. Biol. Chem.*, Vol. 63, pp. 331-337.

SHINGU, S. (1932). *Folio Endocrinol. Japan*, Vol. 8, pp. 7-8. *Chem. Abst.* (1933), Vol. 27, p. 5824.

SMITH, R. G., AND MALCOLM, R. L. (1930). Urinary Sulphur and Thiocyanate Excretion in Cyanide Poisoning. *J. Pharmacol.*, Vol. 40, pp. 457-71.

STEENBOCK, H., AND NELSON, E. M. (1923). Light in its Relation to Ophthalmia and Growth. *J. Biol. Chem.*, Vol. 56, pp. 355-373.

STEYN, D. G. (1929). Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa. 15*th Rept. Dir. Vet. Serv.*, Union of S.A., pp. 777-803.

STEYN, D. G. (1931). The Effects of Sulphur on Merino Sheep. 17*th Rept. Dir. Vet. Serv. and Anim. Indust.*, Union of S.A., pp. 481-492.

STEYN, D. G. (1932). The Effects of Sulphur on Merino Sheep and their Resistance to Potassium Cyanide Poisoning. 18*th Rept. Dir. Vet. Serv. and Anim. Indust.*, pp. 597-610.

STEYN, D. G. (1932). Experiments with Potassium Cyanide on Rabbits. *Ibid.*, pp. 939-945.

STEYN, D. G. (1934). The Effect of Sulphur on Merino Sheep and a Further Experiment to Determine the Safe Dose of Sulphur for Cattle. *Jour. S.A. Vet. Med. Assoc.*, Vol. 5, pp. 18-22.

SUGATA, H., AND KOCH, F. C. (1926). Sulphur Metabolism of Yeast. *Plant Physiol.*, Vol. 1, pp. 337-347.

TODD, W. R., ELVEHJEM, C.A., AND HART, E. B. (1934). Zinc in the Nutrition of the Rat. *Amer. J. Physiol.*, Vol. 107, pp. 146-156.

VEGHELYI, E. (1931). *Mezögazdasági Kutatasok*, Vol. 4, p. 349. *Chem. Abst.* (1932), Vol. 26, p. 2492.

WARTH, F. J. (1932). Sulphur and Sulphate Balance Experiments with Cattle. *Indian J. Vet. Sci. and Anim. Husb.*, Vol. 2, pp. 225-241.

WOODMAN, H. E., AND EVANS, R. E. (1933). The Determination of Total Sulphur and Sulphate Sulphur in Feeding Stuffs. *Jour. Agric. Sci.*, Vol. 23, pp. 459-462.