Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

Sulphur Metabolism.

II. The Distribution of Sulphur in the Tissues of Rats fed Rations with and without the addition of Elementary Sulphur.

By J. H. KELLERMANN, Section of Biochemistry and Nutrition, Onderstepoort.

THE injection and oral administration of elementary sulphur as a prophylactic in various diseases has been practised for many years and the popularity of this drug in medicine seems to increase of late rather than decrease. Yet, curiously enough, little was known about the extent to which this element is absorbed and stored in the animal body. However, a few investigators have studied the storage of some of its metabolic products (sulphide and sulphate) but, unfortunately, their results do not give a true picture of the metabolism of elementary sulphur as such. Nevertheless, their results have some bearing on those to be presented in this article and are, therefore, reviewed.

It was found at the Iowa Experimental Station (1924) that sulphur, when fed to ewes in daily doses of 0·5 oz., was retained in the animal body to a considerable extent, though there was evidence that some had been oxidised to sulphates and excreted in the urine. Support seems to be lent to this point of view by the author's work (1935) on the absorption and excretion of sulphur in rats and sheep. Denis and Reed (1927b) analysed the blood of dogs that had been fed a standard ration to which had been added powdered sulphur to the extent of 0·5 gm. per kilo. per day and found in some cases an increase in the non-protein sulphur compounds and in others a marked decrease. Tasaka and Nakazawa (1931) observed that the change in blood picture after subcutaneous injection of colloidal sulphur is similar to that after subcutaneous injection of insulin, only less marked. According to the work of Kubo (1931) the feeding of sulphur and injection of insulin decidedly increased both the total and neutral sulphur contents of most organs, especially those of the suprarenal capsules where the sulphate content was also raised. Meyer-Bisch and Techner (1931) found no changes in the hydrolyzable and total sulphur contents of the liver in rabbits that received sulphur parenterally.

Denis and Leche (1925b) in a series of experiments in which hypertonic solutions of sodium sulphate were administered to dogs by intravenous injections found by analysis of the blood, muscles, and viscera that there was little absorption of the sulphate iron by

the tissues, although even two hours after injection the sulphate content of the blood might still be ten times its initial value. Arnoldi, Liss and Rosam (1932) fed mineral water containing neutral sulphates to one and alkaline sulphates to another group of rats and found that the average values for total sulphur in the livers of the animals were higher for both the groups given sulphate waters than that of the control animals.

Neither Lehmann (1892) nor Meyer (1898) could find any hydrogen sulphide in the blood of dogs that had inhaled this gas over a long period of time. Denis and Reed (1927a) analysed the blood of dogs that had received sodium sulphide by intravenous injection, or by injection into the intestine and observed that there was sometimes a rise in the inorganic sulphate but no increase in the neutral sulphur fraction. These increased values were noted particularly in the case of animals in whom the kidneys had been tied off.

Taken as a whole the literature given above seems to show that sulphur is stored to a certain extent in the animal body and it was, therefore, the object of this investigation to study more fully the extent to which and the form and tissues in which sulphur is stored in the organism, which information, it was thought, might subsequently throw some light upon the cause of the toxic effect of the oral administration of flowers of sulphur as observed by Steyn (1931), Lewis and Lewis (1927) and Lawson, Redfield and Boyce (1934).

Experimental.

White rats were used in these experiments. The young rats used in the first experiment were the same animals that were employed in another investigation in which the growth of rats fed a modified Osborne-Mendel diet with and without added sulphur was studied. The composition of the rations and the experimental conditions are given in the third paper (Kellermann, 1936), of this series and will, therefore, not be described in detail again. For the sake of clarity, however, it can be mentioned that three groups of young rats were fed a modified Osborne and Mendel low protein ration with and without the addition of 0·8 per cent. of elementary sulphur. Groups II and III received the sulphur supplemented ration. In addition the animals received distilled water except those of group III that were given fresh orange juice *ad libitum* instead of water. On the last day of the experiment referred to above the rats were killed and the tissues removed for analysis. Two groups of 64 and 68 adult rats of about nine to ten months of age were used in the second experiment. They were raised on the stock ration used in this laboratory (Kellermann, 1934) and the only difference in the treatment of these groups was the substitution of three parts by weight of flowers of sulphur for the same amount of yellow maize meal in the ration of one of the groups for the last twenty-one days.

The rats were put under ether anaesthesia and then bled to death after severing one or both carotid arteries. The blood was collected in a beaker that contained lithium citrate as anticoagulant. After the pooled blood was strained through muslin in order to

remove any hairs, it was allowed to cool down to room temperature. The brain, femoral muscles and thoracic and abdominal viscera were removed, and great care was taken not to contaminate them with any hairs. The tissues to be analysed were then pooled and finely ground up.

Only the total sulphur determinations were made on the tissues of the rats (young) in the first experiment. They were made on the dry materials, after the tissues had been dried in an electric oven. The tissues of the rats (adult) in the second experiment were analysed for total sulphur, total sulphate and volatile sulphide. All the determinations were made on the fresh tissues, but for the sake of better comparison the results are expressed in milligrams sulphur per 100 gm. dry material. The moisture determinations were carried out in 35 c.c. weighing bottles kept for about twelve to fifteen hours in an electric oven which was run at 98° C. The total sulphur determinations were made according to the method of Feigl and Schorr (1923). When the determination was made on the fresh material the ground up tissue (2-3 gm.) was thoroughly mixed with the Na_2CO_3-$KMnO_4$ mixture and then dried over a steam bath before the rest of the method was proceeded with. The total sulphate sulphur determinations were made according to a modified method of Denis and Leche (1925a). Whenever enough material was available, 20 gm., instead of 10 gm., of fresh material were autoclaved, as described by Denis and Leche, with 200 c.c. of 25 per cent. Hcl (i.e. 1 vol. of concentrated Hcl made up to 4 vols. with distilled water) in a 400 c.c. Jena beaker. The contents of the beaker were then transferred quantitatively to a 500 c.c. volumetric flask by warm distilled water, cooled and the flask filled to the mark. The liquid, after thorough mixing was filtered through No. 588 of Carl Schleicher and Schull's filter paper. The sulphate in an aliquot (250 c.c.) of the filtrate was then precipitated by the addition of $Bacl_2$ solution and determined further according to the gravimetric method as $BaSo_4$. The volatile sulphide was also determined gravimetrically as $BaSO_4$ as described by Heffter and Hausmann (1904). The results obtained with the young rats are given in Tables I, II and III.

TABLE I.

Distribution of Total Sulphur in Tissues of Young Rats fed Modified Osborne-Mendel Ration for Sixty Days.

(Five animals, three months of age when killed, were used.)

Tissue.	Moisture.	Sulphur in Dry Matter.
	Per Cent.	Mgm. Per Cent.
Brain	77·9	521·1
Lung	77·5	**593·6**
Liver	71·6	582·2
Spleen	75·1	**647·1**
Heart	77·3	856·5
Kidney	74·7	726·4
Muscle	72·3	680·1
Blood	81·6	878·0

TABLE II.

Distribution of Sulphur in Tissues of Young Rats Receiving Elementary Sulphur in Food. (Modified Osborne-Mendel Ration Containing 0·8 per cent. Flowers of Sulphur for Last Sixty Days.

(Five animals, three months of age when killed, were used.)

Tissue.	Moisture.	Total Sulphur in Dry Matter.
	Per Cent.	Mgm. Per Cent.
Brain	77·5	532·6
Lung	76·9	**763·8**
Liver	69·6	563·9
Spleen	73·9	**796·9**
Heart	76·5	925·5
Kidney	74·3	797·9
Muscle	73·3	673·9
Blood	80·7	887·0

TABLE III.

Distribution of Sulphur in Tissues of Young Rats Receiving Elementary Sulphur in Food. (Modified Osborne-Mendel Ration Containing 0·8 per cent. Flowers of Sulphur Plus Orange juice ad libitum *for last Sixty Days.)*

(Five animals, three months of age when killed, were used.)

Tissue.	Moisture.	Total Sulphur in Dry Matter.
	Per Cent.	Mgm. Per(Cent.
Brain	77·5	525·9
Lung	73·6	**644·2**
Liver	72·3	551·6
Spleen	63·5	**747·3**
Heart	72·7	832·4
Kidney	70·8	748·7
Muscle	71·0	665·6
Blood	81·3	804·0

It will be seen that in the first group without the addition of elementary sulphur the blood and heart contained the largest quantity of sulphur. Next in order came the kidney, then the muscle and spleen, and lastly the lung, liver and brain. Comparison of the data in Tables II and III with those in Table I shows that sulphur feeding increased especially the total sulphur content of the lung and spleen, and also to a certain extent that of the heart and kidney in group II. The lungs and spleen of the sulphur fed animals in group II contained respectively 170·2 and 149·8 mgm. and in group III 50·6 and 100·2 mgm. more sulphur per 100 gm. dry material

than the same tissues taken from animals fed the basal ration alone. The hearts and kidneys of the animals in group II only contained 69·0 and 71·5 mgm. per cent. more sulphur than the respective organs of the control group. On the other hand the livers and muscle of the sulphur fed animals contained less sulphur than the same tissues from the control animals. However, these reduced values are so small that they are of no significance. Furthermore, it should be mentioned that on the whole, less sulphur was stored in the tissues of group III than in those of group II. It may be that the extra basic elements introduced with the orange juice caused a faster and more complete excretion of the oxidised sulphur in the urine in view of the fact that Moraczewoki and Sliwinski (1934) have found that the elimination of both thiocyanates and sulphates is rapid on an alkaline diet but slow and somewhat prolonged on an acid one. Unfortunately, the data available are too few and the differences in the amounts of sulphur stored in the various tissues of rats fed sulphur rations with and without orange juice are not marked enough to warrant any definite conclusions and further work is necessary in order to elucidate this point.

It should also be pointed out that the accuracy of the results presented in Tables I to III must be accepted with some reservation in view of the fact that the tissues were first dried before being analysed and it is possible that during the process of drying some of the sulphur escaped as volatile sulphide. In order to test out this point two groups each of five young rats were fed the modified Osborne and Mendel low protein ration, with and without the addition of 0·8 per cent. elementary sulphur, over a period of 60 days after which their tissues were analysed for volatile sulphide. The latter was tested for qualitatively on the various fresh tissues (pooled for each group) by steam distillation as described by Osborne (1928). Notwithstanding the fact that the animals were in a very poor condition, no volatile sulphide could be detected in any of the tissues by this method except in the liver where only a trace was present. Bearing the observations of Osborne in mind, it would seem, therefore, that fresh rat tissues and beef in poor condition, unlike the flesh of sheep and guinea-pigs in a similar condition, do not emit sulphide on boiling. The liver of the sulphur-fed animals emitted about three times as much sulphide as that of the control group, whereas only traces of sulphide were found in the kidney and muscle of the sulphur group. Furthermore, twenty-four hours after killing, the muscle of the control group did not yet emit any sulphide whereas that of the sulphur group showed about twice as much sulphide by that time as the fresh muscle from the same group. With the probable exception of the liver of the sulphur group, it would seem, therefore, that he sulphide lost through drying could not have been of such magnitude that it would have seriously affected the validity of the conclusions drawn.

As a matter of fact the results presented in Tables IV and V show that negligible amounts of H_2S were present in the fresh tissues of adult rats that received a stock diet with and without the addition of sulphur.

TABLE IV.

Distribution of Various Forms of Sulphur in Tissues of Adult Rats Receiving Stock Ration.

(Sixty-four animals, about nine to ten months of age, were used.)

Tissue.	Moisture.	Total S.	Sulphate S.	Hydrogen Sulphide S.	"Undetermined". S.
	Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.
Brain	77·1	751·5	77·87	1·03	672·60
Lung	75·2	**803·9**	30·45	trace	773·45
Liver	72·9	895·6	11·79	0·68	883·13
Spleen	75·5	**1110·0**	**11·21**	trace	1098·79
Heart	77·2	**1364·0**	9·63	,,	1354·37
Kidney	75·8	1062·0	**30·12**	,,	1031·88
Muscle	70·8	933·3	4·24	,,	929·06
Blood	79·2	876·8	10·64	,,	866·16

TABLE V.

Distribution of Various Forms of Sulphur in Tissues of Adult Rats Receiving High Intake of Elementary Sulphur. (Stock Ration containing 3 per cent. of Flowers of Sulphur for last Twenty-one Days.

(Sixty-eight animals, about nine to ten months of age, were used.)

Tissue.	Moisture.	Total S.	Sulphate S.	Hydrogen Sulphide S.	"Undetermined" S.
	Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.	Mgm. Per Cent.
Brain	77·4	764·3	81·41	0·99	681·90
Lung	76·2	**861·8**	27·39	0·25	834·16
Liver	72·3	925·5	16·76	0·50	908·24
Spleen	75·7	**1171·0**	**19·77**	trace	1151·23
Heart	77·1	**1445·0**	15·02	,,	1429·98
Kidney	75·4	1088·0	**50·75**	1·36	1035·89
Muscle	72·4	958·1	7·47	0·30	950·33
Blood	78·6	883·9	18·91	0·36	864·63

A further study of these tables shows that the heart contained the largest quantity of total sulphur. Next in order came the spleen and kidney, then the muscle, liver, blood and lung with brain the lowest. Exactly the same order also holds true for the sulphur group. Comparison of the data in Table V with those in Table IV shows that sulphur feeding increased the total sulphur content of the heart, spleen and lung. These organs of the sulphur fed animals contained, respectively, 81·0, 61·0 and 57·9 mgm. more sulphur per 100 gm. dry matter than the same organs taken from animals raised on the

stock ration alone. This substantiates the data obtained with young rats (Tables I and II) fed the Osborne-Mendel ration with and without added sulphur. However, it should be pointed out that the total sulphur contents of the tissues of the adult rats fed the stock ration alone are all, except for th blood, perceivably higher than those of the same tissues of young rats fed the basal Osborne-Mendel ration. The grand average of the tissues of the eight organs for the adult and young rats were 974·6 and 685·6 mgm. per 100 gm. dry material, respectively. It should be borne in mind that the young rats were fed a low protein, cystine deficient diet that was also very low in sulphur content whereas the adult rats were raised on a normal stock ration, and one of the contributing factors for the low total sulphur content for the tissues of the young rats probably was that the tissues of the latter animals were consequently very low in non-protein sulphur.

The data of Kambayashi (1929) seem to support this theory His figures for the total sulphur, expressed as a percentage of the fresh material, are slightly lower than those of the author, calculated on the same basis, for adult rats. Unfortunately, he only determined the total sulphur in the femoral muscles and liver of male rats weighing 180 to 250 gm. For these tissues he found 242 and 222 mgm. per cent., respectively, whereas the author's figures are 188·4 and 165·3 mgm. for the respective tissues of the young and 272·5 and 250·8 mgm. for those of adult rats. From these figures it is obvious that his data, obtained by means of fusing the dry material with a mixture of KNO_3 and HNO_3 etc., are likewise appreciably higher than the results obtained by the author with the young rats, the cause of which, as mentioned before, should most probably be looked for in the low sulphur intake by these animals.

With regard to the total sulphate content of the tissues in the control group the brain contained by far the largest amount of this salt. Next in order came the lung and kidney, with approximately equal amounts, then the liver, spleen and blood also with approximately equal amounts whereas the heart and muscle contained the least. The high figure for kidney can, as mentioned by Denis and Leche (1925b) probably be explained on the basis of unavoidable inclusion of traces of urine, while it is possible that the high values for the brain and lung were due to the decomposition of some unstable sulphur containing lipoid. Comparison of the sulphate content of the kidney, spleen and blood. These tissues of the sulphur fed animals contained, respectively, 20·63, 8·55 and 8·27 mgm. more sulphate sulphur per 100 gm. dry material than the same organs from animals fed the stock ration alone.

With regard to the distribution of hydrogen sulphide, it is clear that the feeding of sulphur had no effect upon the concentration of this fraction in the tissues. This is to be expected in view of the fact that Haggard (1921) has shown that blood plasma possesses the property of oxidizing hydrogen sulphide rapidly by the withdrawal of oxygen from the blood corpuscles. Although he did not determine the products of oxidation, results obtained by the author (1936) seem to show that they are sulphates. This finding, together with the observation of Denis and Leche (1925b) that sulphates, injected into

the blood stream, are only slowly excreted, explain why the blood and most tissues of sulphur-fed rats contain more of this constituent than the tissues from control animals.

The " undetermined " sulphur values of the tissues of both the sulphur and control groups ran parallel, as will be expected, with the total sulphur contents, and comparison of the former values in Tables IV and V shows once more that feeding of elementary sulphur increased especially the sulphur content of the lung, spleen and heart.

Summary.

(1) The distribution of sulphur was determined in the tissues of three groups of young rats. One was fed a modified Osborne-Mendel low protein basal diet, one the basal diet supplemented with 0·8 per cent. of elementary sulphur, and the remaining one the same supplemented ration plus orange juice *ad libitum*. Sulphur feeding increased especially the total sulphur content of the lung and spleen. Next in order came the heart and kidney. The total sulphur content of the lung of the sulphur group was 170·2 and of the spleen 149·8 mgm. more per 100 gm. dry material than the content of the respective tissues taken from the control group. The average increase in the amount of sulphur in the heart and kidney was about half of the average increase in the lung and spleen. On the whole less sulphur was stored in the tissues of the sulphur group that received orange juice in addition, but the differences are too small to warrant any definite conclusions.

(2) The distribution of various forms of sulphur in the tissues of two groups of adult rats, one fed the stock ration, the other the stock ration supplemented with 3 per cent. elementary sulphur, was also determined. Sulphur feeding likewise increased the total sulphur content of the heart, spleen and lung but not so much of the kidney. The three former organs contained, respectively, 81·0, 61·0 and 57·9 mgm. more sulphur per 100 gm. dry material than the same organs taken from animals raised on the stock ration alone.

(3) Sulphur feeding had little effect upon the sulphate content of rat tissues, thus substantiating the observation of Denis and Leche (1925b). The only tissues in which the sulphate content was slightly increased were the kidney, spleen and blood. The respective tissues of the sulphur fed animals contained 20·63, 8·55 and 8·27 mgm. more sulphate sulphur per 100 gm. dry material than the same tissues taken from the control animals.

(4) There was found to be no difference in the concentration of volatile sulphide in the tissues of animals fed rations with and without the addition of flowers of sulphur.

BIBLIOGRAPHY.

ARNOLDI, W., LISS, G., AND ROSAM, L. (1932). Der Schwefelgehalt der Leber nach Zufuhr einer Sulfat sowei einer Alkaliquelle. *Ztschr. f. d. ges. exp. Med.*, Vol. 80, pp. 633-636.

DENIS, W., AND LECHE, S. (1925a). A method for the determination of total sulphates in tissues. *J. Biol. Chem.*, Vol. 65, pp. 561-563.

DENIS, W., AND LECHE, S. (1925b). On the distribution of injected sulphates in tissues. *J. Biol. Chem.*, Vol. 65, pp. 565-570.

DENIS, W., AND REED, L. (1927a). The action of blood on sulphides. *J. Biol. Chem.*, Vol. 72, pp. 385-394.

DENIS, W., AND REED, L. (1927b). 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.

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

HAGGARD, H. W. (1921). The fate of sulphides in the blood. *J. Biol. Chem.*, Vol. 49, pp. 519-529.

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

IOWA EXPT. STA. REPT. (1924). pp. 38-39. *Expt. Sta. Rec.* (1925), Vol. 53, p. 768.

KAMBAYASHI, Y. (1929). Über den Schwefelgehalt von Muskel und Leber der Ratte. *Biochem, Ztschr.*, Vol. 215, pp. 402-405.

KELLERMANN, J. H. (1934). A well-balanced ration for stock rats. *Onderstepoort J. Vet. Sci. and Anim. Indust.*, Vol. 2, pp. 649-653.

KELLERMANN, J. H. (1935). Sulphur Metabolism I. The absorption and excretion of flowers of sulphur. *Onderstepoort J. Vet. Sci. and Anim. Indust.*, Vol. 4, pp. 199-228.

KELLERMANN, J. H. (1936). Sulphur Metabolism III. The effect of flowers of sulphur on the growth of young rats fed an otherwise well-balanced ration. This Journal.

KELLERMANN, J. H. (1936). Sulphur Metabolism IV. The oxidation and reduction of elementary sulphur by animal tissues *in vitro*. This Journal.

KUBO, S. (1931). *Folia Endocrinol, Japan*, Vol. 7, pp. 176-177. *Chem. Abst.* (1933), Vol. 27, p. 5823.

LAWSON, G. B., REDFIELD, K. T., AND BOYCE, O. D. (1934). The toxic effect of sulphur on guinea pigs and rabbits. *J. Lab. and Clin. Med.*, Vol. 20, pp. 169-171.

LEHMANN, K. B. (1892). *Arch. f. Hygiene*, Vol. 14, p. 135.

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.

MEYER, E. (1898). *Arch, Exp. Path. und Pharmakol*, Vol. 41, p. 325.

MEYER-BISCH, R., AND TECHNER, F. (1931). Über das Schicksal parenteral verabfolgten Schwefels. *Biochem. Ztschr.*, Vol. 231, pp. 110-112.

MORACZEWSKI, W. v., AND SLIWINSKI, R. (1934). Über das Verhalten der Sulfate im Organismus bei verschiedener Kost. *Biochem. Ztschr.*, Vol. 272, pp. 269-276.

OSBORNE, W. A. (1928). A note on the volatile sulphide from muscle. *Biochem. J.*, Vol. 22, p. 1312.

STEYN, D. G. (1931). The effects of sulphur on merino sheep. *17th Rept. Div. Vet. Ser. and Anim. Indust.*, pp. 481-492.

TASAKA, S., AND NAKAZAWA, M. (1931). *Folia Endocrinol, Japan*, Vol. 6, pp. 84-85. *Chem. Abst.* (1932), p. 4877.