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Sulphur Metabolism.

IV. The Oxidation and Reduction of Elementary Sulphur by Animal Tissues *in vitro*.

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In an article on the therapeutic value of sulphur, Bridges (1934) stated that " sulphur is one of the oldest of drugs and still occupies an important place in our *materia medica* . . . Sulphur has played an important part in human medicine, and its use in veterinary medicine is increasing every day ".

Unfortunately, little is as yet known how it functions therapeutically. Power (1930; 1932) studied in some detail the effects of colloidal sulphur injections both in man and in rabbits, and found that one of its most characteristic effects appears to be a leukocytosis of polymorphonuclear cells in the blood stream. Furthermore, he found that these cells exhibit, under *in vitro* conditions, a considerable degree of phagocytic activity. However, Meyer-Bisch and Basch (1921) observed that marked differences exist between the effects of the oral and parenteral administration of sulphur, as determined by the composition of the urine. The fact that the latter form of administering sulphur together with its external application as an ointment in the treatment of eczema, mange, ringworm, etc., is used so extensively in medicine, probably lead to the study of the factors that influence the reduction of elementary sulphur by animal tissues *in vitro*. The reduction of sulphur is most probably the first metabolic change that sulphur undergoes in its sojourn in the organism. Evidently the better the factors that influence this initial step are understood, the easier it will be to study the subsequent transformation of sulphur.

Heffter (1904) and Heffter and Hausmann (1904) found that in the absence of bacteria and enzymes egg white, blood and many other animal tissues, including the intestinal mucosa, could form hydrogen sulphide from elementary sulphur. However, in a strongly acid solution the production of H_2S was completely inhibited. Sluiter (1930) confirmed the results of Heffter and believes the conclusion to be justified that H_2S production in living tissue after addition of sulphur is not due to enzyme activity. She seems to be of opinion that the sulphide production can be explained undoubtedly by the purely chemical process: $2\ G.SH + S = G.S.S.G. + H_2S$, in view of

the fact that tissues, glutathione and a dehydrogenase solution, as three different sources of SH used by her, all yielded H_2S after addition of sulphur. Likewise, Di Capua (1934) observed that sulphur is reduced to H_2S at about 30° in the presence of cystine, and De Rey-Pailhade (1929) found that H_2S is evolved when certain biological materials are mixed with sulphur at 40°, even if the nitroprusside reaction is not given.

The subsequent detoxication of the H_2S seems to be by oxidation to sulphate, and the blood is doubtless the main tissue of the animal body in which oxidation takes place. The results of Denis and Reed (1927a) give support to this theory. Furthermore, Haggard (1921) found that the rate of oxidation of H_2S in the blood is such that in a comparatively short period many times the lethal amount of sodium sulphide may be administered intravenously to animals without any apparent effect. The blood is reduced by the H_2S as a result of the withdrawal of oxygen from the corpuscles for the oxidation of the sulphide. He found that the products of oxidation, though undetermined, combine in part with the sodium of the plasma. Another tissue of importance in the oxidation of sulphur appears to be the suprarenal glands. Loeper, Garcin and Lesure (1926) believe that the suprarenals possess a double function, namely, thiopexic and thio-oxidizing in view of the fact that blood from the suprarenal vein contains much less sulphur than that from the suprarenal artery, whereas blood from the suprarenal vein contains a larger proportion of oxidized sulphur than does that from the suprarenal artery. Moreover, they observed that the suprarenals are richer in sulphur than most other organs, which corroborates the results of Aufrecht and Driesing (1910) who consider the adrenal glands as the regulators of the sulphur metabolism of the animal body just as, for instance, the thyroid gland regulates iodine metabolism.

It is evident from the results of Denis and Reed (1927b), substantiated later by the work of the author (1935) that he absorption of elementary sulphur from the alimentary tract results mainly in its excretion in the urine as sulphates. According to the observations of Meyer-Bisch and Basch (1921) a large fraction of the sulphur injected peritoneally or intramuscularly is also excreted in the sulphate form. Unfortunately, not much could be learned from these *in vivo* studies as to the extent to which certain factors might influence the metabolism of sulphur, and it was, therefore, the object of this paper to investigate some of the factors that might influence the reduction and especially the oxidation of elementary sulphur by animal tissues *in vitro*.

Experimental.

In all of the experiments on the reduction (and oxidation) of elementary sulphur by animal tissues *in vitro*, a control was always run on the same tissue without the addition of sulphur, and under the same experimental conditions, in so far as time and temperature were concerned. This precautionary measure was essential in view of the fact that Osborne (1928) has shown that the flesh of several species of animals, when starved or in an emaciated condition, emitted H_2S immediately after killing whereas the flesh of well-fed animals of the same species did not evolve H_2S until some hours,

preferably twenty-four, after killing. Furthermore, in order to exclude the possibility of any bacterial action, the studies were carried out in an antiseptic solution. With the exception of one experiment where a weak antiseptic of 5 gm. boracic acid per litre of Ringer solution was used, all the experiments were carried out with the use of a very strong antiseptic of 0·83 gm. thymol per litre of Ringer solution (Lutman 1929).

The production of H_2S by animal tissues in T.R. solution* was studied under seven different conditions. The first solution constituted a negative control to which no sulphur was added. The next two served as sulphur controls but the tissue in one of them was first boiled for 30 minutes in order to destroy all enzyme action. To the four remaining solutions were added sulphur plus varying amounts of acid and alkali to give a solution approximately N/4·3 with respect to HCl, N/1·76 with respect to NaOH and N/10 with respect to Na_2CO_3 and $NaHCO_3$ respectively. When phenolphthalein was used as indicator and the solutions containing beef liver were titrated hot to end-point with HCl or NaOH as necessary, it was found that the first three solutions had on the average an acid equivalence of 2·23 c.c. N.HCl, the fourth of 38·14 c.c. N.HCl whereas the last two had an average base equivalence of 10·73 c.c. of N.NaOH. Almost identical figures were obtained with beef muscle. The base equivalence of the sodium carbonate and bicarbonate solutions were determined by adding an excess of acid to the solution which, after being boiled to drive off the carbon dioxide, was then titrated back with alkali. The difference between the amount of N.HCl added and that of N.NaOH used in the back titration constitutes the base equivalence of the solution. The base equivalence of the fifth solution, which was made strongly alkaline by the addition of NaOH, could not be determined in view of the fact that no sharp end-point could be obtained.

The determination of the reduction of elementary sulphur by animal tissues was carried out as follows: 50 gm. of tissue or 50 c.c. of blood plus 150 c.c. T.R. solution together with the necessary chemicals, as the case may be, and to be described shortly, were introduced into a 700 c.c. Pyrex boiling flask and stoppered with a tight fitting rubber stopper which was provided with in and out leading glass tubes. The end of the tube through which air was later to be drawn in, was such that its end dipped under the surface of the solution in the flask. To the ends of the in and out leading tubes were attached tight fitting rubber tubing, the free ends of which were closed tightly with screw pinch-cocks. The flasks were then kept in an incubator, which was run at 37°, for twenty-four hours with occasional shaking after which the free volatile sulphide was determined by the method as described by Heffter and Hausmann (1904). However, an error may possibly be introduced by the reduction of elementary sulphur by the various solutions as such, that is, without the presence of tissue, in view of the fact that several investigators [Geitner, (1864); Boehm, (1883); and Cross and Higgin, (1883), as quoted by Heffter and Hausmann (1904)] have found that

* T.R. solution is an abbreviation for thymol-Ringer solution.

small amounts of H_2S were given off when sulphur was boiled with water. Likewise, Heffter (1904) found that sulphide was formed when sulphur was heated at 40° with either a sodium hydroxide or carbonate solution.

In order, therefore, to see whether elementary sulphur was reduced by the antiseptic solutions as such, these were put through the same routine as described above and determinations made of the amounts of H_2S emitted. Negative results were found for most of the solutions except for the carbonate and bicarbonate ones from which an average of only a small fraction of a milligram of H_2S-S was obtained. As a result of these minimal values in comparison with the large amounts of sulphide formed by liver and muscle, they were simply disregarded in the presentation of the results obtained with tissues. The results are given in Table I.

It will be seen that only traces of H_2S were emitted by the blood in the various solutions. The most probable reason for this phenomenon is that the H_2S was completely oxidized by the oxygen present in the blood corpuscles. However, quite different results were given by the liver and muscle. Here the addition of sulphur alone caused the formation of an average increase of 98·7 mgm. H_2S-S per cent. over and above the negative control. Boiling caused a great average decrease of 59·86 mgm. H_2S-S per cent. compared with the fresh tissue yet still 38·87 mgm. per cent. higher than the value of the negative control. The decrease in H_2S formation after boiling of the tissue can probably be explained on the grounds that the SH group was decomposed by heating as was shown by Sluiter (1930) and the $2\ G.SH + S = G.S.S.G. + H_2S$ reaction could, therefore, no longer take place. In a strongly acid or alkaline solution the amount of H_2S emitted was markedly decreased. This substantiates the observations of Heffter and Hausmann (1904) and Sluiter (1930). In the case of the acid solution the liver and muscle gave an average value of only 9·32 mgm. H_2S-S per cent. whereas practically none was recovered from the strongly alkaline solution. However, under the experimental conditions, it cannot be concluded that no reduction took place in the strongly alkaline medium, in view of the fact that the H_2S formed might have been completely bound by the excess of alkali. Later results seem to give indirect testimony to this effect. In the presence of a weak alkaline solution the production of H_2S was markedly enhanced. The average increases over the sulphur control were 364·2 and 148·7 mgm. H_2S-S per cent. for the sodium carbonate and bicarbonate solutions, respectively.

In order to see to what extent the alkali as such was responsible for the increase in H_2S formed, a control was run, simultaneoulsy, for each of the various solutions. The results given in Table II show that only the $NaHCO_3$ solution, as such, caused an increase in the amount of H_2S formed. In this case the average amount (for liver and muscle) of H_2S emitted was only 36·3 mgm. H_2S-S more than that of the negative control. On the other hand when sulphur was added the amounts of H_2S evolved by the sodium hydroxide, carbonate and bicarbonate solutions were nil, 462·7 and 202·0 mg. H_2S-S per cent. more than those of their respective

Table I.

The Reduction of Elementary Sulphur by Various Beef Tissues in T.R. Solution.

	Tissue.	Moisture.							
		Per Cent.			Boiled for 30 mins.				
Treatment of Tissues:									—
Per cent. S added to fresh tissues.....	—	—	—	3	3	3	3	3	3
Normality of solution with respect to HCl..	—	—	—	—	—	N/4·3	—	—	—
Normality of solution with respect to NaOH	—	—	—	—	—	—	N/1·76	—	—
Normality of solution with respect to Na_2CO_2	—	—	—	—	—	—	—	N/10	—
Normality of solution with respect to $NaHCO_2$	—	—	—	—	—	—	—	—	N/10
Mgm. H_2S. S per 100 gm. dry tissues.....	Blood	81·56	trace	trace	trace	trace	trace	trace	trace
Mgm. H_2S. S per 100 gm. dry tissues.....	Acrated* Blood	79·39	,,	,,	,,	,,	,,	,,	,,
Mgm. H_2S. S per 100 gm. dry tissues.....	Liver	71·12	4·67	96·56	76·79	4·74	,,	812·3	100·7
Mgm. H_2S. S per 100 gm. dry tissues.....	Muscle	76·33	15·34	120·90	20·95	10·90	,,	133·5	414·1

* After the T.R. solution and the necessary chemicals were added to the blood in the boiling flask, the latter was immediately connected up in the apparatus for the determination of hydrogen sulphide and H_2S-free air drawn through for three hours. The H_2S emitted was caught up in an ammoniacal H_2O_2 solution. The boiling flask was partly immersed in a water bath kept at a constant temperature of 40° C.

Table II.

The Effect of Alkali on the Reduction of Elementary Sulphur by Animal Tissues in T.R. Solution.

	Tissue.	Moisture.			Increase in H_2S. S.			Increase in H_2S S.			Increase in H_2S S.			Increase in H_2S S.
		Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.
Per cent. S added to fresh tissue	—	—	—	3	—	—	3	—	—	3	—	—	3	—
Normality of solution with repect to NaOH	—	—	—	—	—	N/1·76	N/1·76	—	—	—	—	—	—	—
Normality of solution with respect to Na_2CO_3	—	—	—	—	—	—	—	—	N/10	N/10	—	—	—	—
Normality of solution with respect to $NaHCO_3$.....	—	—	—	—	—	—	—	—	—	—	—	N/10	N/10	—
Mgm. H_2S-S per 100 gm. dry material.....	Beef Liver	71·12	4·7	96·6	91·9	trace	trace	0	5·4	812·3	806·9	17·9	82·7	64·8
Mgm. H_2S–S. per 100 gm. dry material.....	Beef muscle	76·33	15·3	120·9	105·6	trace	trace	0	15·0	133·5	118·5	74·8	414·1	339·3

controls. It is evident, therefore, that the large amount of H_2S rendered by the carbonate and bicarbonate solutions, containing elementary sulphur, owes its origin chiefly to the sulphur present and only to a very small degree to the reducing effect of the salt (bicarbonate) upon the tissue itself. Nevertheless, the addition of the carbonate and bicarbonate salts increased considerably the reduction of elementary sulphur by animal tissues. This is clear from the fact that the reduction of elementary sulphur, as such, by tissues in the carbonate and bicarbonate solutions exceeded on the average the reduction of sulphur by the same tissues in an ordinary T.R. solution by 364·0 and 103·3mgm. H_2S-S per cent., respectively.

In order to see to what extent various tissues can also oxidize elementary sulphur, the amount of sulphate present in the different solutions, after twenty-four hours of incubation, was also determined. Two different types of solution were employed, one with weak (0·5 per cent. boracic acid) and the other with very strong (0·083 per cent. thymol) antiseptic properties. The experimental conditions were exactly the same as already described under the determination of the reduction of elementary sulphur, except that the solutions with the tissues, chemicals, etc., were kept in 400 c.c. Gena glass beakers and, during incubation, occasionally stirred with glass rods. The negative control solutions, that is, those to which no elementary sulphur was added, were always incubated in a separate incubator in order to prevent the absorption of any volatile sulphide. After incubation the solutions were acidified with HCl, autoclaved and the sulphate determined gravimetrically, as $BaSO_4$, by the method as described by the author (1936). However, caution should be exercised not to ascribe oxidizing properties to tissues that actually belong to the antiseptic solutions as such. For that reason blank determinations were made of the amount of sulphates formed by the various acid and alkaline solutions, to which sulphur was added. In all of the solutions, except for the sodium hydroxide one, the amounts of sulphates formed were either nil or so small that they might be disregarded. However, appreciable amounts of sulphate were formed in the hydroxide solution. For three different determinations values ranging from 0·0368 gm. to 0·0376 gm. with an average of 0·0372 gm. $BaSO_4$ were obtained. This average value had of course to be deducted from the amount of $BaSO_4$ precipitated in each of the tissue-hydroxide solutions.*

The data presented in Table III show that he boracic acid and T.R. solutions gave parallel results. Furthermore, comparison of the sulphate values in Table III with those of the H_2S in Table I reveals a close parallelism between these data. This suggests that the oxidation of elementary sulphur is preceded by its reduction, and that the same factors which influence its reduction will, therefore, also show up in its oxidation, in view of the fact that the greater the concentration of H_2S formed the more of it will be available for oxidation and *vice versa*. For the sake of simplicity only the average values of the various columns will be discussed.

* The values for sulphate-sulphur given under the tissue-hydroxide columns in the tables represent those after the necessary corrections had been made.

TABLE III.

The Oxidation of Elementary Sulphur to Sulphate by Animal Tissues in Weak and very Strong Antiseptics.

In 0·5 per cent. Boric Acid Solution

	Tissue.	Animal.	Moisture.							
			Per Cent.			Boiled for 30 mins.				
Treatment of Tissue:										
Per cent. S added on basis of fresh tissue.....	—	—	—	—	3	3	3	3	3	3
Normality of solution with respect to HCl....	—	—	—	—	—	—	N/4·3	—	—	—
Normality of solution with respect to NaOH....	—	—	—	—	—	—	—	N/1·76	—	—
Normality of solution with respect to Na_2CO_3...	—	—	—	—	—	—	—	—	N/10	—
Normality of solution with respect to $NaHCO_3$..	—	—	—	—	—	—	—	—	—	N/10
Mgm. SO_4–S per 100 gm. dry tissue.....	Blood	Bovine	80·98	8·51	20·81	7·09	6·62	36·87	28·38	25·54
,, ,, ,, ...	Dried (100°C.) liver	Sheep	69·58	3·61	3·61	3·61	3·61	58·23	5·42	6·78
In Thymol-Ringer Solution.										
Mgm. SO_4–S per 100 gm. dry tissue.....	Liver	Beef	70·33	15·74	18·99	17·13	12·50	240·3	95·85	37·51
,, ,, ,, ...	Steamed (3 hrs.) liver	,,	66·37	8·98	8·98	—	trace	247·0	31·03	32·67
,, ,, ,, ...	Dried (70° C.) liver	,,	7·45	7·72	8·31	9·49	6·53	150·2	12·08	10·97
,, ,, ,, ...	Muscle	,,	75·61	1·69	13·39	trace	1·13	212·4	98·02	33·81
,, ,, ,, ...	Blood	,,	81·71	14·71	47·07	32·85	12·75	371·6	194·60	115·20
,, ,, ,, ...	Liver	Rat	70·87	2·83	3·77	2·83	1·89	302·8	43·40	22·64
,, ,, ,, ...	Muscle	,,	72·23	trace	0·49	1·48	trace	137·6	53·43	19·79
,, ,, ,, ...	Small intestine	,,	77·80	3·71	3·71	—	4·95	—	14·86	—
,, ,, ,, ...	Blood	,,	79·11	1·28	60·67	—	—	—	—	—
Average mgm. SO_4–S per 100 gm. dry tissue....	—	—	—	6·25	17·25	9·31	5·0	195·22	57·70	33·88

The addition of sulphur to the tissues resulted in part in its being oxidized to sulphate. This is evident from the fact that the tissue-sulphur solutions (sulphur controls) yielded 11·0 mgm. more sulphate-sulphur per cent. than those (negative controls) to which no sulphur was added. Previous boiling of the tissues, however, reduced the oxidizing capacity of the tissue-solutions because those containing tissues so treated only yielded 3·06 mgm. more sulphate-sulphur per cent. than the negative controls. In a strongly acid medium this oxidizing capacity is completely lost whereas the addition of alkalies, on the other hand, greatly enhanced the oxidation of elementary sulphur by animal tissues. This is evident from the fact that from the sodium hydroxide, carbonate and bicarbonate solutions an average of 177·97, 40·45 and 16·63 mgm. more sulphate-sulphur per cent. were recovered than from the sulphur controls.

In order to see to what extent the alkalies, as such, were responsible for the marked increase in sulphates formed, a control, without the addition of sulphur, was run for each solution. The results are given in Table IV.

It is clear, that the addition of any of the alkalies, increased the sulphate content of tissues when determined after an incubation period of twenty-four hours. On the average for bovine blood and liver the sodium hydroxide, carbonate and bicarbonate solutions yielded, respectively, 46·9, 6·0 and 7·1 mgm. more sulphate-sulphur per cent. than the control solutions to which no alkali had been added. However, when sulphur was added in addition to the alkalies, the average increases in sulphate values, as a result of the sulphur as such, that is, the increases over the alkali controls to which no sulphur was added, were 243·8, 124·0 and 54.0 mgm. sulphate-sulphur per cent. for the sodium hydroxide, carbonate and bicarbonate solutions, respectively. These values still exceed the average net sulphate increase due to sulphur, without the addition of alkali, by 226·0, 106·2 and 36·2 mgm. sulphate-sulphur per cent. for the respective alkaline solutions from which it is evident that, although alkali in itself increases the sulphate content of tissues, it nevertheless augments the oxidation of elementary sulphur by animal tissues to a marked extent.

Exactly how the alkali facilitates the oxidation of elementary sulphur by tissues is not quite clear. It is evident that its action is not secondary to any vital entity in the tissue, in view of the fact that some of the studies were carried out on tissues previously steamed or dried (see Table III) and always under strictly antiseptic conditions. The results suggest, however, that the chief effects of the alkali in the oxidation of sulphur are to increase the reduction of sulphur by the tissue and the subsequent concentration of sulphide in the solution; furthermore, it is well known that both sodium and hydrogen sulphide are rather unstable in solution and gradually take on atmospheric oxygen to form sulphate. It might be, therefore, that the sole effect of the alkali was to increase the reduction and concentration of sulphide in the solution where it was oxidized either quickly by the oxygen or corpuscles, in the case of blood, or slowly by atmospheric oxygen, in the case of " blood free " tissues. If this theory is correct, it sould also follow that the oxidation of a

TABLE IV.

The Effect of Alkali on the Oxidation of Elementary Sulphur by Animal Tissues in T.R. Solution.

	Tissue.	Moisture.			Increase in SO_4S.			Increase in SO_4S.			Increase in SO_4S.			Increase in SO_4S.
		Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.			Mgm. Per Cent.
Per cent. S added to fresh tissue	—	—	—	3	—	—	3	—	—	3	—	—	3	—
Normality of solution with respect to NaOH	—	—	—	—	—	N/1·76	N/1·76	—	—	—	—	—	—	—
Normality of solution with respect to Na_2CO_3	—	—	—	—	—	—	—	—	N/10	N/10	—	—	—	—
Normality of solution with respect to $NaHCO_3$	—	—	—	—	—	—	—	—	—	—	—	N/10	N/10	—
Mgm. SO_4–S per 100 gm. dry material	Bovine blood	81·71	14·7	47·1	32·4	80·8	371·6	290·8	23·0	194·6	171·6	19·1	115·2	96·1
Mgm. SO_4–S per 100 gm. dry material	Bovine liver	70·33	15·7	19·0	3·3	43·5	240·3	196·8	19·4	95·8	76·4	25·5	37·5	12·0

" constant " supply of H_2S by an alkaline-tissue solution should be the same no matter whether the tissue was fresh, boiled or dried. This was found to be actually the case, and demonstrated as follows: a " constant " stream of H_2S was passed for exactly fifteen minutes through one of each two samples of fresh, boiled and dried tissue in 150 c.c. of N/10 (with respect to Na_2CO_3) T.R. solution. The H_2S-treated solutions were then incubated separately from the untreated (control) ones and after an incubation period of twenty-four hours, with occasional stirring, the sulphate in each was determined as described previously. The difference between the sulphate contents of the H_2S-treated and untreated solutions constitutes the amount of sulphate formed from the oxidation of hydrogen sulphide. The results are given in Table V.

TABLE V.

The Oxidation of Hydrogen Sulphide By Animal Tissues in T.R. Solution, N/10 *with respect to Sodium Carbonate.*

State of Tissue.	Tissue.	Fresh.	Boiled. (30 mins.)	Dried. (100° C.)
Mgm. SO_4–S formed from oxidation of H_2S	Beef liver....	1·63	2·06	1·79
	Beef muscle	1·61	1·39	1·29

The similarity of the values obtained with the differently treated tissues is striking. For beef liver they varied from 1·63 to 2·06 mgm. with an average of 1·83 mgm., and in the case of beef muscle from 1·29 to 1·61 mgm. with an average of 1·43 mgm. What is of interest is the fact that the amount of sulphate-sulphur recovered from three blanks of alkaline (N/10) Thymol-Ringer solutions through which H_2S was also passed, etc.,varied from 4·205 to 4·479 mgm. with an average of 4·30 mgm., which is 163·8 per cent. more than the grand average of the liver and muscle values. A probable explanation as to why the addition of tissues reduced the capacity of the alkaline solution to oxidize H_2S, might be that the formation of sodium proteinates reduced the dissociation of the salt with a subsequent reduction in the power of the solution to bind and concentrate the volatile sulphide.

Because of the large amounts of carbonates and buffer salts present in blood where injected (intravenously) sulphur is reduced (Heffter 1904) and then readily oxidized (Haggard 1921), it was thought of interest to study also the influence of these salts, in the absence of tissues, on the oxidation of H_2S. This experiment was carried out as follows: A " constant " stream of H_2S was passed for exactly fifteen minutes through one of each of two 200 c.c. samples of the following solutions: thymol-Ringer solutions with and without the addition (to give N/10 solutions) of sodium hydroxide, carbonate and bicarbonate. Similarly, H_2S was passed through one of each of two samples of the following buffer mixtures, KH_2PO_4—NaOH (Clark 1922); Na_2HPO_4—KH_2PO_4, and sodium borate and

N/10 HCl (Sörensen 1909). The pH values of these mixtures are 7·4, 7·347 and 7·621, respectively, all of which approximate closely that of blood. The H_2S-treated and control solutions were incubated separately after which the amounts of sulphate were determined.

TABLE VI.

The Oxidation of Hydrogen Sulphide to Sulphate in 200 c.c. of Various Alkaline and Buffer Solutions.

In T.R. Solutions.

Normality with respect to NaOH	—		N/10		—		—	
Normality with respect to Na_2CO_3	—		—		N/10		—	
Normality with respect to $NaHCO_3$	—		—		—		N/10	
Saturated with.....	—	H_2S	—	H_2S	—	H_2S	—	H_2S
Mgm. SO_4–S in sample.....	0·08	0·71	0·05	1·39	0·05	0·81	0·069	0·74

In Buffer Solutions.

	KH_2PO_4—NaOH Mixture.		Na_2HPO_4— KH_2PO_4 Mixture.		Na borate and N/10 Hcl Mixture.	
H ion concentration (pH)..	7·4		7·347		7·621	
Saturated with.....	—	H_2S	—	H_2S	—	H_2S
Mgm. SO_4–S in sample....	0·05	1·55	0·15	1·72	0·19	1·25

The data presented in Table VI show that the H_2S-treated thymol-Ringer solution and those made N/10 with respect to sodium hydroxide, carbonate and bicarbonate contained, respectively, 8·87, 27·8, 16·2 and 10·72 times as much sulphate as the control solutions without the addition of H_2S. It is evident, therefore, that the oxidation of H_2S was markedly increased by the alkaline salts added. This was most probably accomplished through the formation of the non-volatile sodium sulphide* which, like the H_2S, in T.R. solution, was slowly oxidized to sulphate when exposed to atmospheric oxygen. For similar reasons the oxidation of H_2S in the buffer solutions was also greatly increased. The H_2S-treated buffer solutions contained, in the order given previously, 31·0, 11·45, and 6·58 times as much sulphate as the H_2S-free solutions.

If alkaline salts can exert such far reaching effects on the reduction and subsequent oxidation of elementary sulphur, by tissues *in vitro*, they should also have the same influence on orally administered sulphur, in view of the fact that sulphur is readily reduced by the bacteria and mucosa of the digestive tract in whose contents oxygen is normally always present. As a matter of fact, it was observed by the author (1935) that the intestinal contents of rats, fed elementary sulphur in addition to a protein-free ration, were much richer in sulphates than the contents of animals fed the

* That solutions of $NaHCO_3$ are capable of binding and concentrating H_2S as Na_2S was, according to Pohl (1886-87), demonstrated experimentally by Diakonow.

same ration without the addition of sulphur. It was suggested at that time that the increase in sulphate " might have been due to a more copious flow of intestinal secretions induced by hydrogen sulphide irritation, or to a greater concentration of sulphates in the secretion of the sulphur-fed rats, or to both ". In the light of knowledge gained later in an *in vitro* study, to be presented directly, it seems as though this theory is only partly true because the greater part of the increase in sulphate content most probably had its origin in the digestive tract through the oxidation of H_2S to sulphate.

The *in vitro* study was carried out as follows: a group of twelve adult rats were fed *ad lib.* distilled water and a protein and salt free ration consisting of dextrinized starch 62, sucrose 20, lard 10, cod liver oil 2, and agar 6 parts by weight for eight days. They were then killed and the contents of the stomach, small intestine and caecum removed. The pooled contents of each section were then divided, after samples for moisture determination had been taken, into three parts as nearly as possible of the same weight. To one part was added elementary sulphur and to another an alkaline salt mixture in addition to the sulphur. The salt mixture was a modification of Steenbock and Nelson salts 40 (1923) in which the $MgSO_4 7H_2O$ was replaced by an equivalent amount of $MgCO_3$. After thymol-Ringer solution was added to each part, they were incubated at 37° for twenty-four hours and their sulphate contents determined. The results are given in Table VII.

TABLE VII.

The Oxidation of Elementary Sulphur to Sulphate by the Intestinal Contents of Rats in T.R. Solution.

Contents.	Stomach.			Small Intestinal.			Caecal.		
Moisture per cent.	49·86			84·89			87·16		
Weight of fresh sample (gm.)..	27	27	27	25	24	21	7·5	8	8
No. of c.c. T.R. solution added	65	65	65	20	20	20	20	20	20
Per cent. S added on basis of dry material.....	—	3·69	3·69	—	4·13	4·73	—	14·56	14·56
Per cent. salts 40* added on basis of dry material	—	—	5·17	—	—	11·04	—	—	33·98
Mgm. SO_4–S per 100 gm. dry material.....	63·13	62·52	75·30	252·3	264·2	287·7	458·0	466·9	547·0

* The inorganic salt mixture added was a modification of Steenbock and Nelson salts 40 (1923) in which the $MgSO_4 \cdot 7H_2O$ was replaced by an equivalent amount of $MgCO_3$.

It will be seen that the addition of sulphur alone to the stomach material had no effect on its sulphate content which was probably due to the acid nature of the stomach contents. On the other hand when 5·2 per cent. of salts were added in addition, the sulphate content increased 12·17 mgm. sulphate-sulphur per cent., as

compared with that of the control solution. The addition of sulphur and sulphur plus salts to the contents of the small intestine increased their sulphate values by 11·9 and 35·4 mgm., and when added to the caecal contents by 8·9 and 89·0 mgm. sulphate-sulphur per cent. respectively, when compared with the sulphate values of their control solutions. Furthermore, the parallelism between the amounts of salts added and the subsequent increases in sulphates formed, as compared with those of the sulphur controls, is striking. The amounts of salts added to the contents of the stomach,small intestine and caecum were 5·17, 11·04 and 33·98 per cent., respectively, and the subsequent increases in sulphate were 12·78, 23·5 and 80·1 mgm. sulphate-sulphur. When the lowest amount of salts added is taken as unity, and the two other values are expressed as multiples thereof, their relation to each other is as 1 : 2·1 : 6·6; similarly, the relation of the subsequent sulphate increases is as 1 : 1·8 : 6·3 to each other which shows how closely the oxidation of elementary sulphur is dependant upon the alkalinity of the medium. The importance, therefore, of an excess of base-forming elements in the diet is evident, especially in cases where a large amount of H_2S is formed in the digestive tract, in view of the fact that these elements help to oxidize and detoxicate part of the sulphide before it reaches the liver. On the other hand, it should be mentioned that alkalies also increase the reduction of elementary sulphur which might largely offset their beneficial effects when sulphur is incorporated in the ration.

Discussion.

The experiments described in this paper show that elementary sulphur is first reduced to sulphide and subsequently oxidized to sulphate by animal tissues *in vitro*, and that both processes are inhibited by acids but enhanced by alkalies and alkaline salts. In view of the fact that all protein (SH) containing tissues can reduce elementary sulphur, it is clear that the only other condition necessary for its subsequent oxidation is that the tissue be bathed in an alkaline (or alkaline buffer) solution which is also rich in oxygen. If the oxidizing and reducing reactions studied in the laboratory are analogous to those taking place in living organisms, it is evident that the essential conditions given above are excellently fulfilled in the blood stream. Nevertheless, by measuring the H_2S content of the expired air, it was found by Forst (1928) that H_2S was formed in the blood in amounts approximating the fatal dose after the intravenous injection of relatively large amounts of colloidal sulphur, and it is probably for that reason that at the present time the injection of sulphur either subcutaneously or intramuscularly is more and more resorted to.

When sulphur is injected in this way, it is probably first reduced to sulphide, as in the case of blood, then partly oxidized and finally, as the results of Meyer-Bisch and Basch (1921) seem to show, excreted largely as sulphates in the urine. The question now arises as to how the oxidation of the sulphide to sulphate is accomplished in the organism. Part of it, no doubt, passes into the blood stream and is oxidized there whereas some of it is most probably oxidized by the tissue into which it is injected without the aid of the blood stream, in view of the fact " that hydrogen peroxide is a

product of cell activity and that phosphates are present in all cells " (Gortner 1929) thereby containing in itself the essential agents for the oxidation of sulphide. However, the tissues into which sulphur is usually injected are much poorer in oxygen and buffer salts than blood, and it is there that one might be able to enhance the oxidation and reduction of elementary sulphur by the simultaneous injection of alkaline salts. If the enhancing effect of alkaline salts on the reduction of sulphur by animal tissues *in vitro* also holds true for its reduction in living organisms, the injection of a colloidal suspension of sulphur in an alkaline solution might find special application in certain cases of acute poisoning. It is revealed in the literature (Kellermann 1935) that colloidal sulphur forms a good antidote for corrosive sublimate and cyanide poisoning in view of the formation of the less poisonous sulphide and thiocyanate salts, respectively. It is evident, therefore, that if the liberation of the labile sulphur (sulphide) could be increased and speeded up, it should be possible to render a dose of poison, several times the lethal amount, quite harmless. If this theory is correct, it would help to explain why Forst (1928) found the administration of such a large amount (three to four times lethal dose) of HCN harmless when followed up within ten to fifteen minutes by the injection of a colloidal suspension of sulphur in a sodium bicarbonate solution.

Summary and Conclusions.

1. Data are presented on the oxidation and reduction of elementary sulphur by animal tissues *in vitro*, and the enhancing or otherwise effects of certain chemicals on these reactions are also shown.

2. All protein tissues seem to be capable of reducing sulphur to H_2S. Acid (HCl) inhibits this reaction whereas alkalies and alkaline salts enhance it.

3. Similarly, acid inhibits and alkalies and alkaline buffers increase the oxidation of reduced sulphur to sulphate. An alkaline medium, rich in oxygen, is therefore essential for the oxidation and detoxication of H_2S. This is readily accomplished in blood where these conditions are excellently fulfilled. Nevertheless, all tissues in the animal organism should be able to oxidize sulphides, in view of the fact that oxygen (e.g. H_2O_2) and phosphates (buffers) are present in all tissues.

4. The chief, if not the sole, effect of alkali on the oxidation of sulphur seems to be to bind and concentrate the reduced sulphur in the solution where it is then oxidized to sulphate by the oxygen present in the corpuscles, in the case of blood, or by atmospheric oxygen, in the case of other tissues. Furthermore, the oxidation of sulphur by tissues under laboratory conditions is not influenced by enzyme activity, in view of the fact that no difference was found in the oxidation of H_2S by fresh, boiled or dried tissue suspensions in an alkaline (Na_2CO_3) thymol-Ringer solution. As a matter of fact the oxidation of H_2S also took place with relative ease in alkaline (and buffer) solutions without the presence of tissues.

5. Similarly, the reduction of sulphur by animal tissues is not due to enzyme activity because the reaction took place in tissues that were boiled previous to their incubation with sulphur in a very strong antiseptic solution.

6. A close parallelism was found to exist, under laboratory conditions, between the increases in the oxidation of sulphur and (as a result of) the different percentages (on the dry basis) of modified Steenbock salts 40 added to the intestinal contents of the rats fed an ash and protein-free ration. This suggests that a diet with an excess of base-forming elements may be of importance in the oxidation and detoxication of sulphides in cases where large amounts of H_2S are formed in the digestive tract.

7. The possibility of beneficial effects arising from injecting (subcutaneously or intramuscularly) a colloidal suspension of sulphur in an alkaline solution as an antidote in certain cases of acute poisoning is discussed.

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