CXLVI. THE OXIDATION OF THIOSULPHATE TO SULPHATE BY TISSUE SLICES *IN VITRO*.

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SCHMIEDEBERG [1867] isolated thiosulphate (as barium thiosulphate) from the normal urine of cats and some dogs; this observation was confirmed by Meissner [1868] and by Salkowski [1886, 2] who also found it in dog faeces. Salkowski, like most later workers, did not isolate the thiosulphate but simply showed that sulphur was formed when the urine was acidified and heated. Strümpell [1876] found thiosulphate in the urine of a patient suffering from typhoid and Spiegel [1901], who suggested that it was a normal oxidation product of cystine in the body, found it in the urine of a cystinuric. Since Mester [1890] had already shown that thiosulphate occurred in the urine after feeding both cystinuric and normal men on milk of sulphur and since several workers have found it in some human urines, the presence of thiosulphate in the urine cannot be looked upon as a characteristic of any disease.

Salkowski [1872; 1873] showed that taurine was not oxidised either to sulphate or to thiosulphate in the dog, the man or the rabbit when injected, but that it was oxidised when fed to the rabbit, and he found considerable quantities of both oxidation products in the urine. He suggested that the sulphate arose secondarily by the oxidation of the thiosulphate, for Trachtenberg [1861] had already demonstrated that the latter was readily oxidised when fed to man. Salkowski later [1876] found that isethionic acid could be oxidised to sulphate by rabbits whether fed or injected but that it only gave rise to thiosulphate when fed. These observations were extended by Heffter [1886] and Rothera [1904] who pointed out the importance of the intestinal flora in the production of thiosulphate. Heffter found that putrid meat led to a greater excretion of thiosulphate when fed to dogs than fresh meat; he also showed that diets rich in carbohydrate increased thiosulphate excretion and suggested that this was due to the increased fermentation in the gut. Some of Heffter's conclusions were harshly criticised by Salkowski [1886, 1] but his main conclusions have been generally accepted.

Inspired by Mörner's demonstration that cystine occurred in proteins Blum [1903] fed cystine to dogs and Wohlgemuth [1904, 1] fed it to rabbits; both detected thiosulphate in the urine, although Goldmann, using smaller doses, had earlier [1885] failed to do so. Blum however found great individual variations between his dogs, as might be expected if the action were due to bacteria in the gut. He also showed that no thiosulphate was excreted when the cystine was given intravenously or subcutaneously. Salkowski, returning after a long interval to his old studies, found [1914, 1] that rabbits excreted more thiosulphate in their urine when fed on cabbage than when fed on other diets; he later [1914, 2; 1917] showed that the substance concerned could be extracted from cabbage by boiling water but that if the cabbage were boiled with acid

before feeding no thiosulphate was found in the urine. He demonstrated, however, that the substance was not thiosulphate itself and that it was probably not inorganic.

It would seem therefore to be a reasonable assumption that thiosulphate is either made by bacteria in the gut or else that it is made by the body from some substance that is made in the gut.

Wohlgemuth [1904, 2] found that cystine gave rise to thiosulphate when incubated with putrid meat infusion and from this argued that it was formed during intestinal putrefaction. Neuberg and Rubin [1914] similarly found that mixed putrefactive bacteria could make thiosulphate from taurine and chondroitinsulphuric acid. A mechanism is therefore available whereby organic sulphur compounds can be converted into thiosulphate but there is little reason to think that it is the only mechanism. Ever since its discovery it has been known that hydrogen sulphide is a product of protein putrefaction. Rothera [1904] found that it was made by *Bact. coli* from cystine while Almy and James [1926] and Tarr [1933, 1] have followed its production by *Proteus vulgaris*. Tarr [1933, 2; 1934] demonstrated that other bacteria, notably *Serratia marcescens* and *C. sporogenese*, had the same power and that sulphur compounds other than cystine could be broken down. The early literature of this subject is reviewed by Wilson [1923].

Hydrogen sulphide has frequently been detected in intestinal gases and this fact, taken in conjunction with McIver, Redfield and Benedict's [1926] amplification of Teschendorf's [1924] demonstration that it is very readily absorbed from body cavities, goes to show that considerable amounts are actually formed in the intestine. This process has not been studied in detail, but Deganello [1931] has shown that there is much more hydrogen sulphide in the intestine and faeces of a rabbit that is fed on cabbage than in that of one fed on other diets. This, on the theory put forward later in this paper, is in good agreement with Salkowski's [1914, 1, 2] observations. Sulphates, and so presumably other sulphur compounds, were shown by Zörkendörfer [1931] and Kochmann [1920] to be reduced when incubated with faeces, while Wilson [1923] has shown that *Bact. typhosum* and other bacteria can reduce sulphites in the same way. Many other bacteria are known which reduce inorganic sulphur compounds to hydrogen sulphide but they have been derived from sources other than the intestinal tract. Finally Lorant and Reimann [1930], who estimated the hydrogen sulphide content of many samples of faeces, have claimed that it is possible to increase the amount of faecal sulphide-sulphur by feeding iron salts and thus, by converting some of the hydrogen sulphide into an insoluble form, minimising the absorption of it. The literature that has been cited so far shows, therefore, not only that thiosulphate may be formed in the gut but that hydrogen sulphide certainly is formed in fairly large quantities and that it is absorbed.

The early literature of the effect of administration of hydrogen sulphide to animals and men has been ably summarised by Mitchell and Davenport [1924]. The recent work that has been done on the pharmacological action of the gas is not relevant from our present point of view. Flint [1887] noticed that animals could withstand a certain critical concentration of the gas without apparent ill effect and suggested that they could oxidise it to some extent. This was confirmed and extended by Wilson [1894] who found that whereas 0·1 % of hydrogen sulphide in the atmosphere would kill a rabbit in half an hour, 0·025 % had no perceptible effect; Haggard *et al.* [1922], using anaesthetised dogs, came to similar conclusions. Haggard [1921], working *in vitro* with dog's blood, found that oxygenated blood destroyed relatively large amounts of hydrogen sulphide;

he did not determine what the end-product was but concluded that it was an acid since the CO_2-binding capacity of the blood was reduced. Becher [1927] stated that all body fluids could oxidise hydrogen sulphide to thiosulphate but he gave no experimental details, nor any data as to the rate at which this oxidation proceeded; he found that boiled blood and deproteinised blood were inactive. While Becher's conclusions are very plausible the absence of experimental details makes it difficult to assess their value. Sulphhaemoglobin, which most recent workers have found to be an artefact that does not occur *in vivo*, has been thoroughly studied by Keilin [1933]. The substance previously known as sulphhaemoglobin he looks upon as a non-dissociable complex made from oxidised haemoglobin and hydrogen sulphide or an oxidation product thereof; a simple addition compound is however made by methaemoglobin with hydrogen sulphide. It is unlikely that haemoglobin itself plays any part in the oxidation of hydrogen sulphide by blood. Denis and Reed [1927] found that sodium sulphide, when injected into an anaesthetised dog in lethal or nearly lethal doses, led to an increase in the inorganic sulphate of the serum. This increase, as might be expected, was larger when the kidneys were ligatured but was always small.

The oxidation of thiosulphate to sulphate when fed to normal men was studied by Trachtenberg [1861] and Nyiri [1923, 1]; they found that a 10 g. dose could be almost completely oxidised and that only about 5 % was excreted unchanged. Nyiri [1923, 2] used the excretion of unchanged thiosulphate after its intravenous injection as an index of kidney function, for he concluded that whereas normally 30–40 % was excreted unchanged, damaged kidneys excreted 0–23 %; he estimated the thiosulphate iodimetrically after treating the urine with animal charcoal. Holbøll [1925] criticised this method and pointed out that sometimes little or no thiosulphate was excreted by people with perfectly normal kidneys. Bacterial contamination of the urine was one cause of this but a more interesting one was increased basal metabolic rate. Holbøll gives figures for the excretion by patients with Graves' disease and other metabolic disturbances or febrile conditions. Höppener [1864] had previously found that less thiosulphate was excreted in the urine when a dose was fed during periods of fever or exercise than during periods of rest. The interpretation of the results obtained by the injection of thiosulphate is therefore difficult and the fact that, as will be shown in this paper, the oxidation can take place in both liver and kidney complicates the position still further.

A study of the processes involved in the oxidation of thiosulphate in the body is of importance, not because there is any reason to suppose that thiosulphate is a normal intermediate in the breakdown of the sulphur-containing constituents of food, such as methionine and cystine, in the body, but rather because it is an instance of the body's capacity to protect itself from poisoning by the products of intestinal putrefaction and because it is the first process of this sort that has been studied *in vitro*. The extent to which sulphur compounds are broken down to thiosulphate or hydrogen sulphide in the gut is not as yet known, but the fact that, in spite of its ready oxidisability, thiosulphate is frequently found in the urine makes it probable that this process accounts for a considerable fraction of the sulphur metabolism of the animal. This type of metabolism should be especially prominent with a very luxuriant intestinal flora, and it is significant that the rabbit has been found to excrete extra sulphate in its urine after the feeding of a much larger group of sulphur compounds than is effective in this way with the dog [Sherwin *et al.*, 1927; Pirie, 1932].

Experimental.

The technique used in the series of experiments already published [Pirie, 1934] has been altered in several respects in this series of experiments. It is unnecessary, when studying the oxidation of thiosulphate, to cut the tissue slices as thin as Warburg recommends, for there is no detectable difference in the amount of sulphate formed per mg. dry weight of tissue when the slices are cut as thinly as possible and when relatively coarse (0·5–0·7 mm.) slices are used. It is unnecessary also to use the complicated Ringer solution used by Krebs [1932], for this oxidation takes place as readily in a mixture of isotonic sodium chloride solution and isotonic sodium bicarbonate of the correct p_H. The p_H generally used was 7·6 but, as will be shown later, this need not be adhered to closely.

The method used for the estimation of sulphate is still somewhat unsatisfactory. In my previous paper the method of Cuthbertson and Tompsett [1931] was modified, and the precipitate of benzidine sulphate was washed on a filter instead of on the centrifuge. This change was made because of the difficulty experienced in making a small pellet of benzidine sulphate adhere to the end of a centrifuge-tube. This difficulty has now been overcome by the use of a faster centrifuge (3500 R.P.M.) and by washing the precipitate with 80 % acetone instead of with absolute acetone. In some recent experiments a relatively large amount of cystine and of certain tissue extractives has been precipitated along with the benzidine sulphate; this precipitate is very readily washed free from absorbed benzidine on the centrifuge but less easily on a filter. In the method of estimation used at present the technique of precipitating the benzidine sulphate and of estimating the benzidine colorimetrically is that described before, while the method of washing is that of Cuthbertson and Tompsett.

Benzidine forms a thiosulphate that is similar in many respects to its sulphate [Wahl, 1902]. This precipitates when there is more than 0·7 mg. of sodium thiosulphate in the 2 ml. sample on which sulphate is estimated. The thiosulphate is however much more soluble in 80 % acetone than benzidine sulphate and so is dissolved during the washing. The concentration of sodium thiosulphate may therefore be raised to 1 or 1·5 mg. in the 2 ml. sample without affecting the sulphate estimations. It is well known that thiosulphates are not stable in acid solution but break down to give free sulphur and a not very clearly defined group of sulphur acids; fortunately these breakdown products do not appear to precipitate with benzidine in dilute solution. Sulphate can therefore be estimated in the usual way in a solution from which the protein has been removed with trichloroacetic acid. The sulphite that is formed during the acid decomposition of thiosulphate is fairly rapidly oxidised to sulphate. The sulphate values will therefore be too high unless the estimations are started not more than half an hour after the removal of the protein. It would appear that there is no oxidation after the addition of the acetone and benzidine, for the same sulphate figures are obtained whether the estimations are left at this stage for 2 or 20 hours.

Oxidation of thiosulphate by rat tissues.

Some experiments on the oxidation of sodium thiosulphate by slices of rat tissue are collected in Table I *a*. Only liver, kidney and chorion have been found to bring about the oxidation rapidly under the conditions used, and the first is the most active. Brain, testis, spleen, blood, muscle (diaphragm) and placenta are inactive or only very feebly active. The livers used in Exps. 95 and 12

(Table I *a*) were from foetal rats about 2 and 5 days from term respectively. Clearly, foetal liver is active but less so than adult liver. It is obvious from Table I *a* that liver varies markedly in activity, and so far it has not proved possible to correlate this variation with differences in age, sex *etc.* in the rats.

The effect of inhibitors on this oxidation has not yet been studied in any detail, but the effect of a number of physical agents is recorded in the hope that at a later stage it may lead to an understanding of the mechanism of this oxidation.

Heating for 10 minutes to 60° inactivates the slices completely, and slices that have been kept at 0° for more than 2 days are inactive. These facts, taken in conjunction with the inactivity of organs other than liver and kidney, tend to confirm the theory that we have here to do with a real enzyme action and not simply with a catalytic oxidation. The enzyme system responsible for this oxidation is more stable than that concerned in the oxidation of cysteine to sulphate. Liver, for example, whether whole or sliced, will keep its activity almost unimpaired for 8–10 hours if kept cold, and the same slices which have already been used in one oxidation experiment lasting 4 hours may be used over again and show only a partial falling off in their activity. The liver used in Exp. 85 (Table I *a*) is a case in point for it had already been shaken for 4 hours at 37° with thiosulphate before Exp. 85 was put up.

A further instance of the relative stability of this system is given by its behaviour towards hypotonic solutions. Table I *b* illustrates this, for slices of the same organ are put up at the same p_H and for the same time but with the sodium chloride solution replaced by distilled water in some cases so that in these cases the tissue is suspended in a fluid with only one-sixth the normal osmotic pressure. In Exp. 75 both the oxidations were carried out under isotonic conditions but the slices used in the one marked "hypotonic" had been left with occasional shaking in distilled water for 3 hours before use. If a large volume of cold water is used and it is changed frequently the inactivation is greater. For example, the 60 mg. dry weight of slices used in Exp. 19 were washed for an hour with 250 ml. of ice-cold distilled water; after a second and a third washing with fresh cold water the activity was found to be only one-third of that of the untreated slices. Exposure to hypertonic solutions, *e.g.* 9·0 % sodium chloride solution, causes at 0° a rapid diminution in the activity of liver slices. The dry weights of tissue slices that have been shaken isotonically and hypotonically are not strictly comparable, for in the former case the wet weight is about 8·3 times the dry whereas in the latter it is about 10 times but variable. This difference in the amount of material soluble under the two conditions also shows up as a marked increase in the amount of material precipitable by trichloroacetic acid in the hypotonic fluid. Measurements of the dry weight of liver are also more or less invalid in all experiments done at a greater p_H than 8 or for periods longer than 2 hours. In spite of its apparent stability all attempts to make a preparation of this enzyme have so far been unsuccessful. Fine mincing of the liver causes a marked fall in the activity, as does freezing, while thorough grinding with sand leads to an almost complete loss of activity. It seems, therefore, that any damage to the cell structure leads to partial inactivation, and this is borne out by the fact that treatment with toluene or chloroform reduces markedly the capacity of liver to perform this oxidation. Mere subjection of the slices to high pressure on the other hand has little effect, and a sample that had been exposed to a pressure of 40 atmospheres of nitrogen for 90 minutes had Q 4·4 whereas the control Q was 5·9.

The kinetics of the oxidation of thiosulphate by rat-liver have been studied in some detail. Exps. 62 and 73 in Table I *a* show that, as might be expected,

the amount of sulphate formed is proportional to the time of action and to the amount of liver present. This regularity justifies the introduction of the symbol Q to indicate thousandths of a mg. of sulphur appearing in the form of sulphate per hour per mg. dry weight of tissue. Since Q varies with the concentration of substrate as well as with the nature of the substrate it is necessary to introduce a suffix to represent the concentration (expressed in thousandths of a mg. of sulphur per ml.) and nature of the substrate. The symbol for the conditions most commonly used in this paper, *i.e.* 3·5 mg. of hydrated sodium thiosulphate in 8 ml. of solution, is therefore $Q_{112\ \mathrm{thio}}$.

Table I *a*.

Exp. No.	Wt. of thiosulphate in 8 ml. (mg.)	Dry wt. of tissue (mg.)	Time of action min.	Wt. of sulphur oxidised to sulphate (γ)	Q
62	3·7	101 liver	30	140	2·7
	3·7	107	60	275	2·6
	3·7	83	120	420	2·5
73	4·1	10	120	55	2·7
	4·1	26	120	100	1·9
	4·1	53	120	225	2·2
85	3·5	100 (old)	120	265	1·3
90	3·5	33	64	130	3·7
95	3·5	23 } foetal	120	73	1·6
12	3·5	35 } liver	120	72	1·0
97	3·5	52	60	185	3·5
	3·5	73	60	225	3·1
63	3·7	82 kidney	120	245	1·5
85	3·5	40 kidney	120	200	2·5
12	3·5	20 chorion	113	78	2·1
15	3·5	17 chorion	90	64	2·5

Table I *b*.

62	3·7	Isotonic 83 liver	120	420	2·5
		Hypotonic 63	,,	400	3·2
75	3·3	Isotonic 45	120	175	2·0
		Hypotonic 76	,,	200	1·3
84	3·5	Isotonic 47	120	225	2·3
		Hypotonic 43	,,	166	1·9
85	3·5	Isotonic 40 kidney	120	200	2·5
		Hypotonic 25	,,	105	2·1
90	3·5	Isotonic 33 liver	60	130	3·7
		Hypotonic 35	,,	145	4·1
94	3·5	Isotonic 56	60	180	3·2
		Hypotonic 56	,,	120	2·1
19	3·5	Isotonic 45	60	270	6·0
	3·5	Hypotonic 60	,,	114	1·9

Table I *c*.

62	3·7	83 liver	120	420	2·5
	1·8	77	120	310	2·0
73	4·1	5·3	120	225	2·2
	2·0	75	120	200	1·3
	0·8	56	120	105	0·9
97	3·5	52	60	185	3·5
	2·7	43	60	148	3·4
	1·8	40	60	110	2·7
	0·9	42	60	65	1·5

The effect of changes in the concentration of thiosulphate is rather less simple, for the amount of sulphate formed is not quite doubled when the thiosulphate concentration is doubled. This is shown in Table I *c*. It is clear from this table that in any experiment in which much of the thiosulphate initially present has been oxidised the Q value will be smaller than it should be on account of the fall in substrate concentration. For this reason and also on account of the progressive inactivation of slices at 37° it is advisable to calculate Q values from short (1 hour) experiments.

At p_H values between 7·3 and 8 the rate of formation of sulphate is approximately constant but it falls off suddenly on either side of this range. On the acid side this diminution in activity is complicated by the spontaneous oxidation of the thiosulphate at a slow rate in even weakly acid solution. Two typical experiments on rat-liver are recorded in Table II in one of which only the

Table II.

p_H	Wt. of thiosulphate (mg.)	Dry wt. of rat-liver (mg.)	Time mins.	Wt. of sulphur oxidised to sulphate (γ)	Q
6·7	3·5	72	60	120	1·6 (1·8)
7·0	,,	69	,,	120	1·7 (2·6)
7·3	,,	65	,,	190	2·9 (2·9)
7·6	,,	73	,,	225	3·1 (3·0)
7·9	,,	62	,,	230	3·7 (3·1)
8·2	,,	(51)	,,	185	—
		Guinea-pig-kidney			
6·6	3·5	66	60	75	0·8
6·8	,,	61	,,	72	0·9
7·1	,,	95	,,	105	0·8
7·7	,,	79	,,	148	1·4
8·0	,,	57	,,	140	1·8
8·3	,,	(43)	,,	75	—

Q values are given. The p_H was controlled by varying the ratio of isotonic sodium bicarbonate to isotonic sodium chloride and, in the case of the more alkaline solutions, by using 97·5 % oxygen and 2·5 % carbon dioxide in place of the usual 95 % oxygen and 5 % carbon dioxide mixture.

In a few experiments the amount of thiosulphate disappearing was measured as well as the amount of sulphate formed. As can be seen in Table III the

Table III.

Wt. of thiosulphate in 8 ml. (mg.)	Dry wt. of rat-liver (mg.)	Time of action (mins.)	Sulphur in thiosulphate oxidised (γ)	Sulphur in sulphate formed (γ)	Q
3·5	71	240	520	430	1·5
,,	60	120	248	230	1·9
,,	56	60	146	180	3·2
,,	50*	60	180	170	3·4
,,	56†	60	130	120	2·1

* Slices cut very thinly. † Hypotonic.

agreement is reasonably good. The thiosulphate was estimated by removing the protein from a sample of the reaction mixture with zinc sulphate and sodium hydroxide as in the blood-sugar method of Hagedorn and Jensen. An excess of an acid solution of iodine in potassium iodide was then added and the excess

titrated with standard thiosulphate solution. Tissue extractives such as glutathione will of course interfere, but under the aerobic conditions used in these experiments such substances will be largely in the oxidised state, and in any case they are quantitatively insignificant. A more serious error is caused by the fact that only one-third or one-fourth of the thiosulphate is oxidised in most experiments and one is therefore measuring, rather inexactly, a small change in a fairly large quantity. It is clear from Table III that, allowing for the probable errors, there is no evidence for the existence, under these conditions, of any process leading to the destruction of appreciable amounts of thiosulphate except the formation of sulphate. This demonstration raises the question of the possible rôle of the best known oxidation product of thiosulphate, namely, sodium tetrathionate, in this oxidation. Sodium tetrathionate was prepared in the usual way by pouring a strong aqueous solution of sodium thiosulphate into an alcoholic solution of iodine. The precipitated tetrathionate was washed well with alcohol, taken up in water and precipitated again with alcohol; it had the theoretical sulphur content. As Table IV *a* shows, sodium tetrathionate is oxidised to sulphate by rat-liver and kidney but at a very much slower rate, considering the amount of sulphur that is present in 1 ml. of solution, than sodium thiosulphate, *i.e.* $Q_{143\,\mathrm{tetra}}$ is 1·4–3·2 whereas $Q_{112\,\mathrm{thio}}$ is 2·5–5·9. It is therefore unlikely that tetrathionate is an intermediate in the oxidation of thiosulphate to sulphate. It is possible that oxidation to sulphate is preceded by a reduction of the tetrathionate to thiosulphate. I showed in the earlier paper that such a preliminary reduction took place in the case of the oxidation of cystine but so far I have been unable to demonstrate it in this case. It is unlikely that such a reduction takes place, for the ratio $Q_{\mathrm{thio}}/Q_{\mathrm{tetra}}$ appears to be constant irrespective of the treatment that the slices of liver have undergone. In the case of cystine, on the other hand, it was found that the capacity of liver slices to reduce cystine to cysteine was strictly limited and that this limited the production of sulphate. The analogy is of course not wholly satisfactory, since thiosulphate, unlike cysteine, cannot undergo aerobic re-oxidation, and so it might be expected that it would be easier for the liver to maintain the concentration of the reduced substance. When incubated anaerobically with liver slices, sodium tetrathionate does not give rise to any appreciable amount of thiosulphate. Some further experiments illustrating this type of oxidation are given in Table IV *b*. All the experiments lasted for an hour and were carried out in 97·5 % O_2, 2·5 % CO_2, but the slices used in Exps. *c* and *d* are those that had already been used in *a*, while those used in *e* and *f* had already been used in *b*. Clearly the $Q_{\mathrm{thio}}/Q_{\mathrm{tetra}}$ ratio is approximately constant, and previous exposure to tetrathionate leads to no greater loss of activity than exposure to thiosulphate. The other thionic acids have not been tried, and sodium sulphite is so rapidly autoxidised in the absence of tissue, and still more in the presence of boiled tissue, that a study of its enzymic oxidation is not possible by this technique.

The falling off in the activity of a tissue during the second hour of shaking is especially marked in the case of livers which are initially very active. Such a fall is clearly seen in Tables III and IV *b* and is analysed more fully in Table IV *c*. There are three possible explanations of this phenomenon; firstly it may simply be the normal ageing process accelerated by the higher temperature at which the liver has been kept; secondly it may be due to the using up of a metabolite during the course of the oxidation of thiosulphate; and thirdly it may be due to the loss by diffusion of a soluble substance, either a metabolite or a co-enzyme, essential for sulphate formation. The results recorded in Table IV *c* are strongly

Table IV *a*.

Exp. No.	Wt. of tetrathionate (mg.)	Dry wt. of tissue (mg.)	Time of action (mins.)	Wt. of sulphur oxidised to sulphate (γ)	Q
84	2·4	56 liver	120	165	1·4
	2·4	45 liver heated to 70°	,,	5	—
85	2·4	42 kidney	,,	95	1·1
	2·4	28 kidney heated	,,	6	—
	2·4	No tissue	,,	0	—
90	2·7	42 liver	64	92	2·0
	5·4	40	,,	125	2·9
	1·3	41	,,	70	1·6
	2·7 (hypotonic)	43	,,	108	2·3
3	2·4	91 liver	60	160	1·8
4	2·4	37 liver	60	45	1·2

Table IV *b*.

7 *a*	3·5 thio.	53 liver	60	315	5·9
b	2·4 tetra.	57 liver	,,	185	3·2
c	3·5 thio.	24 liver	,,	73	3·0
d	2·4 tetra.	29 liver	,,	45	1·5
e	3·5 thio.	22 liver	,,	75	3·2
f	2·4 tetra.	35 liver	,,	55	1·6

Table IV *c*.

11 *a*	3·5 thio.	43 liver	60	240	5·6
b	3·5	43	120	400	4·7
c	3·5	43	60	185	4·3
d	3·5	37	,,	160	4·3
e	3·5	29	,,	115	4·0
f	3·5	45	75	120	2·1
g	3·5	28	,,	55	1·6
12 *a*	3·5	59	60	95	1·6
b	3·5	52	,,	102	2·0

in favour of the first theory. Exps. *a* and *b* were carried out in the usual way for periods of 1 and 2 hours respectively. Exp. *c* is a 1 hour experiment on the slices that had already been used in Exp. *a*. This gives $Q=4{\cdot}3$ instead of the initial 5·6. The Q values found in Exps. *d* and *e* should be compared with this value for in each case the slices underwent a preliminary shaking for one hour in thiosulphate-free bicarbonate-saline. After this, thiosulphate was added to the one (*d*) while the slices were removed from the other, rinsed and added to fresh solution containing thiosulphate. Clearly the values of Q in Exps. *c*, *d* and *e* are very similar; this shows that the presence of thiosulphate leads to no increase in the rate of disappearance of essential metabolites, for if it did Q in Exp. *c* would be smaller than in Exps. *d* or *e*. Similarly there is little loss by diffusion for if there were *e* should be smaller than *d*. Exps. *f* and *g* were carried out in the same way but with 2 hours' preliminary shaking at 37°. In Exp. *f* the oxidation was carried out in the fluid in which the slices had previously been shaken for 90 minutes, while in *g* fresh fluid was used. The difference, though definite, is small enough to indicate that this is not an important cause of loss of activity. If a gas mixture consisting of 95 % N_2 and 5 % CO_2 is used for the preliminary shaking, it is found that the slices are completely inactivated in an hour whether thiosulphate is present or not. Anaerobic incubation for half an hour causes nearly complete inactivation. Other oxidising systems are

already known which are stable only in the presence of oxygen, but this one is specially interesting since, as will be shown later, the oxidation of thiosulphate does not lead to any increase in the oxygen consumption of the slices. On account of this inactivation there is no anaerobic formation of sulphate. The presence of $M/4000$ methylene blue and $M/200$ nitrate leads neither to the production of sulphate, although the methylene blue is kept in the oxidised state by the nitrate-reducing system in the liver, nor to an increase in the stability of the enzyme system. Methylene blue and nitrate, whether singly or together, have also no effect on the aerobic sulphate production.

Manometric experiments with rat-liver.

In collaboration with Dr H. A. Krebs of this laboratory some experiments on the influence of thiosulphate on the oxygen uptake of liver have been carried out. These have led to the unexpected result that, under conditions where oxygen is appearing in the form of sulphate at a rate corresponding to one-third of the total respiration, there is no apparent increase in the oxygen consumption. Table V gives the results of one such experiment. The fluid used was Ringer

Table V.

Wt. of tissue (mg.)	Wt. of thiosulphate in 3 ml. Ringer (mg.)	Wt. of sulphur oxidised to SO_4 per hour (γ)	Q_{thio}	Oxygen equivalent of this sulphur reduced to 40 mins. (μl.)	Oxygen consumption in 40 mins. (observed) (μl.)	Q_{O_2}
7·9	1·3	27	3·4	15·7	44·7	8·5
9·7	1·3	31	3·2	18·0	56	8·7
16·1	None	—	—	—	95	8·85
7·9	None	—	—	—	45·5	8·6

solution made up according to Krebs [1932] but with magnesium sulphate replaced by magnesium chloride and with only 2 ml. of 1·2 % sodium bicarbonate per 100. Such a solution maintains a suitable p_H when used in conjunction with pure oxygen and with a side-bulb to absorb CO_2. Sulphate estimations were done on the reaction fluid in the usual way and the oxygen equivalent is calculated on the basis of 5 oxygen atoms to 2 atoms of sulphur as required by theory. This value is multiplied by 2/3 since the oxygen absorbtion was only followed for 40 minutes while the tissue was in contact with thiosulphate at 37° for an hour. The constancy in the values of Q_{O_2} in the four experiments recorded shows that no extra oxygen can have been taken up during the oxidation of the thiosulphate.

Oxidation by the tissues of other animals.

A few experiments have been made with other animals. It is unnecessary to give the results in detail and the Q values only are recorded.

	Q
Ox-kidney (2–3 hours after slaughter)	1·3
Rabbit-liver...	1·3
Rabbit-kidney	0·65
Guinea-pig-kidney	1·1–2·7
Pig-liver (2–3 hours after slaughter)	1·0
Dog-kidney (after chloroform)	0·6
Dog-liver (after chloroform)	0·8

All these experiments were done at p_H 7·6 and with 112γ sulphur present in the form of thiosulphate per ml. The effect of changes in p_H on the activity of

guinea-pig-kidney is recorded in Table II whence it is clear that the enzyme system found there is, from this point of view, similar to that found in rat-liver. The other tissues have not been studied further.

Oxidation by goose-kidney.

Goose-kidney resembles rat-liver closely in its ability to oxidise thiosulphate to sulphate. The results of a few experiments are given in Table VI; these were

Table VI.

Exp. No.	Wt. of thiosulphate (mg.)	Wt. of goose-kidney (mg.)	Time of action (mins.)	Wt. of sulphur oxidised to SO_4 (γ)	Q
78	4·0	90	120	650	3·6
79	4·0	55	120	360	3·3
	4·0	25	120	160	3·2
	2·4	68	120	280	2·1
	1·2	70	120	180	1·3
88 p_H 6·7	3·5	72	120	385	2·8
p_H 7·3	3·5	46	120	300	3·3
p_H 7·7	3·5	65	120	410	3·2
p_H 8·0	3·5	54	120	380	3·5

carried out in the usual way at p_H 7·6 but using a mixture of 1·2 % sodium bicarbonate and 1·1 % sodium chloride as the reaction fluid. As with rat organs no sulphate is made by slices that have been heated to 60° nor if the slices are shaken with thiosulphate anaerobically. Freezing and grinding cause a very marked diminution in the activity while the effect of treatment with distilled water is definite though less marked.

Goose-kidney also resembles rat-liver and kidney in its ability to oxidise cysteine and methionine to sulphate; this oxidation will be described in a later paper.

Discussion.

Although the oxidation of thiosulphate by bacteria was first observed many years ago the mechanism of the process is still obscure. Several species of bacteria are known which have this power. Lieske [1912] and Guittonneau [1925, 1, 2] have isolated such organisms from pond mud and soil but they did not study the oxidation in any detail. Nathanssohn [1902] and Trautwein [1921; 1924] have studied similar bacteria and find that the oxidation proceeds through tetrathionate and dithionate. Beijerinck [1904], on the other hand, working with *Thiobacillus thioparus*, found that tetrathionate was less readily oxidised than thiosulphate. The behaviour of this organism is therefore analogous to that of liver. A comparative study of the properties and stability of the enzyme systems derived from these bacteria and from liver would be very interesting.

Thiosulphate can scarcely be oxidised directly, for in that case 5 atoms of oxygen would have to be present simultaneously for the oxidation of one molecule of thiosulphate; furthermore, if the oxidation were direct it would be hard to see why it should cause no increase in the oxygen consumption of liver slices. The irreversible inactivation of liver slices on exposure to nitrogen is especially interesting in this connection. It is known that the respiration of cells is, in general, irreversibly inhibited by anaerobic incubation and Warburg *et al.* [1926] have found that even tumour tissue will not respire after 4 hours without oxygen or glucose. These facts suggest that the oxidation of thiosulphate

is a phenomenon which parallels closely the fundamental oxidation processes of the cell, but the connection is incomplete since some organs, with relatively high respiration rates, cannot bring about this oxidation. All attempts to reactivate liver that has been inactivated in this way have so far been unsuccessful but further work on this problem is in progress.

Summary.

Rat-liver, kidney and chorion, and goose-kidney, can oxidise sodium thiosulphate to sulphate.

This oxidation is accompanied by no perceptible increase in the oxygen consumption of the tissue slices, but it will not proceed anaerobically. The slices are irreversibly inactivated by anaerobic incubation.

Sodium tetrathionate is also oxidised, but reasons are given for thinking that it is not an intermediate.

The reason for the loss of activity by the slices on keeping and the effects of some physical agents on the enzyme system have been investigated.

The liver and kidney of some other animals have been tested and found to be active but relatively weak.

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