CLXXXIV. THE AUTOCATALYTIC OXIDATION OF SULPHYDRYL COMPOUNDS.

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IN previous papers by Warburg and Sakuma [1923], by Toda [1926] and by the author [Harrison, 1924, 1927], it was shown that minute traces of certain catalytic metals such as iron are capable of producing a great acceleration in both the aerobic and the anaerobic oxidation of sulphydryl compounds, such as cysteine and glutathione. When this catalytic effect of the metals was prevented, either by careful purification of the sulphydryl compounds from traces of metallic impurities, or by forming a non-catalytic complex of the metals with potassium cyanide, the rate of uptake of oxygen or the rate of reduction of methylene blue by the sulphydryl compounds was found to be very much reduced. It had been claimed by Dixon and Tunnicliffe [1923] that the oxidation of sulphydryl compounds was autocatalytic. By measuring the rate of reduction of methylene blue by sulphydryl compounds, they had obtained the S-shaped curves typical of autocatalytic reactions. Further, they had shown that the aerobic and anaerobic oxidation of a sulphydryl compound was accelerated by the addition of the oxidised, *i.e.* disulphide, form, either of the same or of some other sulphydryl compound. When it was found, as indicated above, that the presence of metallic impurities ordinarily present in sulphydryl compounds exerted such a marked effect in determining the velocity of both the aerobic and anaerobic oxidation of the sulphydryl compounds, the possibility suggested itself that the accelerating effect on the aerobic and anaerobic oxidation of the SH group observed by Dixon and Tunnicliffe when a disulphide was added to the solution might in reality be due to the catalytic action of traces of metals such as iron and copper present in the added disulphide, rather than to a catalysis by the disulphide *per se*. The criticism of such a theory that it would not explain the autocatalytic curves obtained by Dixon and Tunnicliffe from measurements of the rate of reduction of methylene blue by sulphydryl compounds, does not necessarily carry much weight, since Dixon and Tunnicliffe, in a note at the end of their paper, stated that the autocatalytic nature of the curve was due partly to an initial retardation of the reaction by traces of oxygen present in the vacuum tubes. This oxygen tended to reoxidise the methylene white and led to a retardation in the initial velocity of the decoloration of the methylene blue. On taking greater precautions to remove

traces of oxygen, the curves were found to become more linear, though their autocatalytic nature never completely disappeared. It was suggested by the author [Harrison, 1924] and by Oppenheimer [1926] that traces of iron impurities might be playing a part in the catalytic effects observed by Dixon and Tunnicliffe. When, on studying the catalytic action of metals on the oxidation of the SH group, it was found that a marked acceleration was produced by concentrations of iron and copper of the order of 10^{-5} mg. per cc., or even less, it seemed worth while to attempt to find whether the catalysis observed by Dixon and Tunnicliffe was a catalysis by the disulphide itself, or was due to the catalytic action of metallic impurities present in the disulphide compound.

The following experiments suggested themselves as means of deciding this question.

(a) To prepare a pure disulphide compound taking every possible precaution to remove traces of metallic impurities, and to try whether this purified compound were still capable of catalysing the oxidation of sulphhydryl compounds.

(b) To find whether the catalysis were affected by the addition of cyanides, which form non-catalytic complexes with catalytic metals.

(c) To destroy the —S—S— linkage by acting on the carefully purified disulphide with a suitable reagent, and then to test whether the resulting product still possessed catalytic properties. If, after this treatment, the catalysis were still produced, it would be evident that it could not be a property of the disulphide group.

EXPERIMENTAL.

The preparation of metal-free dithiodiglycollic acid.

Dithiodiglycollic acid was chosen as the most suitable disulphide, since it could be prepared by oxidising thioglycollic acid with iodine [Bilmmann, 1905] and both the thioglycollic acid and the iodine could be obtained metal-free by distillation in quartz vessels. The iodine was purified by distillation from quartz in a current of steam. (This method was used in preference to the sublimation method of Toda [1926], since the risk of traces of metallic halides distilling over in the iodine vapour was minimised.) All the solvents used in the preparation were first distilled from quartz. 7.3 g. of thioglycollic acid (previously distilled 3 times from quartz at 16 mm. Hg pressure) was dissolved in 10 parts of pure water, and pure iodine was slowly added with shaking until a very slight excess was present. The solution was shaken with 3 volumes of ether, the ether layer being then carefully poured off and shaken with a little water. After standing, the ether layer was again poured off, the ether was evaporated and the residue dried at 100° in a quartz dish until nearly all the excess of iodine had been removed. The residue was dissolved in the minimum quantity of hot ethyl acetate, treated with 9 volumes of benzene

and allowed to crystallise. After being washed by decantation three times with a mixture of ethyl acetate and benzene, the crystals were dissolved in hot ethyl acetate and the solution poured into a quartz dish, the solvent being evaporated in a vacuum desiccator. 2.9 g. of perfectly colourless, crystalline dithiodiglycollic acid were obtained, melting at 106–107°.

The catalytic action of metal-free dithiodiglycollic acid.

Experiments were then carried out to find whether the purified dithiodiglycollic acid behaved like the unpurified disulphides in being capable of accelerating the reduction of methylene blue by sulphydryl compounds. The glass vacuum tubes employed in these experiments were well cleaned each time before use in the manner previously described [Harrison, 1927]. In all the experiments on the reduction of methylene blue, 0.3 cc. of a 1 in 5000 aqueous solution of the dye was used in each vacuum tube. The dithiodiglycollic acid was dissolved in pure water and neutralised with dilute ammonium hydroxide, previously distilled in quartz vessels. The alkali was added from a quartz burette, the amount required being determined by a separate titration. The required volume of neutralised dithiodiglycollic acid was added from the quartz burette to a mixture of 0.3 cc. of 1 in 5000 methylene blue solution, 0.1 cc. of neutralised sulphydryl compound (cysteine, thioglycollic acid or glutathione), and sufficient phosphate buffer of the required p_H to make the total volume of solution in each tube equal to 2.0 cc. In the control tubes, instead of the solution of dithiodiglycollic acid, an equal volume of pure water was added. In many of the experiments employing dithiodiglycollic acid, the latter was neutralised with sodium hydroxide instead of ammonia, since it was found that by using the former reagent the solution could be more easily maintained at the required p_H . The sodium hydroxide used was a very pure specimen made from sodium, and in every experiment the same amount of alkali, neutralised with quartz-distilled hydrochloric acid, was added to the control tube. A second control tube was used in which pure water replaced the neutralised alkali. In every case the two controls became decolorised at the same time, showing that the sodium hydroxide used to neutralise the disulphide did not contain impurities which in any way affected the rate of reaction. No special precautions were taken to obtain the sulphydryl compounds, methylene blue or phosphate buffer free from the last traces of metal impurities, since the same amount of these substances was added to both the experimental and the control tubes. The tubes were all evacuated together by means of the water-pump and filled with nitrogen (washed with alkaline pyrogallol). This was repeated and the tubes were finally evacuated completely and immersed in a water-bath maintained at the required temperature. The p_H was checked at the end of each experiment by introducing a few drops of the appropriate indicator through the side tube of the vacuum tube. The tubes in the bath were illuminated as uniformly as possible and protected from direct sunlight, since the latter appears to

accelerate the reaction. The experiments were carried out in duplicate, and the reduction times of duplicate tubes showed good agreement.

Table I gives a comparison of the time required for the reduction of methylene blue by thioglycollic acid and glutathione in the absence and in the presence of metal-free dithiodiglycollic acid (R.S.S.R.).

Table I.

R.S.S.R. = metal-free dithiodiglycollic acid. Temp. = 37°.

No.	Sulphydryl compound	p_H	Time in minutes for decoloration of methylene blue	
1	Thioglycollic acid (1.2 mg.)	7.4	Without disulphide	145
			+ 4 mg. R.S.S.R.	50
2	Thioglycollic acid (1.2 mg.)	7.6	Without disulphide	110
			+ 4 mg. R.S.S.R.	45
3	Thioglycollic acid (1.6 mg.)	7.6	Without disulphide	63
			+ 4 mg. R.S.S.R.	23
4	*Thioglycollic acid (2.4 mg.)	7.4	Without disulphide	280
	" "	"	+ 4 mg. R.S.S.R.	27
	" "	"	+ 8 mg. R.S.S.R.	10
5	Glutathione (1.2 mg.)	7.6	Without disulphide	53
	" "	"	+ 4 mg. R.S.S.R.	29

* Freshly distilled thioglycollic acid.

The experimental results given in Table I are typical of a large number which have been obtained. It will be observed that a large acceleration in the rate of reduction of methylene blue by the sulphydryl compounds is produced by the addition of the purified dithiodiglycollic acid. What appears still more significant is the fact that the acceleration produced by a given amount of the metal-free disulphide is of the same order as that recorded by Dixon and Tunnicliffe using the unpurified disulphide. If the acceleration produced by the purified disulphide were due to minute traces of catalytic metals which had escaped removal, it would have been expected that the catalysis observed on adding the purified disulphide would have been of a much lower order than that produced by the unpurified, metal-containing disulphide. The fact that no appreciable reduction in catalytic power occurs, even after very careful purification, would appear to afford strong evidence that the catalysis observed is a property of the disulphide itself. The fact that the metallic impurities in the disulphide used by Dixon and Tunnicliffe caused no appreciable additional catalytic effect is probably due to the quantity of catalytic impurities already present in their unpurified —SH compounds. Still stronger evidence on these lines is given in Exp. 4 in Table I, where, using a freshly distilled specimen of thioglycollic acid with a relatively slow reducing power, the relative acceleration produced by the purified dithiodiglycollic acid was very much greater than that observed by Dixon and Tunnicliffe, using unpurified compounds. The relatively slow rate of reduction of methylene blue by the freshly distilled thioglycollic acid alone is probably due not only to removal of catalytic metals, but also to the removal, by distillation, of any traces of disulphide which might be present owing to slow oxidation of the thioglycollic acid. This would explain the relatively greater effect

produced by added disulphide. Two different preparations of dithiodiglycollic acid were made by the method given above, and both accelerated to a similar extent. This is in itself evidence that the catalysis is not due to impurities, since it is unlikely that two different preparations of the disulphide would contain the same amount of impurity.

Table II shows the effect of addition of pure dithiodiglycollic acid to sulphydryl compounds in presence of cyanide. In experiments employing potassium cyanide, a fresh solution of the latter was made up every day, and neutralised with HCl immediately before use.

Table II.

R.S.S.R. = metal-free dithiodiglycollic acid.

No.	Sulphydryl compound	p_H	Temp.	KCN	Time in minutes for decoloration of methylene blue
1	Thioglycollic acid (1.2 mg.)	7.6	37°	M/500	Without disulphide 102 + 4 mg. R.S.S.R. 62
2	*Thioglycollic acid (2.4 mg.)	7.4	37°	M/1000	Without disulphide 410 + 8 mg. R.S.S.R. 17
3	*Thioglycollic acid (2.4 mg.)	7.4	37°	M/500	Without disulphide 420 + 4 mg. R.S.S.R. 35
4	Cysteine (0.8 mg.)	7.6	25°	M/400	Without disulphide 110 + 4 mg. R.S.S.R. 65
5	Glutathione (1.2 mg.)	7.6	25°	M/500	Without disulphide 173 + 4 mg. R.S.S.R. 46

* Freshly distilled.

It is evident from Table II that the acceleration produced by pure dithiodiglycollic acid can still take place even in the presence of cyanide. Moreover, using freshly distilled thioglycollic acid as the —SH compound, the acceleration produced by the disulphide is relatively greater in the presence of cyanide than in its absence. This again affords strong evidence that the acceleration by dithiodiglycollic acid is a true property of the disulphide group itself. Potassium cyanide is well known to form non-catalytic complexes with catalytic metals such as iron and copper, and, if the observed acceleration were due to minute traces of metallic impurities which had escaped removal during purification of the disulphide, one would expect the addition of cyanide to prevent such an acceleration.

The effect of destroying the —S—S— group by bromine.

An attempt was now made to destroy the —S—S— group in the pure dithiodiglycollic acid, which, as has been shown above, is capable of catalysing the oxidation of the sulphydryl group. If, after destroying the disulphide group, the resulting product no longer catalysed the reduction of methylene blue by sulphydryl compounds, additional strong evidence would be afforded that the catalysis is a property of the disulphide group itself. Bromine was chosen as the reagent for destroying the —S—S— group in dithiodiglycollic acid by oxidation. Preliminary experiments showed that an aqueous solution of dithiodiglycollic acid readily reacts with bromine in the cold, the amount of bromine required corresponding with the formation of sulphoacetic acid.

This is analogous to the oxidation by bromine of cystine to cysteic acid.

The product no longer gave a nitroprusside reaction after treatment with zinc dust and HCl, showing that all the disulphide had been destroyed.

A dilute aqueous solution of pure dithiodiglycollic acid containing 4 mg. per cc. was treated with a slight excess of quartz-distilled bromine, the product being warmed to 110° in a quartz dish till the HBr was removed. The product was then neutralised with pure NaOH, water being added until 1 cc. of the solution contained the oxidised product from 4 mg. of dithiodiglycollic acid. The effect of 1 cc. of this solution (in which the —S—S— linkage has been destroyed by bromine) on the rate of reduction of methylene blue by thioglycollic acid was then compared with (a) the rate of reduction of methylene blue by thioglycollic acid alone; and with (b) the rate of reduction of methylene blue by thioglycollic acid in presence of 4 mg. of dithiodiglycollic acid (not treated with bromine).

The following results were obtained.

Time for reduction of methylene blue by thioglycollic acid (1.2 mg.)								
$p_{\text{H}}=7.4$ Temp. = 37°								
(a)	Alone ...	...	...	...	...	...	...	143 min.
(b)	4 mg. dithiodiglycollic acid (—S—S— linkage intact) ...							
(c)	Product of 4 mg. of dithiodiglycollic acid treated with							
	bromine (—S—S— linkage destroyed) ...	...	...	...	...	...	...	145 ..

These results show that after destroying the disulphide linkage in dithiodiglycollic acid with bromine, the product has no power whatever to catalyse the anaerobic oxidation of the sulphydryl group. Had the catalysis been due to metallic impurities, these would have remained in the solution after treatment with bromine, and the catalytic power of the solution would not have been destroyed. The fact, that after destruction of the —S—S— group by bromine the catalysis is no longer manifested, clearly indicates that the catalysis is a function of the disulphide group itself.

Table III shows the effect of increasing concentrations of pure dithiodiglycollic acid on the rate of reduction of methylene blue by freshly distilled thioglycollic acid.

Table III.

Added dithiodiglycollic acid (mg.)	Temp. = 37°	$p_{\text{H}}=7.9$
	Time for decoloration of methylene blue by thioglycollic acid (2.4 mg.), min.	
—	275	
0.4	150	
1.0	80	
2.0	30	
4.0	27	

It is evident that marked acceleration is produced in the rate of anaerobic oxidation of thioglycollic acid by relatively small concentrations of pure

dithiodiglycollic acid. The fact that 4 mg. of the disulphide produces very little more acceleration than 2 mg. is probably explained by the effects of mass action, the catalytic action of the disulphide being opposed by the tendency of the latter to drive the reaction in the opposite direction. A similar effect was observed by Dixon and Tunnicliffe.

Table IV shows the catalytic effect of pure dithiodiglycollic acid at different hydrogen ion concentrations.

Table IV.

Temp. = 37°

p_H	Dithiodiglycollic acid (mg.)	Time for decoloration of methylene blue by thioglycollic acid (2.4 mg.), min.
6.0	—	255
„	4	125
6.8	—	255
„	4	45
7.4	—	150
„	4	20

The results show that as the solution becomes more acid there is a falling off of the relative acceleration produced by the disulphide. Dixon and Tunnicliffe also mention this.

The catalysis produced by dithiodiglycollic acid cannot be attributed to an appreciable increase in the concentration in the —SH group, brought about by hydrolysis at the —S—S— linkage in the weakly alkaline solutions, since it was found that dithiodiglycollic acid alone showed no ability to reduce methylene blue even on prolonged standing in the bath at 37°. Further, in some of the experiments, using tubes with and without added disulphide, the tubes were opened, 0.1 cc. of concentrated HCl was quickly added to each (to prevent further oxidation), and the solutions were titrated with *N*/100 iodine, using starch as an indicator. The tubes containing added disulphide were found to reduce practically the same amount of iodine as those containing no added disulphide. This shows that any hydrolysis of the disulphide which occurs is of so slight an extent that the amount of —SH formed, in comparison with that already present, is too small to be detected by the above experiment. Even in the presence of KCN, which is known to increase the hydrolysis of the —S—S— group, no decoloration of methylene blue was observed. (5.2 mg. dithiodiglycollic acid, *M*/200 KCN, methylene blue and phosphate buffer of p_H 8.0.)

Ample justification for undertaking the work described in this paper is afforded by the fact that ordinary specimens of so-called pure substances chosen at random, differing as widely as succinic acid and ammonium chloride, were found to be capable of causing an acceleration in the anaerobic oxidation of thioglycollic acid of a similar order to that produced by an equal weight of dithiodiglycollic acid. After subliming the succinic acid as succinic anhydride into a quartz dish and neutralising with pure sodium hydroxide, it was found to be no longer capable of producing any acceleration in the reaction.

The acceleration produced by ammonium chloride did not disappear after sublimation into quartz vessels, but a specimen prepared by neutralising quartz-distilled hydrochloric acid with quartz-distilled ammonia did not produce any acceleration in the reduction of methylene blue by thioglycollic acid. (Probably the failure to destroy the catalytic power of ammonium chloride by sublimation was due to the simultaneous sublimation of the halides of catalytic metals in the atmosphere of dry HCl produced by the dissociation of the ammonium chloride.) It is not surprising that so-called pure substances should be capable of producing such an acceleration in the oxidation of sulphydryl compounds, when one considers the powerful catalytic effect produced on the reaction by extraordinarily minute traces of such metals as copper and iron.

The catalytic action of metal-free dithiodiglycollic acid on the aerobic oxidation of sulphydryl compounds.

Experiments were carried out to find whether the metal-free dithiodiglycollic acid was capable of catalysing the oxidation by oxygen of thioglycollic acid and cysteine. The oxygen uptake was measured by means of the Barcroft microrespirometer, the apparatus being shaken in a water-bath at 37°. The bottles of the apparatus were made of quartz, and were thoroughly cleaned with acids before use. The total volume of liquid in each bottle was 3.0 cc., this volume being made up with phosphate buffer. The dithiodiglycollic acid was neutralised with pure NaOH, an equal amount of the alkali, neutralised with quartz-distilled HCl, being used in the control apparatus.

Fig. 1 shows the catalytic effect produced on the aerobic oxidation of cysteine by the addition of pure dithiodiglycollic acid. It is evident that a large acceleration is produced by the addition of the pure disulphide, and, further, by comparing curves *B* and *C*, it can be seen that an acceleration is brought about by the disulphide even in presence of *M*/900 cyanide. It is very significant that curves *A* and *B* show distinctly the S-shaped, sinuous form characteristic of an autocatalytic reaction. Dixon and Tunnicliffe failed to obtain any such evidence of the autocatalytic nature of the reaction in any of their experiments on the aerobic oxidation of sulphydryl compounds. In the case of cysteine, they explain this by the fact that the disulphide formed, namely, cystine, is insoluble, and, being precipitated from the solution, is unable to catalyse the oxidation of the remaining cysteine. In the present experiment, such a precipitation of cystine was observed during the oxidation of the control, containing no added disulphide (Curve *C*), but it was interesting to find that in the two solutions containing added disulphide (Curves *A* and *B*), no precipitation of cystine could be observed, even at the end of the reaction. This ability of dithiodiglycollic acid to prevent the precipitation of cystine is probably due to a mutual interaction of the two disulphides, cystine and dithiodiglycollic acid, of the nature:

leading to the formation of a soluble product. Hence, during the reaction, the concentration of soluble disulphide is continuously increasing, and one observes not only the catalytic effect of added dithiodiglycollic acid, but also the autocatalytic effect of the increasing concentration of cystine formed during the reaction and held in solution by the dithiodiglycollic acid. The fact that such an autocatalytic curve has now been obtained for the *aerobic* oxidation of a sulphhydryl compound affords almost conclusive evidence that the oxidation of the sulphhydryl group is catalysed by disulphides.

The control curve *C* appears to be very slightly autocatalytic in shape. This may be due to non-precipitation of cystine in the earlier stages of the reaction, owing to slight solubility or to supersaturation of the solution (such a retention in solution of small quantities of cystine has been observed in the methylene blue experiments, in which no precipitation of cystine could be observed at the end of the reaction); or it might possibly be due to removal

Fig. 1. A. 8 mg. cysteine + 16 mg. pure dithiodiglycollic acid
 B. " " + M/900 KCN " " + M/900 KCN
 C. " " + M/900 KCN " " + M/900 KCN
 $p_H = 7.4$.

of a small amount of cyanide by distillation into the upper part of the bottle, or even by coupled oxidation or decomposition during the reaction. This might allow a slight increase in the activity of traces of catalytic metals present in the cysteine. A similar effect might account for a very small part of the autocatalysis exhibited by curve *B*, but the autocatalytic nature of curve *A* must be due entirely to a catalysis by disulphide since cyanide was not present.

It may be mentioned that in an unpublished experiment carried out by the author in 1924 it was found that the addition of 45 mg. of oxidised glutathione (freed as completely as possible from catalytic metals by the method described [Harrison, 1924]) to 13.5 mg. of pure cysteine hydrochloride, brought about a marked acceleration in the rate of oxidation of the latter (temp. = 37°; $p_H = 7.6$; vol. = 3 cc.). Here again it was observed that no precipitation of cystine occurred in the bottles containing added oxidised

glutathione, though in this case, owing to the rapid oxygen uptake, the readings taken were not sufficiently accurate to determine whether or not the reaction was autocatalytic.

It has been found that the aerobic oxidation of thioglycollic acid is also catalysed by the addition of pure dithiodiglycollic acid. 8 mg. of thioglycollic acid in 3 cc. of water (temp. = 37°, $p_H = 7.4$), took up 13.7 mm.³ of oxygen in the first 11 hours, while an equal amount of thioglycollic acid to which 16 mg. of pure dithiodiglycollic acid had been added took up 72.4 mm.³ of oxygen over the same period of time.

DISCUSSION.

It has been shown by the experiments described that the catalytic effect produced in both the aerobic and anaerobic oxidation of sulphydryl compounds by the addition of disulphides is not due to catalytic impurities present in the disulphide, but is a true property of the disulphide itself. The question arises as to explanation of this catalytic effect. Dixon and Tunncliffe advance the hypothesis that an addition compound between the sulphydryl compound and the disulphide is formed, and that the compound oxidises more rapidly than the sulphydryl compound itself. The evidence they give in support of this hypothesis is very slender, and definite reasons can be given against the likelihood of the formation of such an addition compound. Sidgwick [1927] has recently pointed out that certain substances, such as water and the alcohols, show many abnormal physical properties, owing to the formation of complexes by molecular association. When the oxygen in these compounds is replaced by sulphur, the resulting compounds show normal physical properties, indicating that association has not taken place. Unlike oxygen compounds, sulphur compounds do not show a tendency to complex formation.

It seems possible that the catalytic effect of the disulphide compounds on the oxidation of the sulphydryl compounds may be explained by the following mechanism. Firstly, the disulphide, when in solution, is, to a very slight extent hydrolysed to a mixture of the corresponding sulphydryl compound and the sulphenic acid:

Secondly, the sulphenic acid takes up oxygen to form the sulphinic acid:

Thirdly, if a sulphydryl compound (R'SH) is present, it reduces the sulphinic acid:

The sulphydryl compound thus becomes oxidised to the disulphide, and the sulphenic acid is regenerated. If the oxidation of the sulphenic acid to the sulphinic acid, and the re-reduction of the latter by the sulphydryl compound, together take place more rapidly than the direct oxidation of the sulphydryl

compound by the air (and experiments have shown that the latter reaction in the absence of metals is very slow), then a positive catalysis of the oxidation will be expected on the addition of disulphide.

That such reactions as those suggested above can actually take place appears likely from the following considerations. Reaction (1), namely, the hydrolysis of the —S—S— group to a mixture of —SH and —SOH is given by Smiles [1911] as the way in which the breakdown of aromatic disulphides in acid or alkaline solution takes place. Evidence is given by Smiles and Stewart [1921] that in the case of the hydrolysis of *m*-dithiobenzoic acid (which takes place on treatment with cold aqueous NaOH) the primary products are the sulphydryl compound and the sulphenic acid.

The isolation of the sulphenic acids has not apparently been achieved so far, except in the case of anthraquinonesulphenic acid [Fries, 1912], but derivatives can be isolated. Smiles points out that the hydrolysis (1) is reversible and that the equilibrium is greatly in favour of the disulphide. As regards reaction (2), it has been shown that anthraquinonesulphenic acid in solution readily takes up oxygen from the air to form the corresponding sulphinic acid. (Indeed the occurrence of sulphinic acid in alkaline solutions of a disulphide [Fromm and Wittmann, 1908] was one of the reasons for suggesting the existence of sulphenic acids.) There is little doubt that reaction (3), namely, the reduction of the sulphinic acid by the sulphydryl compound can take place, for Otto [1888] has shown that phenylsulphinic acid is reduced by hydrogen sulphide, and Gilman, Smith and Parker [1925] find that even toluenesulphonyl chloride is reduced by R.SH. Many of the aliphatic sulphinic acids have been isolated, and the fact that the existence of the aliphatic sulphenic acids is probably only transitory does not in any way mean that they are necessarily incapable of taking part in such a catalysis as that pictured above.

In the case of the anaerobic oxidation of sulphydryl compounds in which methylene blue replaces oxygen, the catalysis by disulphides may be assumed to take place on exactly similar lines, the reversible reaction

being substituted for reaction (2). Reaction (2a) will proceed in a forward direction leading to complete reduction of the methylene blue, provided that the sulphinic acid R.SOOH is continuously removed by re-reduction by the sulphydryl compound.

It is not essential that the actual compounds concerned in the intermediate reactions should necessarily be those mentioned. It is merely suggested that the formation of compounds of such a type may be responsible for the catalysis by disulphides.

If the catalysis by disulphides takes place by the mechanism described, in which the first stage involves hydrolysis of the disulphide, it might be expected, since this hydrolysis takes place to a greater extent in alkaline solution, that increasing the p_{H} of the solution would bring about an increase

in the catalytic power of the disulphide. That such is actually the case is evident from the experiment shown in Table IV. This increase of catalysis in alkaline solution thus affords some evidence for the theory suggested.

Previously quoted experiments indicate that near neutrality, if hydrolysis of the —S—S— group occurs, it does so to a very small extent. In other words, reactions (1) and (2) proceed in a forward direction only very slightly. This does not in any way preclude the possibility that hydrolysis may occur to a sufficient extent to bring about the catalysis.

Considering the experiments as a whole, there seems no doubt that the catalytic effect of disulphides on the oxidation of sulphhydryl compounds is a true property of the —S—S— group and is not due to metal impurities.

SUMMARY.

- (1) Both the aerobic and anaerobic oxidations of sulphhydryl compounds are catalysed by pure (metal-free) dithiodiglycollic acid.
- (2) This catalysis is not inhibited by cyanide.
- (3) By destroying the —S—S— linkage in pure dithiodiglycollic acid, its catalytic action disappears.
- (4) A mechanism is suggested by which the catalysis may take place.

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