generalized denaturant or coagulant effect on the skin proteins. Although the evidence presented above is insufficient to warrant a firm conclusion that vesication is initiated by this respiratory defect, yet, since pyruvate oxidation is known to be a fundamental stage in the energy-yielding reactions of many animal cells, it is felt that inactivation of carbohydrate metabolism at this stage may represent a biochemical lesion sufficiently severe to account for grave alterations in the functions of the capillary endothelial cells. It should be pointed out that the defect in pyruvate metabolism is certainly present at an early stage in the development of the skin lesion, although no evidence has as yet been obtained that it actually precedes the development of visible oedema, since, by the time respiratory measurements were actually begun, using the skin removed 5 min. after contamination, it might be argued that oedema would also have been present had the skin still been on the animal.

SUMMARY

1. The effect of low concentrations of sodium arsenite and lewisite on the respiration of rat skin

slices in the presence of pyruvate and succinate has been studied.

2. Under the conditions used, addition of these arsenicals caused a marked inhibition of the small extra oxygen consumption due to pyruvate (86–100 %), but only a slight effect on succinate oxidation (0–19 %).

3. The respiration of skin contaminated *in vivo* with lewisite has been compared with that of normal skin slices. In the contaminated skin there was again a marked inhibition of pyruvate oxidation but no significant inhibition of succinate oxidation.

4. The significance of these changes to the pathogenesis of vesication is briefly discussed.

Most of the work described here was contained in a report submitted to the Ministry of Supply in 1940 (Thompson). It was carried out as part of a programme of extra-mural research for the Ministry of Supply under the direction of Prof. R. A. Peters, M.C., F.R.S. My thanks are due to the Chief Scientific Officer, Ministry of Supply, for permission to publish, and to Miss M. R. Kempson for technical assistance.

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British Anti-Lewisite

1. ARSENIC DERIVATIVES OF THIOL PROTEINS

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The basic mechanism by which arsenic injures living cells and exerts its toxic effect on the whole organism, whether protozoal or metazoal, has been for many years the subject of extensive research. To a large degree this has been the outcome of the introduction of organic compounds of arsenic for chemotherapeutic purposes. Subsequently arsenic

was introduced for offensive purposes against man, in the form of the arsenical smokes and vesicants, once more focussing interest on the problem of its mode of action and on the prevention or treatment of its effects.

Although arsenic has in the past been described as a 'general protoplasmic poison' it is not an

active protein precipitant. This fact alone suggests an action on the functional activities rather than on the structural basis of living cells. It is natural, therefore, that the action of arsenic on the enzyme systems of cells should have received attention, and it is now known from earlier investigations carried out in this laboratory and elsewhere (Peters, Sinclair & Thompson, 1946; including bibliography of earlier work) that sodium arsenite, lewisite and other arsenicals are highly selective in the toxic effects exerted by them on a number of enzymes. The pyruvate oxidase system in brain, for example, is markedly inhibited by low concentrations of arsenic, whereas the lactate dehydrogenase, the α -glycerophosphate dehydrogenase and the cytochrome oxidase systems of brain, for example, are relatively unaffected by very much higher concentrations. It seemed in fact that the action of arsenic on the enzymes studied showed a certain similarity to the action of iodoacetate, since pyruvate oxidation is also markedly inhibited by this poison (Peters, Rydin & Thompson, 1935), while lactate and α -glycerophosphate dehydrogenases are almost unaffected even after incubation with $M/50$ iodoacetate for 1 hr. (Hopkins, Morgan & Lutwak-Mann, 1938).

Since the demonstration by Dickens in 1933 that iodoacetate combines with the thiol groups in cysteine and reduced glutathione, results published by many authors have led to the view that whenever iodoacetate inhibits the activity of a biological system it does so by reaction with thiol groups present in that system and necessary for its normal functioning. In direct support of this, from the standpoint of enzyme function, Rapkine (1933) has shown that iodoacetate reacts readily with the thiol groups in proteins.

If this similarity between the actions of iodoacetate and the arsenicals on respiratory enzyme systems is real it might therefore be expected that arsenic also exerts its toxic action by combining with the thiol groups in certain enzymes. The earlier work of Voegtlin and his associates had indeed led them also to the view that the toxic action of arsenoxides on living cells involves a reaction with thiol groups, although these workers were for the most part concerned with small molecular thiols such as reduced glutathione. Thus, in 1923 Voegtlin, Dyer & Leonard showed that the trypanocidal action of 3-amino-4-hydroxyphenyl-arsenoxide ('arsenoxide') could be counteracted by the addition to the trypanosome suspensions of cysteine, reduced glutathione, or other simple monothiois. Further, the toxic action of arsenoxide in rats could be diminished or even prevented by the intravenous injection of glutathione immediately before the administration of the arsenical (Voegtlin, Dyer & Leonard, 1925). In both trypanosomes and

rats, however, a large excess of glutathione was required to abolish the effects of the arsenoxide, a ratio of 10 g.mol. of glutathione to 1 of arsenoxide being necessary, while in the case of sodium arsenite it was found that a ratio of 40:1 was required to abolish trypanocidal activity (Rosenthal & Voegtlin, 1930). These workers also showed that the reduction in oxygen consumption of animal tissues caused by arsenoxide could be prevented by the addition to the system of reduced glutathione in a ratio of 10 g.mol. to 1 of arsenoxide (Voegtlin, Rosenthal & Johnson, 1931); but again, when sodium arsenite was used in place of arsenoxide, 40 g.mol. of glutathione were required, a ratio of 10:1 being entirely without effect.

More recently this work has been extended by Eagle (1939), who has shown that glutathione is also capable of abolishing the antispasmodic action of arsenoxide; in this case also a large excess of glutathione was required (GSH:As=10:1); cysteine was found to be less effective, even 12-14 g.mol. failing to abolish the activity entirely.

It seems clear, therefore, that under the various conditions used by these workers an excess of a simple thiol compound, such as glutathione, is able to protect a variety of biological systems against the toxic effects of arsenoxide and to a lesser extent against the effects of sodium arsenite.

That the protection afforded by these simple monothiois is only partial and in some cases inadequate from the physiological standpoint is, however, clearly shown by the work of Schmitt & Skow (1935), who investigated the ability of monothiois to prevent the toxic action of arsenite on the medullated nerves of frogs. They found that while cysteine and reduced glutathione delayed the extinction of the nerve action potential produced by arsenite, in no case were they able to prevent the eventual extinction of the potential, regardless of the ratio SH:As. Nor was it possible by the addition of monothiois to produce any recovery once the action potential had been abolished by arsenite. They also claimed that a large excess of cysteine (150:1) was unable to prevent the inhibition of respiration of nerve produced by arsenite.

Similar evidence of partial but inadequate protection was provided by Walker (1928), who reported that sodium thioglycollate had an inhibitory effect on the toxic action of diphenylchlorarsine on glaucoma; and that if monothioethylene glycol was added at the end of 3 min. to a culture that had received a 3 min. lethal dose of the arsenical, rapid revival of the organisms occurred, but was followed by death in 1-2 hr.

Finally, Sinclair (quoted by Peters, 1940) has shown that a variety of monothiois, even when present in large excess (up to 200 equivalents),

failed to protect the brain pyruvate oxidase system from the toxic action of lewisite.

Moreover, the evidence from this work relating the toxicity of arsenic to its reaction with essential tissue thiols is indirect. As early as 1909 Ehrlich had suggested that the 'chemo-receptors' for arsenic might be hydroxyl or thiol groups, but the earliest direct evidence in support of this appears to have been provided by Walker (1924), who, working with Prof. R. A. Peters, showed that the addition of certain arsenicals such as diphenylchloroarsine to dried muscle preparations (Hopkins & Dixon, 1922) containing so-called 'fixed' SH groups (i.e. thiol groups in protein molecules) led to the inhibition of certain catalytic effects shown by these preparations, this inhibition being accompanied by the rapid disappearance of the fixed SH reaction of the muscle powder. In Walker's earliest work there was no proof that the inhibition of the SH reaction was due to combination of the thiol groups with the arsenical, but after the development of his cyanide test for disulphide groups (Walker, 1925) it became clear that after removal of the SH reaction by the addition of the arsenical no recovery was brought about by treatment with cyanide, suggesting that true combination might have taken place.

Although Voegtlin *et al.* (1925) had suggested that in addition to an ability to combine with simple thiols such as glutathione, arsenic might also react with thiol groups present in proteins, it was not until 1932 that Rosenthal demonstrated that trivalent arsenic could not be ultra-filtered from suspensions of arsenic-treated heat-coagulated proteins containing thiol groups. Further, the nitroprusside reaction of these protein suspensions and of washed muscle powder and washed minced liver suspensions was caused to disappear by treatment with the arsenoxides.

In order to investigate further the hypothesis that arsenic, like iodoacetate, exerts its toxic effects by combination with tissue thiols, including 'thiol proteins', it was decided to study *in vitro* the reaction occurring between arsenicals and a soluble thiol protein, and to examine the degree of dissociation at different hydrogen-ion concentrations of any compound formed between the reactants. Ideally, in view of the inhibitory effect of trivalent arsenicals on pyruvate oxidation, and the evidence obtained showing that disorganization of this system exists in the bodies of animals poisoned with arsenic (Peters *et al.* 1946; Thompson, 1946), it would be of the greatest interest to examine the reaction with the isolated purified pyruvate enzyme; unfortunately, this enzyme has not yet been obtained from animal sources in a purified state.

Preparations of urease, myosin and crystalline lens protein were investigated but since, for the

work envisaged, it was necessary to have available relatively large amounts of protein, kerateine, a derived protein, obtained by rupture of the disulphide bonds of keratin, was finally chosen.

The general procedure adopted in the experiments about to be described was to add the arsenical to buffered solutions of kerateine until the nitroprusside reaction of the solution disappeared. The protein was then precipitated at pH 4.6 with acetic acid, washed and dried. The nitrogen, sulphur and arsenic contents of the derivatives were then estimated, and the stability of the proteins investigated by analysis after re-precipitation or dialysis.

METHODS

Analyses. Nitrogen analyses were carried out in duplicate on the various preparations by the method of Conway & Byrne (1933); sulphur by the macro-Carius method. Arsenic was estimated by the titrimetric micro-method of Allcroft & Green (1935). After digestion of the material with perchloric and nitric acids, the subsequent reduction to arsine was carried out by immersion of the generating flask in a water-bath at 80–100°. Using 5 ml. of $N/75$ -AgNO₃ in each of the three absorption towers, 97–100% recovery of amounts of arsenic varying from 50–300 µg. was obtained.

Preparation of kerateine. Kerateine was prepared from washed human hair by treatment with alkaline cyanide at 38° according to the method of Goddard & Michaelis (1934). The solution obtained by this treatment was filtered, precipitated at pH 4.6 with glacial acetic acid, washed with dilute acetic acid, and either dried or redissolved in buffer at pH 7.0 and used directly for the preparation of arsenical derivatives.

In contrast to the preparations obtained by Goddard & Michaelis from wool, the dried kerateine from human hair was soluble in water and neutral salt solutions. The solutions gave a strong nitroprusside reaction. The nitrogen and sulphur contents of three different preparations are given in Table 1.

Table 1. Nitrogen and sulphur content of kerateine from human hair

N (%)	S (%)
14.9	1.37
13.9	1.17
14.9	1.39

RESULTS

Arsenic derivatives of kerateine. The preparation of the lewisite (chlorovinylchloroarsine) derivative of kerateine was carried out as follows: kerateine derived from 50 g. hair was precipitated at pH 4.6, filtered off and redissolved in 200 ml. of 0.4M-phosphate buffer (pH 7.0). The solution was then titrated with 19% (w/v) lewisite in ethanol with vigorous stirring until the nitroprusside reaction in dilute ammonia became negative. At the end of the titration the solution was clear, and showed no

evidence of any precipitation of lewisite oxide; the pH was maintained at 7.0 throughout the addition of the arsenical. Glacial acetic acid was then added until the precipitation of the protein was complete (pH 4.6). The precipitate was then filtered off, washed with water and dried.

The arsenite derivative was prepared in a similar manner, 4.95% (w/v) neutralized sodium arsenite being used to titrate the kerateine solution. A large excess of sodium arsenite was required to abolish the nitroprusside reaction, and in some preparations the nitroprusside reaction never completely disappeared. One preparation of the ethyldichloroarsine derivative was also made and analyzed.

The analytical figures on the various dry preparations obtained by the treatment of fresh solutions of kerateine with the arsenicals are shown in Table 2.

Table 2. *Analysis of arsenic derivatives of kerateine*

Derivative	N (%)	S (%)	As (%)	
Lewisite	—	1.36	0.58	Mean As = 0.52%
	—	—	0.48	
	15.6	1.32	0.63	
	14.8	1.41	0.46	
	14.8	1.31	0.47	
	—	—	0.53	
Ethyldichloroarsine	—	—	0.52	
Sodium arsenite	15.2	1.49	0.56	Mean As = 0.41%
	13.5	1.25	0.33	
	14.8	1.62	0.46	
	14.2	1.26	0.30	

It will be seen that treatment of kerateine with lewisite or arsenite yields derivatives containing approximately 0.5 and 0.4% arsenic respectively. The arsenic content of the derivatives was found to be independent of the amount of arsenic used to titrate the kerateine solution; thus, in the case of the lewisite derivatives, amounts varying from 0.6 to 3.9 ml. of 19% (w/v) lewisite per g. kerateine were used in the different preparations, i.e. from 8 to 55 times the amount of arsenic found in the protein. All the proteins prepared gave clear solutions below pH 4 and above pH 5.4, and showed maximal flocculation at pH 4.6.

The stability of these arsenic-kerateine derivatives was next investigated by determining the arsenic contents after reprecipitation at pH 4.6, the precipitate being once more washed and dried, and after dialysis against distilled water at room temperature for 20 and 44 hr. The results of these experiments are shown in Table 3.

It will be seen that the lewisite derivative is very much more stable to reprecipitation or dialysis than the arsenite derivative, 84–87% of the arsenic

Table 3. *Arsenic content of kerateine derivatives after reprecipitation or dialysis*

Derivative	As after			
	Original As (%)	one re-precipitation (%)	As after 20 hr. dialysis (%)	As after 44 hr. dialysis (%)
Lewisite	0.63	0.53 (84%)	0.35 (56%)	0.30 (48%)
Lewisite	0.46	0.40 (87%)	0.30 (65%)	0.21 (46%)
Arsenite	0.56	0.17 (30%)	0.07 (13%)	—
Arsenite	0.33	0.18 (55%)	0.04 (12%)	—
Arsenite	0.46	0.20 (44%)	0.09 (19%)	—

(The numbers in brackets give the percentage remaining of the original arsenic content.)

remaining attached to the protein after reprecipitation in the case of the lewisite-kerateine, as compared with only 30–55% in the case of the arsenite-kerateine. The dialyzed samples show an even more striking difference between the two derivatives.

Goddard & Michaelis (1935) have shown that kerateine can readily be oxidized from the thiol to the disulphide form at neutral reaction by atmospheric oxygen: they have called this disulphide form metakeratin, and have pointed out that in spite of the reformation of disulphide bonds in the molecule the protein is still soluble and its isoelectric point is unchanged. In view of this ready transformation from the thiol to the disulphide form without any other apparent change in the molecule or its physical properties, solutions of metakeratin have also been treated with the same amounts of lewisite and arsenite that were used in the preparation of the corresponding kerateine derivatives. Such an experiment should help to decide whether the arsenic contained in the kerateine derivatives is present in true combination with the thiol groups or merely non-specifically attached to the protein molecule and precipitating with it.

The metakeratin was prepared by passing oxygen through solutions of kerateine until the nitroprusside reaction had completely disappeared. After treatment with the arsenicals the metakeratin was then precipitated at pH 4.6, washed and dried in the same manner as was used for the kerateine derivatives. The analytical figures obtained on the dried preparations and after a further reprecipitation at pH 4.6, are given in Table 4.

Table 4. *Analysis of metakeratin after treatment with lewisite, arsenite and arsenate*

Derivative	S (%)	As (%)	As after one reprecipitation (%)
Lewisite	1.22	0.04	0.02
Lewisite	1.37	0.07	—
Arsenite	1.48	0.09	0.03
Arsenate	1.13	0.04	0.04

A further experiment was carried out in which the kerateine was incompletely oxidized, the final solution used for the titration with lewisite giving a very faint nitroprusside reaction. The arsenic content of this preparation was 0.12%.

Treatment of metakeratin with arsenicals therefore results in the formation of derivatives containing only a small fraction of the arsenic present in the corresponding kerateine derivatives. In order to show that the small amount of arsenic present in these lewisite and arsenite derivatives was probably due to adsorption, an experiment was carried out using sodium arsenate as the reagent, since, if no thiol groups are present, this cannot be reduced to the reactive trivalent form, and any arsenic present in the derivative would therefore most likely be due to adsorption. It will be seen that, taking into account experimental error, no significant difference exists between the arsenate and the other derivatives.

Since it has been shown by Cohen, King & Strangeways (1931) that the combination of pentavalent arsenicals with thiols takes place according to the equation

it was anticipated that if kerateine was treated with sodium arsenate the amount of arsenic found in the product would be intermediate between those of the arsenite-kerateine and arsenate-metakeratin.

The reaction between sodium arsenate and kerateine was therefore carried out under the same conditions as for the arsenite-derivative, and, as will be seen from Table 5, the products had the expected intermediate arsenic contents.

Table 5. *Analysis of kerateine treated with excess of sodium arsenate*

N (%)	As (%)	S (%)
14.1	0.19	—
14.9	0.11	1.31
14.3	0.06	1.30

Mean As = 0.12%.

These results still further support the view that the arsenic present in the lewisite and arsenite-kerateine derivatives is present in true combination with the thiol groups and not associated non-specifically with the protein.

If this is true, the arsenic content of these derivatives should be dependent on the thiol content of the parent proteins. A variety of compounds (I_2 , $K_3Fe(CN)_6$, phenylmercuric chloride, dichlorophenolindophenol and cystine) has been used in the past for the estimation of thiol groups, but none has given entirely satisfactory results.

More recently Kuhn & Desnuelle (1938) and Greenstein (1938) have suggested porphyrindin as a more suitable reagent. This was, therefore, synthesized according to the method of Kuhn & Franke (1935), and was standardized against cysteine ester hydrochloride. It was found necessary to use carefully controlled conditions in order to avoid over-titration, and these conditions were rigidly adhered to both in the standardization against cysteine ester and the titration of the protein solutions. From these titrations it was possible to calculate the amount of arsenic which would combine with the protein, assuming the ratio of 1 As : 2 SH. The lewisite derivative was next formed from a sample of the protein solution; after washing and drying in the usual way the arsenic content of the dry preparation was estimated.

Table 6 shows the arsenic content as found and as calculated from the porphyrindin titration in four different preparations.

Table 6. *Estimated As content and content calculated from porphyrindin titration (lewisite-kerateine)*

	Found (%)	Calc. from thiol titration (1 As:2 SH) (%)
	0.49	0.45
	0.63	0.46
	0.58	0.47
	0.60	0.46
Mean	0.58	0.46

From the results of the four experiments shown in Table 6 it will be seen that, when calculated from the porphyrindin titration of the thiol groups originally present in the parent kerateine on the assumption that 1 mol. of lewisite combines with two thiol groups, the mean arsenic content of the lewisite-kerateine compound would be 0.46%. The arsenic content as determined by analysis in these four experiments ranged from 0.49 to 0.63%. The value calculated for the ratio 1 As : 2 SH accounts therefore for 73–92% of the arsenic actually found.

It must be realized of course that since the experimental conditions involved an excess of lewisite it was possible that some of the molecules of lewisite may have reacted with only one thiol group so that the ratio 1 As : 2 SH would not account for all the arsenic found.

DISCUSSION

The main evidence for Voegtlin's view that the toxic action of arsenic is due to its combination with thiol groups is based on the ability of simple thiols, when present in excess, to protect certain systems from the effects of certain arsenicals. This protection, however, is not found in all cases, and therefore

such evidence cannot be held to be conclusive. The explanation of this inconsistency possibly lies in the dissociability of the As—S link (Cohen *et al.* 1931; Strangeways, 1937) since it has been shown that arsenoxides and thiols react thus:

The final equilibrium established depends on the hydrogen-ion concentration, acid shifting it to the right and alkali to the left. The equilibrium can also be altered by the addition of a second thiol whose affinity for arsenic is greater than that of the first. Such an explanation may account for the high toxicity of tricysteinyarsine (Voegtlin *et al.* 1931) which has been shown to have as great a toxicity as arsenite on the respiration of living cells, the thiol groups of some essential enzymes, by reason of a possible high affinity for arsenic, combining perhaps with the arsenite released by hydrolysis from the tricysteinyarsine, thus driving the reaction to the left.

It seemed important therefore to investigate the nature of the interaction occurring between arsenic and thiol groups in proteins. Owing to the impracticability of obtaining, at the time, adequate amounts of an arsenic-sensitive enzyme in a purified state kerateine was chosen as a model; it is fully realized that this was not an ideal protein for the investigation in view, but it was felt that it would provide a suitable thiol protein for initial experiments towards this end. So far as we are aware no arsenical derivatives of soluble proteins have yet been prepared under physiological conditions. The nearest approach to such an investigation is that of Wölfling & Möller (1935), who treated acid hydrolysates of keratin with hydroxyphenylarsenoxide. These hydrolytic products, however, were of high sulphur content and proteose in nature. We also have obtained preparations with high sulphur and arsenic contents (As, 4.0%; S, 5.65%) by using the pH 4.6 filtrate, but these contained proteoses and smaller molecular compounds, and hence were abandoned as being unsuitable for our purpose.

The experiments described above are interpreted as showing that under physiological conditions of temperature and hydrogen-ion concentration lewisite and arsenite combine with kerateine to give water-soluble protein derivatives, lewisite forming a more stable compound than arsenite. The arsenicals combine solely with the thiol groups of the kerateine. The evidence for concluding that the arsenic is present in these derivatives in true combination with the thiol groups, and is not merely present in non-specific physical association with the proteins, is as follows:

(1) Treatment of kerateine with lewisite or arsenite results in the formation of compounds

whose arsenic content is independent of the amount of arsenic added.

(2) The arsenic content of the derivatives bears a direct relationship to the thiol content of the parent protein.

(3) The thiol content of kerateine, as determined by porphyrindin titration, is sufficient to account for all the arsenic found in the derivatives.

The lewisite appears to be relatively firmly combined with the protein, as judged by the arsenic content after reprecipitation and dialysis. Moreover, from the porphyrindin titrations it appears that at least 75% of the arsenic found in the lewisite-protein is in combination with two thiol groups. In view of the ease with which kerateine can be re-oxidized to the disulphide form it seems probable that the arsenic has combined with two SH groups closely placed in space on the same molecule, to form a ring. On physico-chemical grounds ring compounds of the type

would be expected to be more stable than compounds of an arsenical with 2 mol. of a monothiol, i.e. of the type

There emerged from these experiments therefore the conception that the high toxicity of trivalent arsenicals might be due to their ability to combine with two closely situated essential SH groups on certain tissue proteins to form relatively stable arsenical rings. If this conception is correct it follows that efficient protection against these arsenicals would only be afforded by the presence of a competing dithiol capable of forming a ring compound at least as stable as the compound of arsenical with the tissue 'acceptor'. An account of the further development of this theory, and of the elaboration of a therapeutically successful dithiol, is given in succeeding papers. A brief, preliminary review of the whole work has already appeared (Peters, Stocken & Thompson, 1945).

SUMMARY

1. The preparation of derivatives of kerateine with lewisite, ethyldichloroarsine, sodium arsenite and sodium arsenate is described.

2. The stability of these arsenical proteins has been investigated.

3. It appears that under the conditions used in this work arsenic combines with proteins by reacting with the thiol groups, and no evidence has been

obtained that any significant combination occurs with other groups in the kerateine molecule.

4. At least 75% of the arsenic found in the lewisite-derivative is in combination with two thiol groups, and the hypothesis has consequently been put forward that dithiols may prove more effective antidotes to arsenic than the mono-thiols used in the past.

This work was carried out as part of a programme of extra-mural research for the Ministry of Supply under the direction of Prof. R. A. Peters, M.C., F.R.S., and was communicated to the Ministry in 1940 (Stocken & Thompson). We wish to express our gratitude to Prof. N. V. Sidgwick, F.R.S., and Mr J. St L. Philpot for their helpful advice, and to the Chief Scientific Officer, Ministry of Supply for permission to publish the facts. Our thanks are also due to Mr C. Dear and Mr E. Facer for skilled technical assistance.

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British Anti-Lewisite

2. DITHIOL COMPOUNDS AS ANTIDOTES FOR ARSENIC

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In a previous paper the preparation of arsenic derivatives of kerateine has been described (Stocken & Thompson, 1946). In the course of that work it was found that the arsenicals had combined with the SH groups present in the kerateine, and that at least 75% of the arsenic in the lewisite derivative was present in the proportion 1 As : 2 SH. It seemed probable therefore that the arsenic had combined with 2 SH groups closely placed in space on the same molecule to form a ring compound, and it was suggested that efficient protection against trivalent arsenicals would only be afforded by the presence of a dithiol capable of forming a relatively stable ring compound with the arsenical, instead of the much more dissociable 'open chain' com-

pounds formed between arsenic and the monothiol groups investigated in the past.

Much of the earlier work (Voegtlin, Dyer & Leonard, 1923, 1925; Walker, 1924; Rosenthal & Voegtlin, 1930; Voegtlin, Rosenthal & Johnson, 1931) demonstrating the limited capacity of simple monothiol groups to protect certain biological systems from the toxic action of the therapeutic arsenicals was also reviewed, but it was pointed out that the protection afforded by compounds of this type was much less complete when exerted against sodium arsenite than against the therapeutic arsenoxides, and that in some cases the protection was inadequate from the physiological standpoint (Walker, 1928; Schmitt & Skow, 1935).