XXXIX. CHEMICAL CONSTITUTION AND TOXICITY.

By ERNEST WALKER.

From the Department of Biochemistry, Oxford.

(*Received November 28th, 1927.*)

In many of the previous attempts to ascertain whether any definite relationship exists between chemical constitution and toxicity the principal difficulty has lain in the selection of a suitable biological standard. In the following investigation protozoa have been used for the measurement of toxicity. This choice was based upon two considerations: firstly, the uniformity (of biological material) obtainable with protozoa cultivated in a synthetic medium was regarded as valuable; secondly, it was felt that the killing-power towards such a primitive organism afforded a measure of general protoplasmic toxicity and avoided specialised toxic effects. Some preliminary work by McCleland and Peters [1919] on the toxicity of certain arsenic compounds to *Paramecium* afforded an introduction to this investigation. Flury [1921] has, in a similar manner, utilised unicellular organisms as a means of comparing the toxicities of arsenic and other irritant compounds. Apart from these instances, however, there appears to be a general recognition of the usefulness of protozoa in the measurement of pharmacological action.

Method of experimentation.

Principle. The principle of the method adopted has lain in the determination of the concentration of substance to be tested which killed all the organisms in a given amount of culture in a fixed time. The unicellular organism used was *Colpidium colpoda*, a culture (derived from a single organism) of which was in the first place supplied by Prof. R. A. Peters. This organism was cultured in the manner described by Peters [1921], in a simple synthetic medium, consisting of one organic and five inorganic salts; the ammonium glycerophosphate (0·06 %) of Peters's medium was replaced by sodium glycerophosphate (0·05 %) and ammonium chloride (0·01 %) owing to the greater degree of purity obtainable by recrystallisation of the sodium glycerophosphate. The culture was originally believed to be a pure one, but during the whole course of this work there has been present an apparently symbiotic bacterium. The possible influence of the presence of this foreign organism upon the toxicity results will be considered later. The cultures

were used for toxicity purposes some 10 or 12 days after inoculation, when the organisms numbered from 3000 to 5000 per cc. At a later stage of this work a second protozoon, which was identified as *Glaucoma scintillans*[1], was introduced. This organism was cultivated in a similar medium to that in which *Colpidium* was grown and it was used for toxicity determinations in parallel with the latter organism.

The concentration required to kill in a given time was chosen as the measure of toxicity, in preference to the time required to kill at a given concentration, since it furnishes a direct value for toxicity as compared with a relative value. There are, however, other objections to the selection of time required to kill for the measuring of toxicity and the foremost of these is the prolongation of the death process, which itself introduces several undesirable features. Firstly, it magnifies the variations in resistance of individual organisms. In the second place, it allows the interference of possible secondary effects arising from decomposition of the toxic agent and the bringing into play of the protective mechanism by the organism. In the third place, it assumes that the effect of a long exposure to a dilute solution is the same as a short exposure to a more concentrated solution; or, in other words, it is assumed that there is a straight line relationship between time and concentration required to kill. Experiments have shown that this is not the case and it is apparent that there is a limiting dilution at which the toxic agent ceases to exert any detectable effect upon the organism. For these reasons it was decided to adopt an arbitrary time limit and the 3-minute period was chosen as being the shortest time compatible with accurate observation.

The death point of the organisms was regarded as occurring when all movement had ceased. The selection of this criterion was based upon the fact that, whatever the initial cause of the cessation of movement, the organisms were never observed to have resumed movement after having once stopped. Owing to the variety of ways in which death may occur, according to the nature of the toxic agent employed, cessation of movement was regarded as being the most satisfactory standard. Prior to death, the movements of the protozoa grew more and more sluggish and finally ceased. Sometimes there was a period of increased activity and occasionally the organism rotated rapidly about its own axis immediately before death; this was especially noticeable in cases where death was due to acidity or to changes in osmotic pressure. Death was followed ultimately by disintegration of the organism.

Technique. The actual toxicity determinations were carried out as follows. Ten drops of the culture were transferred to a clean watch-glass, by means of a Dreyer pipette fitted with rubber teat. The number of organisms per drop showed very good agreement. To the culture were added drops of the toxic solution (of known strength) by means of a similar pipette. During the addition of the solution the watch-glass was gently shaken in order to avoid high local concentration. The effect upon the culture was observed under a

[1] Thanks are due to Dr Helen Goodrich for identifying this organism.

low power microscope (1-inch objective). From the number of drops of toxic solution required to kill the organisms in exactly 3 minutes the concentration of toxic substance was calculated and is referred to hereafter as the toxic concentration. If x be the number of drops which kills in 3 minutes, then the toxic concentration is given by the expression $\frac{fx}{fx+10} \cdot \frac{M}{y}$, where f is a factor used in correcting for the difference in volume of the drops delivered by the two pipettes and $\frac{M}{y}$ is the molecular concentration of toxic agent. In practice it was found simpler to select two pipettes which delivered drops of the same volume, thus obviating the introduction of the factor f.

Considerable difficulty was experienced at first in making aqueous solutions of known strength of the large number of arsenic compounds examined. As a rule these were but slightly soluble in water. In the end, the following procedure was adopted. A weighed amount of the toxic compound was dissolved in 2–3 cc. of pure pyridine. A measured volume of the pyridine solution was then pipetted into a known large volume of water, accompanied by vigorous shaking of the flask. In this way various solutions of the toxic agent were made up until one was obtained which killed in 3 minutes. The strength of the pyridine in the solution was never allowed to exceed 0·1 %. Control experiments showed that pyridine in 10 times this strength did not affect the organisms in periods exceeding the 3-minute period normally used. Solutions made in this manner could only be used for toxicity purposes when freshly prepared, for it was found that many of the tervalent arsenic compounds dissolved in pyridine or alcohol displayed a marked fall in toxicity on standing. This fall was quite definite after the solution had stood for 24 hours, and was attributed to the absorption of moisture and oxygen by the solution, with the formation of the corresponding arsinic acid. Thus, diphenylchloroarsine in alcohol became transformed, on standing, into diphenylarsinic acid:

$$(C_6H_5)_2As.Cl + H_2O + O \rightarrow (C_6H_5)_2As.O.OH + HCl.$$

Standardisation of the method. Before it was possible to place reliance upon this method of toxicity determination it was important to know what conditions would lead to an apparent variation in the toxicity of the substances examined. For this purpose the toxicities of two standard solutions, nickel chloride and mercuric chloride, were followed upon three growing cultures of *C. colpoda*. Tables I, II and III contain the results. It will be seen that there were variations in the toxicities of both solutions amounting to some 40 %. It will also be seen that there was a definite increase in the resistance of the organisms with increasing age and that after a certain period—12 to 14 days—the cultures became less subject to variation and tended more towards a uniform resistance. This period coincides almost exactly with the period required for the cultures to reach their maximum growth, as found by Peters [1921]. For this reason cultures were taken for toxicity measurements, in the subsequent investigations, as nearly as possible

at the time they had reached their maximum growth. Nevertheless, the varying number of organisms was alone not sufficient to account for the 40 % variation obtained, although, perhaps, the most important factor. Diluting the cultures did not result in any significant change in the amount of toxic agent required to kill the organisms. Furthermore, the number of organisms (3000–5000) per cc. represents a comparatively poor growth, so that the proportion of organisms, by bulk, to the medium was exceedingly small (of the order 1 : 100,000).

Table I.

Age in days	Temperature ° C.	Toxic concentration $NiCl_2$	Toxic concentration $HgCl_2$
8	17·6	*M*/583	*M*/27,000
9	17·2	*M*/627	*M*/25,000
10	17·2	*M*/800	*M*/27,000
12	17·4	*M*/583	*M*/17,000
13	15·6	*M*/538	*M*/19,500
15	16·1	*M*/460	*M*/18,100
16	15·6	*M*/490	*M*/13,000
17	17·2	*M*/490	*M*/16,200
19	15·8	*M*/490	*M*/17,000
20	16·2	*M*/490	*M*/18,100
Variation		44 %	41 %

Table II.

Age in days	Temperature ° C.	Toxic concentration $NiCl_2$	Toxic concentration $HgCl_2$
6	17·2	*M*/800	*M*/30,000
7	16·7	*M*/875	*M*/27,000
8	17·6	*M*/875	*M*/27,000
9	17·2	*M*/707	*M*/30,000
10	17·2	*M*/707	*M*/27,000
12	17·4	*M*/490	*M*/23,000
13	15·6	*M*/490	*M*/21,400
15	16·1	*M*/432	*M*/19,500
16	15·6	*M*/432	*M*/17,000
17	17·2	*M*/410	*M*/17,000
19	15·8	*M*/410	*M*/17,000
20	16·2	*M*/432	*M*/17,000
Variation		46 %	43 %

Table III.

Age in days	Temperature ° C.	Toxic concentration $NiCl_2$	Toxic concentration $HgCl_2$
10	17·2	*M*/800	*M*/27,000
12	17·4	*M*/583	*M*/21,400
13	15·6	*M*/583	*M*/21,400
15	16·1	*M*/538	*M*/22,500
16	15·6	*M*/538	*M*/19,000
17	17·2	*M*/490	*M*/19,000
19	15·8	*M*/490	*M*/19,000
20	16·2	*M*/460	*M*/19,000
Variation		44 %	30 %

Possible sources of error.

Measurement of drops. The drop method, in the first place, does not give results more accurate than one drop of the solution used for killing. In the case of the two standard solutions used, an error upon this account might have amounted to some 5 %, but was probably less. In the measurement of drops from the capillary pipette there may have been an error of not more than 10 %. There was thus a total possible error of not more than 15 %, in partial explanation of the 40 % variation.

Variation in temperature. It was anticipated that variations in temperature would exert some influence upon the apparent toxicity of a solution. Toxicity measurements were therefore carried out, using the two standard toxic solutions, upon cultures at widely different temperatures. Two organic compounds were also tested for toxicity at different temperatures. For this test use was made of the warm (and cold) microscope stage, and both culture and toxic solution were kept immersed in baths at the same temperature as the stage. The results are given in Table IV.

Table IV. *Toxic concentration at various temperatures.*

Substance	9·5°	15·5°	17·5°	22·0°	27·0°
Mercuric chloride	*M*/22,500	—	*M*/24,300	*M*/27,000	—
Nickel chloride	*M*/475	—	*M*/583	*M*/667	—
p-Chlorophenol	—	*M*/317	—	—	*M*/317
Formaldehyde	—	*M*/262	—	—	*M*/283

It will be seen that increased temperature was accompanied by decreased resistance on the part of the organisms. The variation in the case of nickel chloride amounted to some 33 % and in the case of mercuric chloride to some 18 %, both over a range of 12·5°. Clearly the slight variations in temperature (1° to 2°) experienced under normal conditions could have exerted no appreciable effect upon the toxicity measurements.

Variation in hydrogen ion concentration. Variations in p_H may affect toxicity measurements either by influencing the rate of penetration of the toxic agent or by affecting the organisms directly. The former possibility has been subjected to investigation in recent years. Brooks [1925] has examined the effect of p_H upon the penetration of arsenic into living cells (*Valonia*) and Crane [1921] has shown that the toxicity of certain alkaloids to *P. caudatum* varies with the p_H attributable to variations in the degree of dissociation of the alkaloidal salt. Table V shows the effect of variation in p_H upon the toxicity of quinine sulphate to *Colpidium* and serves to confirm Crane's conclusion.

Table V. *Toxicity of quinine sulphate.*

p_H	7·7	7·4	6·9	6·4	5·9
Toxic concentration	*M*/1438	*M*/970	*M*/930	*M*/890	> *M*/890

The effect of p_H upon the organisms themselves has been investigated by Dale [1913], who showed that *Paramecium* cannot live in a solution more acid than p_H 5·0 for more than 5 minutes. Experiment showed that *Colpidium* could only live within the limits p_H 5·0 to 9·5. Most of the substances tested for toxicity in this investigation have been presented in amounts too small to affect the p_H to any significant extent.

Contamination of cultures. The possibility of toxicity measurements being affected by the presence of some foreign organism was not considered in the above standardisation, for the culture showed no evidence of bacterial growth. It was not until a grossly infected culture chanced to be used, giving markedly different toxicity figures, that the importance of contamination was realised. The effect of contamination was always to reduce the toxicity of the substance tested. There can be little doubt that this effect is due entirely to the presence of débris, resulting from bacterial growth. In the course of this work many contaminated cultures have been encountered and the application of different forms of filtration has made it quite clear that it was the débris that was affecting the toxicity figures, presumably by absorbing a significant amount of the toxic agent. So long as the culture remained clear, although bacteriologically contaminated, it gave consistent results. In this respect *Glaucoma scintillans* has proved superior to *C. colpoda*, having maintained its clarity throughout, over a period of 2 years. A similar effect has been observed by Morgan and Cooper [1924, 1] and Cooper *et al.* [1925]; they state that the bactericidal power of quinones and certain other compounds is greatly reduced by the presence of organic matter.

None of the above sources of error appeared to be serious and it was felt that, provided cultures were used about the time of their maximum growth, reliable toxicity measurements could be made, in which the margin of error would not exceed 40 % and would probably be less. The following tables contain the results of the examination of a large number of arsenic compounds and a lesser number of miscellaneous compounds, reputed to be toxic. In the first column, opposite each compound, is given the toxicity figure, expressed in the molecular concentration which kills in 3 minutes. In the second column, a figure is given expressing the toxicity relative to the arbitrary toxic value of 100 given to diphenylchloroarsine, which was adopted as a standard. Corresponding figures are also given for the toxicity to *G. scintillans* wherever tests have been carried out with that organism. It should be noted that the symbol $>$, when used in the first column, means that the *toxic concentration* is greater than the figure given; the actual toxicity of that compound is therefore *less* than the figure given in the second column.

It has been impossible to include in these tables all the compounds examined. Those given should be regarded as representative of a far larger number in each series, a fact which has been taken into account when formulating conclusions.

Tervalent arsenic compounds.

Name	Toxicity to *Colpidium*: Concentration which kills in 3 mins.	Toxicity to *Colpidium*: Toxicity relative to diphenylchloroarsine =100	Toxicity to *Glaucoma*: Concentration which kills in 3 mins.	Toxicity to *Glaucoma*: Toxicity relative to diphenylchloroarsine =100
Primary derivatives:				
p-Chlorophenyldichloroarsine	*M*/3300	2	—	—
o-Nitrophenyldichloroarsine	>*M*/4460	<2	*M*/5870	1
o-Bromophenyldichloroarsine	*M*/5460	3	*M*/23,470	6
Phenylarsenious oxide	>*M*/9160	<5	*M*/16,000	4
o-Benzarsenious oxide	>*M*/50	0	—	—
Benzophenone-*o*-arsenious oxide	>*M*/42,000	<23	—	—
Benzophenone-*o*-arsenious chloride	*M*/1100	1	—	—
o-Hydroxyphenylarsenious oxide anhydride	>*M*/11,000	<6	—	—
Methyldichloroarsine	>*M*/2000	0	>*M*/2000	0
Butyldichloroarsine	*M*/900	1	—	—
Secondary derivatives:				
Diphenylchloroarsine	*M*/180,000	100	*M*/420,000	100
Diphenylbromoarsine	*M*/228,000	127	—	—
Diphenylcyanoarsine	*M*/182,700	101	—	—
Diphenylarsenious sulphide	*M*/2100	1	—	—
Diphenylarsenious oxide	*M*/224,000	124	—	—
10-Cyanophenoxyarsine	*M*/261,300	145	*M*/466,200	117
10-Chloro-5 : 10-dihydrophenarsazine	*M*/102,000	57	*M*/166,500	42
10-Bromo-5 : 10-dihydrophenarsazine	*M*/71,500	40	*M*/151,500	38
10-Iodo-5 : 10-dihydrophenarsazine	*M*/80,000	44	*M*/69,000	17
10-Aceto-5 : 10-dihydrophenarsazine	*M*/256,300	142	*M*/493,300	123
10 : 10-Sulpho-5 : 10-dihydrophenarsazine	>*M*/15,000	<8	>*M*/15,000	<4
10 : 10-Oxy-5 : 10-dihydrophenarsazine	*M*/115,000	64	*M*/332,500	83
10 : 10-*Bis*-5 : 10-dihydrophenarsazine	*M*/40,000	22	*M*/57,400	14
5-Acetyl-10-chloro-5 : 10-dihydrophenarsazine	*M*/21,000	12	*M*/45,560	11
5-Propionyl-10-chloro-5 : 10-dihydrophenarsazine	*M*/17,400	10	*M*/34,140	8
3-Amino-10-chloro-5 : 10-dihydrophenarsazine hydrochloride	*M*/16,000	9	*M*/33,000	8
2-Amino-10-chloro-5 : 10-dihydrophenarsazine hydrochloride	*M*/10,000	6	*M*/14,400	4
3-Carboxy-10-chloro-5 : 10-dihydrophenarsazine	>*M*/50,000	<28	>*M*/50,000	<12
4-Carboxy-10-chloro-5 : 10-dihydrophenarsazine	>*M*/16,000	<9	>*M*/16,000	<4
2 : 10-Dichloro-5 : 10-dihydrophenarsazine	*M*/21,000	12	*M*/34,000	8
3 : 10-Dichloro-5 : 10-dihydrophenarsazine	>*M*/75,000	<42	*M*/92,000	48
2 (or 8)-Methyl-10-chloro-5 : 10-dihydrophenarsazine	*M*/110,000	61	*M*/320,000	80
N-Acetyl-2-(or 8)-methyl-10-chloro-5 : 10-dihydrophenarsazine	*M*/36,000	20	*M*/102,300	26
2 : 8-Dimethyl-10-chloro-5 : 10-dihydrophenarsazine	>*M*/120,000	<67	>*M*/120,000	<30
12-Chloro-12 : 7-dihydrobenzophenarsazine	>*M*/100,000	<55	>*M*/100,000	<25
10-Methyl-12-chloro-12 : 7-dihydrobenzophenarsazine	>*M*/100,000	<55	>*M*/100,000	<25
7-Chloro-7 : 14-dihydrodibenzophenarsazine	>*M*/100,000	<55	>*M*/100,000	<25
14-Chloro-7 : 14-dihydrodibenzophenarsazine	>*M*/50,000	<28	>*M*/50,000	<13
Phenylchloroarsine acetic acid	*M*/160	0	—	—
Phenylethylmethylchloroarsine	*M*/12,700	7	—	—
Phenylethoxychloroarsine	>*M*/4800	<3	*M*/13,000	3
Cacodyl chloride	*M*/2260	1	—	—
Cacodyl iodide	*M*/2260	1	—	—
Cacodyl oxide	*M*/1550	1	—	—
Cacodyl cyanide	*M*/47	0	—	—

Quinquevalent arsenic compounds.

Primary derivatives:				
Diphenyl-4 : 4′-diarsinic acid	*M*/4000	2	*M*/4000	1
o-Benzarsinic acid	*M*/140	0	—	—
o-Tolylarsinic acid	*M*/450	0	—	—
o-Arsanilic acid	*M*/570	0	—	—
Benzophenone-*o*-arsinic acid	*M*/920	1	—	—
Azobenzene-*o* : *o*′-diarsinic acid	>*M*/3500	<2	—	—
Secondary derivatives:				
Diphenylarsinic acid	*M*/1500	1	—	—
α-Naphthylarsinic acid	*M*/620	0	*M*/850	0
o : *o*′-Dicarboxydiphenylarsinic acid anhydride	>*M*/11,000	<6	—	—
Tertiary derivatives:				
Triphenylarsine dichloride	*M*/670	0	—	—

Antimony compounds.

Name	Toxicity to *Colpidium*: Concentration which kills in 3 mins.	Toxicity to *Colpidium*: Toxicity relative to diphenylchloroarsine =100	Toxicity to *Glaucoma*: Concentration which kills in 3 mins.	Toxicity to *Glaucoma*: Toxicity relative to diphenylchloroarsine =100
Diphenylchlorostibine	*M*/183,000	101	—	—
Triphenylstibine dichloride	*M*/700	0	—	—
Dimethylbromostibine	*M*/3400	2	—	—
Trimethylstibine dichloride	*M*/1140	1	—	—
Trimethylstibine hydroxybromide	>*M*/80	0	—	—

Mercury compounds.

Phenyl mercuric chloride	*M*/1,152,000	640	—	—
Allyl mercuric iodide	*M*/599,000	333	*M*/442,200	110

Miscellaneous compounds.

Diphenylbromobismuthine	*M*/270,000	150	—	—
Triphenylbismuthine	*M*/11,000	6	—	—
Tri-*p*-tolylbismuthine	>*M*/2000	<1	—	—
Triphenylbismuthine difluoride	*M*/7100	4	—	—
Methyl chloroacetate	*M*/8	0	—	—
Chloromethyl chloroacetate	*M*/300	0	*M*/400	0
Chloromethyl trichloroacetate	*M*/240	0	*M*/400	0
Trichloromethyl trichloroacetate	>*M*/550	0	>*M*/550	0
m-Methoxybenzyl bromide	*M*/1560	1	—	—
Dicyanofuroxan	*M*/800	0	*M*/950	0
Allyl isothiocyanate	*M*/100	0	—	—
Hexachlorodimethyl carbonate	*M*/5000	3	*M*/4400	1
Chloroaceto-*p*-bromoanilide	>*M*/2500	<1	>*M*/2500	<1

Discussion.

Before proceeding to discuss the results detailed in the preceding tables, it should be mentioned that a preliminary investigation was carried out in order to ascertain whether the protozoal test gave results concordant with the general knowledge available concerning toxicity. That investigation is reported elsewhere [Walker, 1922].

The results, for both organic and inorganic compounds, indicated that unicellular organisms provide a useful means of measuring general protoplasmic toxicity. The protozoal test, however, fails to give any indication of poisons exerting a specific action upon mammals; for example, the cyanides, certain amines and the alkaloids.

Consideration of present results.

The present results afford striking confirmation of the conclusions reached by McCleland and Peters [1919]. These conclusions were "for similar compounds:

(*a*) primary compounds are less toxic than secondary,
(*b*) aliphatic compounds are less toxic than aromatic,
(*c*) arsenic compounds are less toxic than antimony."

With regard to conclusion (*a*) the evidence in its favour brought forward here is overwhelming. Conclusion (*b*) is amply supported and is in accordance with the general rule concerning aliphatic and aromatic compounds. The

evidence in favour of conclusion (*c*) is definite but too slight to render a sweeping assertion possible. McCleland and Peters, however, only examined tervalent derivatives of arsenic. In the present work a large number of both ter- and quinque-valent arsenic derivatives have been examined, with the result that it is possible to make a fourth generalisation; viz. tervalent derivatives are more toxic than quinquevalent derivatives of arsenic. This generalisation is commonly implied in the explanation of the trypanocidal action of quinquevalent arsenic compounds; for such compounds are but slightly toxic to trypanosomes *in vitro* and it is assumed that they are reduced *in vivo* to the tervalent state prior to exerting their trypanocidal action [Durham, Marchal and King, 1926; Voegtlin *et al.*, 1923]. The number of tertiary derivatives of arsenic which have been examined is small, but if, as appears to be justified, the results for antimony compounds be taken into account as well, it will be seen that yet a fifth generalisation is possible; viz. that tertiary derivatives are less toxic than secondary derivatives of arsenic. The evidence also suggests that tertiary are less toxic than primary derivatives. Thus, there are four conclusions well established:

1. Aromatic derivatives are more toxic than aliphatic derivatives of arsenic.

2. Tervalent derivatives are more toxic than quinquevalent derivatives of arsenic.

3. Secondary derivatives are more toxic than primary and tertiary derivatives of arsenic.

4. Primary derivatives are more toxic than tertiary derivatives of arsenic. Whilst the evidence in favour of a fifth is suggestive.

5. Antimony compounds are more toxic than the corresponding arsenic compounds.

There is little doubt that conclusions 1–4 are as applicable to antimony (and probably bismuth) as to arsenic. Conclusion 2 may be expressed in another manner: amongst arsenic compounds the greater the susceptibility to oxidation, the greater the toxicity. Whether such conclusion is capable of extension to other metals is at least an open question; Morgan and Cooper [1924, 1] state that tellurium in bivalent combination is far superior to quadrivalent tellurium as a bactericidal agent.

An outstanding feature of the results is the high toxicity of tervalent secondary derivatives of arsenic; and within this class those compounds in which the arsenic atom is associated with the diphenyl group (or some modification of it) are characterised by the highest toxicity. So long as the arsenic is linked up with the diphenyl group it seems to be of little significance what element or group engages the third valency of the arsenic, provided that such group is capable of being readily replaced by an hydroxyl group—an important point. Thus diphenylchloroarsine, diphenylbromoarsine, diphenylcyanoarsine and diphenylarsenious oxide are all of the same high order of toxicity—and all can, in aqueous medium, give rise to diphenylarsenious

hydroxide. Linking up the diphenyl groups alters the toxicity but little. This is best illustrated by the examples of diphenylchloroarsine, $C_6H_5.AsCl.C_6H_5$, M/180,000, cyanophenoxyarsine, $C_6H_4\begin{smallmatrix}O\\AsCN\end{smallmatrix}C_6H_4$, M/261,300, and 10-chloro-5 : 10-dihydrophenarsazine, $C_6H_4\begin{smallmatrix}NH\\AsCl\end{smallmatrix}C_6H_4$, M/102,000.

The above examples form an interesting group, from which it appears that the exact type of linkage of the two phenyl groups has but little effect upon the high toxicity of these compounds. But any other modification of the structure inevitably leads to a fall in toxicity, in some cases very marked. Thus, substitution of one or both phenyl groups by naphthyl groups leads to a fall in toxicity, and substitution of one or both phenyls by aliphatic radicles again leads to a fall in toxicity. Substitution in the benzene ring at once reduces the toxicity; this has been tested by the insertion in the ring of the following groups: Cl, —COOH, $—NH_2$ and $—CH_3$. Ample evidence in support of the above statements is to be found in the tables.

As already mentioned, the toxicity seems but little affected by the third group attached to the arsenic, provided it is hydrolysable; for there can be little doubt that these compounds, administered as they are in such dilute solution, exert their toxic effect in the hydrolysed form. The rate of hydrolysis of diphenylchloroarsine, for instance, is described by Rona [1921] as "momentan." It may be mentioned that there can be no question of acid effect in these toxicity measurements, since there was always more than enough pyridine present to neutralise the acid liberated. Further proof that these compounds exert their toxic action in the hydroxide form is that the corresponding oxides are all rather more toxic than the halogen derivatives, as would be expected since one molecule of the oxide can give rise to two molecules of the hydroxide:

$$[(C_6H_5)_2As]_2O \xrightarrow{H_2O} 2(C_6H_5)_2As\text{-}OH.$$

Such variations in toxicity as occur among a series of diphenylarsine derivatives, in which the third hydrogen atom is replaced by different groups (Cl, Br, I, CN, O), are probably due to concomitant variations in solubility and rate of hydrolysis. One further point concerning the secondary derivatives of arsenic is worthy of note: all these highly toxic compounds are readily lipoid-soluble and but slightly soluble in water. Whether this is an essential condition to high toxicity or whether it is merely coincidental is a matter of opinion.

The mercury compounds examined are of considerable interest in view of their general high toxicity. The substitution of a phenyl (or allyl) radicle for one of the chlorine atoms of mercuric chloride enhances the toxicity enormously. It has already been noted that the highly toxic arsenic compounds are all readily lipoid-soluble and it seems probable that these organic derivatives of mercury owe their enhanced toxicity (over mercuric chloride)

mainly to their ready solubility in lipoids. It is not suggested that lipoid-solubility is the only factor influencing toxicity in this series of compounds, but the absence of information concerning the precise mode of action of mercury compounds prevents the appreciation of whatever other factors are at work.

Amongst the miscellaneous compounds examined, those containing bismuth suggest that this metal ranks with arsenic in toxicity, whilst tellurium belongs to a lower order of toxicity. The remaining miscellaneous compounds were tested because they have been reported, at different times, in the current literature as possessing toxic properties. It will be seen that they are generally of a low order of toxicity, at least as protoplasmic poisons, and no feature of interest is raised by them.

Application of results to higher animals.

Many of the compounds examined for toxicity to protozoa, particularly the secondary directives of arsenic, are known to be powerful respiratory irritants. It is a matter of some interest therefore to ascertain whether any relationship exists between toxicity as measured against protozoa and respiratory irritant power.

The work of Flury [1921] and of Tattersfield and Roberts [1920] suggests that such a relationship does exist and the results recorded in this paper point to a similar conclusion. There are, however, notable exceptions which prevent the promulgation of the simple rule that toxicity to *Colpidium* is a measure of irritancy. The question cannot be discussed here, but this much may be said: if it be permitted to suppose that high (sensory) irritant power resides in an optimum combination of

(*a*) a primary irritant action (directly upon nerve endings), and

(*b*) a weak but prolonged irritant action (indirectly, through the swelling of protoplasm and consequent pressure upon nerve endings), then all the results are capable of interpretation.

Toxicity to protozoa would give a measure solely of (*b*) and therefore would give, when high enough, an apparent correspondence between toxicity to *Colpidium* and sensory irritant power.

Mode of action of arsenicals.

The precise mode of action of arsenic in its various combinations has been investigated intermittently for many years and throughout there appears to have been an appreciation of the significance of the two states, the ter- and the quinque-valent, in which arsenic can exist. The pioneer work in this field was done by Ehrlich and Hata [1910], particularly in relation to therapeutical measurements. A brief résumé of the position is to be found in a report by Voegtlin *et al.* [1923]. Attempts have been made [Rona *et al.*, 1922; Flury, 1921] to show that arsenic owes its intrinsic toxicity to the power of inhibiting enzyme action, but little success has attended these efforts. The

most substantial advance is that made by Voegtlin *et al.* [1923], who have presented a very strong case for the view that arsenic specifically attacks the sulphydryl group of the cell (and, in particular, reduced glutathione). In the course of this present investigation it has been fully demonstrated that, of all the arsenic compounds, the tervalent secondary derivatives are the most toxic. It was observed that all these highly toxic compounds seemed to possess the common property of being susceptible to oxidation, even to atmospheric oxidation. This property is best demonstrated by dissolving the compound in turpentine; if a trace of water be added to ensure hydrolysis, the corresponding arsinic acid crystallises out in the course of an hour or two. An account of a typical case of this oxidation is given elsewhere [Walker, 1925]. This case of oxidation suggested that this type of arsenic compound might interfere in the oxidation-reduction system of the cell. Accordingly, the effect of a typical compound (diphenylchloroarsine) upon the washed muscle—glutathione system of Dixon and Hopkins [1922]—was investigated. Evidence was obtained of a definite interference with the oxidation of the tissue, attributable to an inhibition of the glutathione by the arsenic. It was difficult to give precision to this line of investigation, owing to the very low solubility of diphenylchloroarsine in water. Recourse was therefore had to a direct investigation of the effect of sulphydryl compounds upon the toxicity of diphenylchloroarsine to protozoa. To this end, the effect was examined of varying concentrations of sodium thioglycollate upon the toxic effect of a solution of diphenylchloroarsine. The sulphydryl compound was added to the solution of arsenic compound prior to the addition of the latter to the culture in the normal way. Table VI contains the results; the two concentrations refer to one and the same solution.

Table VI.

Concentration of diphenylchloroarsine mg. per litre	Concentration of sodium thioglycollate mg. per litre	Time required to kill all the organisms mins.
2·5	0	9½
2·5	2·5	24
2·5	6·25	33
2·5	12·5	62
2·5	25·0	175
2·5	50·0	>180

This illustrates the very marked detoxicating effect of a simple sulphydryl compound upon diphenylchloroarsine. The investigation was extended further to show that the organisms could be made to survive considerable periods (30–60 minutes), by the addition of a small amount of sodium thioglycollate 2 minutes after the addition to the culture of a dose of diphenylchloroarsine which was lethal normally in 3 minutes. Finally it was found that by the addition of a few drops of a suitable sulphydryl solution, at the end of 3 minutes, to a culture which had received a 3-minute lethal dose of arsenic, the organisms could be revived. The sulphydryl solution was added immediately

all movement of the organisms had stopped; revival was rapid and in one minute some 90 % of the organisms were rapidly swimming about again. Death took place ultimately after 1–2 hours. These are the only circumstances in which the organisms have been seen to resume movement after having once stopped under the influence of an arsenic compound. For this last test the sulphydryl compound used was monothioethylene glycol, prepared by Bennett's [1922] method. A 0·1 % solution in water, neutralised, was used. This compound is superior to most sulphydryl compounds in being more stable and in being readily soluble both in water and organic solvents (and therefore presumably lipoid-soluble). It is clear, therefore, that sulphydryl compounds possess the power of counteracting the toxic effect of arsenic compounds to a marked degree and it is of interest that this conclusion was reached independently, and by a different route, to the similar conclusion of Voegtlin.

Whatever significance may be attached to this result it would appear that the susceptibility of the tervalent arsenic compounds to oxidation can only be regarded as a measure of the combining power of the compound, an indication of the potential of the arsenic in the molecule. For it is in the molecule as a whole that the toxicity resides, not in the arsenic alone, or in the constituent groups. The radicles attached to the arsenic are only important in so far as they affect the reactivity of the arsenic and it seems probable that in the diphenylarsine structure the optimum degree of reactivity is to be found.

Summary.

1. The relationship between chemical constitution and toxicity has been studied, toxicity being measured against the unicellular organism, *Colpidium colpoda*, and, to a lesser extent, against *Glaucoma scintillans*.

2. The bulk of the compounds examined are derivatives of arsenic; a lesser number of antimony, bismuth and mercury compounds have also been examined.

3. The following general rules, appertaining to arsenic compounds, have been established :

(*a*) aromatic derivatives are more toxic than aliphatic derivatives;
(*b*) tervalent derivatives are more toxic than quinquevalent derivatives;
(*c*) secondary derivatives are more toxic than primary and tertiary derivatives;
(*d*) primary derivatives are more toxic than tertiary derivatives.

4. There is suggestive evidence that antimony compounds are more toxic than the corresponding arsenic compounds.

5. The most toxic arsenic compounds are the tervalent secondary derivatives and, within this class, compounds of the diphenylarsine type of structure are the most toxic.

6. It is considered that unicellular organisms only afford a measure of general protoplasmic toxicity.

7. Evidence is brought forward to show that the toxicity of arsenicals is intimately concerned with the sulphydryl constituents of the cell. Organisms which have ceased all movement under the influence of arsenic can be revived at once, and continue to live for periods up to 2 hours, by the addition of a suitable sulphydryl compound.

In conclusion I should like to record my gratitude to Prof. R. A. Peters for his advice and interest throughout this work.

REFERENCES.

Bennett (1922). *J. Chem. Soc.* **121**, 2139.
Brooks (1925). *Amer. J. Physiol.* **72**, 222.
Cooper, Woodhouse and Forstner (1925). *J. Soc. Chem. Ind.* **44**, 134 T.
Crane (1921). *J. Pharm. Exp. Ther.* **18**, 319.
Dale (1913). *J. Physiol.* **46**, 29.
Dixon and Hopkins (1922). *J. Biol. Chem.* **54**, 526.
Durham, Marchal and King (1926). *J. Pharm. Exp. Ther.* **28**, 341.
Ehrlich and Hata (1910). Die experimentelle Chemotherapie der Spirillosen.
Flury (1921). *Z. exp. Med.* **13**, 523.
McCleland and Peters (1919). *J. Physiol.* **53**, *Proc.* xv.
Morgan and Cooper (1924, 1). *J. Soc. Chem. Ind.* **42**, 352 T.
—— —— (1924, 2). *J. Soc. Chem. Ind.* **43**, 304 T.
Peters (1921). *J. Physiol.* **55**, 1.
Rona (1921). *Z. exp. Med.* **13**, 16.
Rona, Airila and Lasnitzki (1922). *Biochem. Z.* **130**, 582.
Tattersfield and Roberts (1920). *J. Agric. Sci.* **10**, ii. 199.
Voegtlin, Dyer and Leonard (1923). *U.S. Pub. Health Rep.* No. 860.
Walker (1922). *Thesis, Univ. Liverpool.*
—— (1925). *J. Chem. Soc.* **127**, 1491.