CXXXI. THE TOXICITY OF PHENYLARSINIC
ACIDS FOR *BALANTIDIUM COLI* MALM.
IN CULTURES, IN RELATION
TO THEIR CHEMICAL
CONSTITUTION.

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IN the course of an examination into the effect of a variety of drugs on cultures of *Balantidium coli* it was found that out of three organic arsenic compounds used, only two showed any considerable degree of toxicity. As this seemed to indicate that a relationship might exist between toxicity and chemical constitution, the effect of a series of selected arsenicals was examined *in vitro*. The results obtained appear to be of sufficient interest to merit a brief record.

Since the discovery of the nature of atoxyl by Ehrlich in 1907, numerous papers have appeared on the use of organic arsenic compounds for protozoal diseases. Many derivatives of both ter- and quinque-valent arsenic compounds have been employed in these researches, but as yet little has been brought to light regarding the laws correlating toxicity and chemical constitution. In this connection a noteworthy feature is that different results may be obtained in toxicity tests on organisms according to whether the experiments are carried out *in vivo* or *in vitro*. Thus while sodium *p*-aminophenylarsinate has proved to be a most effective remedy for sleeping sickness, *in vitro* it is almost without action on trypanosomes.

In order to explain this discrepancy between *in vitro* and *in vivo* experiments, it has been suggested that the arsenicals undergo some change in the animal body, and that it is the resulting product and not the original substance which displays toxicity. These divergencies, however, may be more apparent than real, for toxicity tests on microscope slides may have little significance—this has been clearly shown in the case of *E. histolytica* by Dobell and Laidlaw [1926, 1]—and although trypanosomes can be readily cultivated they are in cultures so far removed from their natural conditions that little weight would attach to results from drugs tested in this way. In the present investigation this difficulty does not appear to be so marked, for it has been found possible to grow *B. coli* under conditions which seem to approximate to natural ones very closely. Particulars of this will shortly be published elsewhere.

Technique.

The strains of *B. coli* used for this work were obtained from the caecum of pigs. So far as can be determined from morphological evidence this is the same ciliate as is found in man, in which host it causes an intractable dysentery. It is possible, however, that there are really two species in the pig as described by McDonald [1922]—*B. coli* and *B. suis*—but in the material used for the toxicity tests the *B. coli* form predominated and in any case both forms are affected by drugs in exactly the same way so that we shall regard the cultures as being those of *B. coli*.

Material from the caecum of freshly killed pigs was planted in Dobell and Laidlaw's [1926, 2] medium consisting of an inspissated horse-serum slope covered with Ringer solution containing the whites of two eggs to one litre of solution. In addition a small quantity of sterile rice starch is essential. In this medium *B. coli* grows with great freedom and shows no signs of degeneration—a feature so common in the cultivation of protozoa. Indeed it seems probable that *in vitro* results on intestinal protozoa grown in this medium are much more reliable than is the case with blood-protozoa on account of the more nearly natural conditions obtained in the authors' tubes. No strain was used for experimental purposes until growth had been well maintained for several weeks.

1 % solutions of the drugs were made in distilled water and the higher dilutions, for use in the toxicity experiments, were prepared by dilution with the requisite quantity of Ringer egg-white solution. In cases where dilutions lower than 0.1 % were required, a 1 % solution was made up in the Ringer egg-white solution. In this way, the liquid medium was never diluted to any appreciable extent by addition of the drug solution. About 5–6 cc. of fluid medium containing the required drug were used in each tube and to this one drop of inoculum, obtained from a mixture of healthy 24 and 48 hour cultures was added. The tubes were incubated at 37° and examined on the second and third days after inoculation, growth in cultures always being at its best on these days. The effect of the drug was estimated by observing the numbers of living and dead organisms, the increase or decrease in these numbers, and especially by the condition of the organisms, such as the rate of ciliary movement, the size and shape of the body and the presence or absence of degenerative changes in the endoplasm. A record of life alone in cultures is not sufficient, as, in two cultures showing living organisms, one might be quite normal and the other degenerate. For this reason the tables showing our findings are annotated to show, as far as possible, the condition of the organisms in the culture tubes. Control tubes are not given in the tables in order to economise space but in all experiments at least one control tube was put up for every four drug solution tubes and usually one control for three drug solution tubes. No experiment is recorded in which the controls were unsatisfactory.

Table I. *Numbers of living (L) and dead (D) cultures on 3rd day of incubation.*

		Dilutions ...		... 1:100		1:250		1:500		1:750		1:1000		1:3000		1:5000		1:7500	
				L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
A	Sodium <i>o</i> -hydroxyphenylarsinate	—	—	—	—	—	—	—	—	—	—	0	3	—	—	—	—	—	—
B	Sodium <i>o</i> -aminophenylarsinate	—	—	—	—	—	—	—	—	—	—	0	3	—	—	—	—	—	—
C	Sodium <i>p</i> -aminophenylarsinate (Atoxyl)	—	—	—	—	—	—	—	—	—	—	0	3	—	—	0	4	0	9
D	Sodium <i>p</i> -hydroxyphenylarsinate	—	—	—	—	—	—	—	—	—	—	0	7	—	—	0	3	—	—
E	Sodium <i>m</i> -aminophenylarsinate	—	—	—	—	—	—	—	—	—	—	0	8	—	—	0	5	—	—
F	Sodium 3-hydroxy-1:4-benzisoxazine-6-arsinate (Cyclosan)	—	—	—	—	0	5	0	5	0	5	0	14	4*	2	8	6	3	2
G	Sodium <i>N</i> -glycineamidophenylarsinate (Tryparsamide)	—	—	—	—	0	10	1	10	1	13	7*	7	10	3	7	0	—	—
H	Sodium 4-hydroxy-5-acetylaminophenylarsinate (Sodium Stovarsol)	0	5	0	4	1	6	1	7	9†	3	5	0	3	0	—	—	—	—
J	Sodium 2-hydroxy-5-acetylaminophenylarsinate (Sodium Troposan)	3‡	0	3‡	0	5	0	5	0	5	0	—	—	—	—	—	—	—	—
K	Sodium 8-acetylmino-3-hydroxy-1:4-benzisoxazine-6-arsinate (Sodium Parosan)	3‡	0	3	0	5	0	5	0	6	0	—	—	—	—	—	—	—	—

		Dilutions ...		... 1:10,000		1:15,000		1:20,000		1:30,000		1:40,000		1:50,000		1:60,000		1:70,000	
				L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
A	Sodium <i>o</i> -hydroxyphenylarsinate	0	4	—	—	0	4	1§	3	3§	9	6	3	5	0	3	0	—	—
B	Sodium <i>o</i> -aminophenylarsinate	0	4	—	—	0	4	1§	3	0	4	5	3	5	0	4	0	—	—
C	Sodium <i>p</i> -aminophenylarsinate (Atoxyl)	4*	9	3	8	5	11	6	3	7	0	6	0	3	0	—	—	—	—
D	Sodium <i>p</i> -hydroxyphenylarsinate	0	8	0	4	5	2	7	0	4	0	4	0	—	—	—	—	—	—
E	Sodium <i>m</i> -aminophenylarsinate	0	9	3†	8	4	3	6	0	4	0	—	—	—	—	—	—	—	—
F	Sodium 3-hydroxy-1:4-benzisoxazine-6-arsinate (Cyclosan)	6	0	6	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G	Sodium <i>N</i> -glycineamidophenylarsinate (Tryparsamide)	3	0	6	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H	Sodium 4-hydroxy-5-acetylaminophenylarsinate (Sodium Stovarsol)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
J	Sodium 2-hydroxy-5-acetylaminophenylarsinate (Sodium Troposan)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K	Sodium 8-acetylmino-3-hydroxy-1:4-benzisoxazine-6-arsinate (Sodium Parosan)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Organisms few and sluggish.

† Organisms sluggish.

‡ Slightly deformed.

§ Organisms few and degenerate.

THE RELATION BETWEEN TOXICITY AND CHEMICAL CONSTITUTION.

Of the recent work on the relation between toxicity and chemical constitution of organic arsenic compounds, researches on trypanosomes have claimed by far the greater part. In this connection reference may be made to papers by Fournneau and his collaborators [1923, 1926], King and Murch [1925], Hewitt and King [1926], and Ewins and Everett [1927]. Although it seems probable that the relation between toxicity and structure may depend to a certain extent on the specific organism experimented upon, one or two generalisations appear to be permissible from the work on trypanosomes.

(1) The aminophenylarsinic acids show considerable toxicity but the position of the amino-group is of importance, the *ortho*-isomer being most highly toxic.

(2) Marked toxicity is exhibited by the hydroxyphenylarsinic acids but the actual position of the hydroxyl grouping is less important.

(3) Fournneau found that in the treatment of trypanosomiasis, the trypanocidal power of *p*-aminophenylarsinic acid was not diminished by acetylation, but under the same treatment, the *meta*- and *ortho*-isomers showed decreased toxicity.

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The compounds considered in the present investigation were examined as sodium salts and fall into three groups.

(a) Amino- and hydroxy-phenylarsinates: toxic to *B. coli* in dilutions above 1/20,000.

(b) Cyclosan, tryparsamide and stovarsol: toxic in dilutions below 1/10,000.

(c) Troposan and parosan: not toxic in dilutions of 1/500 and only very slightly so in 1 % dilutions.

It will be seen from our tables that the results obtained by *in vitro* work on a species of intestinal protozoa are to some extent in agreement with the generalisations given above for *in vivo* work on trypanosomes.

(1) The aminophenylarsinates show considerable toxicity, the *ortho*-isomer being most highly toxic. The position of the amino-group has a very definite effect.

(2) The substitution of a hydroxy-group in place of the amino-group does not greatly alter the toxicity, unless the degree of hydration is taken into account—see below—when the *ortho*-hydroxy salt is the most toxic examined. The position of the group seems to have a considerable effect on toxicity.

(3) Of cyclosan and tryparsamide little can be said beyond that, in the latter case, substitution in the amino-group of *p*-aminophenylarsinate has lowered the toxicity considerably.

(4) In all cases examined, addition of an acetyl-amino-group in a position *meta* to the arsenic grouping has resulted in a marked decrease in the parasitocidal effect. This is most marked in troposan where, by addition of the $\text{CH}_3\text{.CO.NH-}$ grouping, *o*-hydroxyphenylarsinate has been rendered non-toxic.

All the toxic sodium phenylarsinates dealt with in this paper, with the exception of sodium *o*-aminophenylarsinate, are hydrated, and, when the degree of hydration is taken into account, the figures given for the lowest dilution at which the salt is not toxic are changed to those given in Table II.

Table II.

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>
Lowest dilution at which the salt is completely non-toxic	1	1	1	1	1	1	1	1
	73,500	60,000	55,000	41,250	38,800	11,800	7,727	3,920
Molecules of water	3	0	5	5	3	3	$\frac{1}{2}$	5

After making this correction certain points are clarified. In Table I *A* and *B* appear to be nearly equally toxic with a slight balance in favour of greater toxicity on the side of the amino-salt. The same is true of *D* and *E*. In Table II, on the other hand, there are well-defined intervals between the different toxic points and the *ortho*-hydroxy salt is definitely the more toxic.

The order of the toxicity of the compounds, however, still remains as given above. Previous workers do not appear to have taken the matter of hydration into account.

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