I

COMPARATIVE STUDIES IN ORGANIC ARSENIC DERIVATIVES

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I

ACTION ON *TRYPANOSOMA EQUIPERDUM*, WITH ESPECIAL REFERENCE TO ORAL ADMINISTRATION

THE epoch-making discovery by Ehrlich of the product 3 : 3' diamino 4 : 4' dihydroxy-arsenobenzene (Salvarsan "606") which, together with its immediate derivative the methylene sulphonylate (Neosalvarsan, "914") has proved of such inestimable value in the treatment of syphilis, stimulated research in the field of organic derivatives of arsenic to an enormous extent. The result has been the production of a vast number of such compounds, some few of which have proved to be of therapeutic value in diseases other than syphilis, notably yaws and certain forms of trypanosomiasis. Among the more important drugs of this series may be mentioned tryparsamide, atoxyl, stovarsol, etc. There still remain, however, innumerable possibilities for the production of new compounds of this type, while it would appear that many of the derivatives already produced have been only cursorily examined and may prove on more careful consideration to be worthy of more extended investigation.

The present communication is a preliminary report embodying results obtained during investigations which arose from some of the problems met with in the manufacture of products such as neosalvarsan, stovarsol, etc., from which we were led to the study of a number of more or less closely related compounds.

As is well known, products of the salvarsan type cannot be controlled solely by chemical means, but require to be examined biologically for toxicity and therapeutic activity. The tests now generally employed are carried out on mice or rats infected with *Trypanosoma equiperdum*. We have chosen this method of testing as our standard

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for comparison of a considerable number of products which we have had under investigation, and we publish these results in the hope that they may be of value to those engaged in similar research and may possibly stimulate interest in certain aspects of the general problem which we desire to emphasise.

Salvarsan and its more largely employed neutral derivative, neosalvarsan, have been employed in the therapy of syphilis by intravenous or, in some cases, subcutaneous or intramuscular injection. Oral administration of these products is considered to be valueless, the curative effects being negligible and the risk of secondary toxic effects, gastritis, jaundice, etc., being greatly increased. Nevertheless, Ravaut, in a recent publication,¹ has reported consistently good results obtained in the treatment of amœbic dysentery by oral administration of salvarsan, and suggests its employment in this manner as a prophylactic and a curative-agent. It is stated to be well tolerated in doses of from 0.2 to 1.0 gm. per day.

The employment of stovarsol by oral administration in the treatment of amœbic dysentery first suggested by Dr. Marchoux, of the Pasteur Institute of Paris², has had results of the most promising kind, and stovarsol appears to be almost, if not quite, equal in value to emetine, hitherto the most efficient drug employed in the treatment of the disease.^{3,4,5} It is to be noted that the amount of stovarsol constituting a course of treatment amounts to about 7 gm. spread over an interval of a week only, and there is a general consensus of opinion, borne out by the increasing popularity of the drug for this particular purpose, that no unpleasant sequelæ arise from its use in this manner, but that the drug has a well-marked tonic effect upon the system in general, and is extremely well tolerated by the patient under treatment.

Furthermore, it must be remembered that stovarsol, when administered orally, has been demonstrated by Levaditi and his co-workers⁶ to have a well-marked prophylactic or preventive action against syphilitic infection, not only in rabbits, but also in the human subject.

Again, in the very exhaustive series of papers by Brown and Pearce⁷ on the action of tryparsamide, mention is also made of the oral administration of this compound. From these, it appears that, while doses of 1 gm. and 1.25

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gm. per kilo in the rabbit produced symptoms of delayed arsenical poisoning, doses of 0.75 gm. per kilo were well tolerated, and were effective in curing a severe infection of *Tr. brucei* in one rabbit, and infections of *Treponema pallidum* in others. The number of experiments was too small, however, to draw any definite conclusion as to the value of this mode of administration.

Quite recently also Levaditi and his co-workers have shown⁸ that certain arsinic acids, *e.g.*, tryparsamide, 2-hydroxy 4-acetylamino-phenyl arsinic acid, etc., show definite prophylactic and curative effects against *Tr. nagana* in rabbits when administered *per os*.

There appear, therefore, ample grounds for assuming that some, at any rate, of the numerous organic compounds of arsenic now known, or which may be produced as the result of further research, may be of considerable interest and importance when given by the mouth. It will be admitted that, other things being equal, the oral method of administration has everything to recommend it, more especially in the treatment of tropical diseases in districts far removed from the ordinary amenities of civilisation where intravenous or intramuscular injection may be impracticable. The results submitted in this communication are, therefore, concerned in the main with this particular aspect of the question.

DESCRIPTION OF THE METHOD EMPLOYED IN CARRYING OUT THE TESTS FOR TOXICITY AND THERAPEUTIC EFFICIENCY

As is well known, all arsenical preparations employed in this country for the treatment of syphilis, such as salvarsan, neo-salvarsan, and the variously named manufactured products of the same type, are required to be biologically tested before issue.

They are tested by a special department set up for the purpose by the Medical Research Council, toxicity tests being carried out on normal mice, while the therapeutic efficiency is determined by their curative action upon mice infected with *Trypanosoma equiperdum*, an organism which experience has shown gives consistent indications of the activity of such preparations, and forms a reliable basis of comparison.

In order, therefore, to determine the minimum cura-

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tive doses of the various compounds included in the present communication, mice infected with a strain of *Trypanosoma equiperdum* were employed. The strain is maintained in rats which are housed at some distance from the laboratories in order to avoid any possible accidental absorption of arsenic which might tend to increase the resistance of the parasite to arsenic derivatives in general. The mice are infected by an intraperitoneal injection of 0.1 c.c. of diluted blood from an infected rat, the dilution being made with 1 per cent. sodium citrate solution, so that the count is of the order of 56,000 trypanosomes per cubic millimetre. After forty-eight hours the mice are examined, and those showing an infection of from 100,000 to 200,000 trypanosomes per cubic millimetre of blood are employed for the tests. It is found that infections below or above these figures are likely to give erratic results. The drug under investigation is then administered at the required dose and the blood examined twenty-four, forty-eight, and seventy-two hours afterwards. In order that the drug may have effected a "cure," the blood must be free from trypanosomes before or at the end of this period of time. As a rule, for each dose investigated, five mice are employed, and a cure is considered to have been effected when the peripheral blood of all five mice is completely freed from trypanosomes at the end of seventy-two hours. By varying the dose the minimum amount required to effect a "cure" under the above conditions can be determined. It is to be noted that in the majority of these "cures," relapses occur after varying intervals of time, but for the purpose of the present communication this aspect of the question is not taken into account.

Tests by intravenous injection are carried out by injecting a solution of the product into the most prominent tail vein of the mouse. Injection is facilitated by warming the tail by immersion in water at about 40° C.

Tests by subcutaneous injection are made by injecting a solution of the compound slowly under the skin of the back.

The strength of the solution employed for injection is so adjusted that only a fraction of 1 c.c. is employed for the dose required.

For oral administration, the required amount of the solid product is weighed out and transferred to a small

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saucer. A little water and about 0.6 gm. of bread crumb are added, and the whole worked up into a more or less homogeneous mass. This is placed, with an infected mouse, in a separate cage which is quite clean and free from sawdust, etc. Twenty-four hours is allowed for consumption of the meal, and there is usually no difficulty in ensuring that the material is eaten within this period. The mice are kept under observation exactly as described above.

The toxicity tests are carried out in precisely the same manner except that in these cases normal mice are, of course, employed, and for the oral tests the mice are starved for from twenty-four to thirty-six hours previous to administration, to ensure consumption of the rather large doses frequently necessary. At least four mice out of the five must consistently survive for seven days on the maximum tolerated dose.

The products which have been examined so far may be conveniently grouped under three headings:—

(1) *Arseno Compounds*.—These may be considered as compounds of the salvarsan type in that they contain arsenic in the tervalent condition. They are produced generally by the reduction of arsinic acids in which the arsenic is in the pentavalent condition. Two molecules of the arsinic acid on reduction link up to form one molecule of an arsenobenzene derivative. Thus the base of which salvarsan is the hydrochloride, is produced by the reduction of 3-amino 4-hydroxy-phenyl-arsinic acid.

Table I. summarises the results obtained by oral

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administration with a number of compounds of this type. Those compounds which are marked with an asterisk have not hitherto been described. A full account of the chemistry of these bodies will shortly be published elsewhere.

TABLE I

Base.	M.T.D. mgs. per gm.	M.C.D. mgs. per gm.	Ratio $\frac{\text{M.T.D.}}{\text{M.C.D.}}$
4 : 4' diamino arsenobenzene	0.10	0.02	5
*4-amino 4' acetylamino arsenoben- zene	0.3	0.02	15
*4 : 4' diacetylamino arsenobenzene	1.5	0.10	15
*3-amino 4 : 4' dihydroxy arseno- benzene	0.4	0.02	20
3 : 3' diamino 4 : 4' dihydroxy arsenobenzene (salvarsan)	0.9	0.03	30
*3-amino 3' acetylamino 4 : 4' di- hydroxy arsenobenzene (acetyl salvarsan)	4.0	0.05	80
3 : 3' diacetylamino 4 : 4' dihydroxy arsenobenzene (diacetyl salvar- san)	8.00	0.08	100
5 : 5' diamino 2 : 2' dihydroxy arsenobenzene	0.15	0.02	7.5
*5-amino 5' acetylamino 2 : 2' di- hydroxy arsenobenzene	0.20	0.02	10
*5 : 5' diacetylamino 2 : 2' dihydroxy arsenobenzene	7.00	0.02	350
*3 : 3' diamino 4 : 4' dihydroxy 5-acetylamino arsenobenzene	1.5	0.06	25
*3 : 3' diamino 4 : 4' dihydroxy 5 : 5' diacetylamino arsenobenzene	1.2	> 1.00	< 1.2
*4-glycinamido 4' hydroxy 3'-amino arsenobenzene	1.5	0.08	19
*4-glycinamido 4' hydroxy 3'-acetyl- amino arsenobenzene	6.0	0.15	40
*3 : 3' dihydroxy 6 : 6' arseno- 1 : 4 benzisoxazine	1.00	0.05	20

(2) *Arsinic Acids*.—These compounds are those in which the arsenic is in the pentavalent condition, as for example in stovarsol.

Their activity *in vivo* is generally regarded as dependent on their reduction in the tissues to the corresponding oxides (see Table III. below), in which the arsenic is again tervalent, as in the arseno bodies. The arsinic

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acids are known to be excreted with considerable rapidity, and it is, no doubt, largely owing to this fact that relatively large doses are required for efficient curative action and that very large doses are well tolerated by the organism.

TABLE II

Base.	M.T.D. mgs. per gm.	M.C.D. mgs. per gm.	Ratio M.T.D. M.C.D.
3-acetyl-amino 4-hydroxy phenyl arsinic acid (stovarsol) . . .	4.5	0.4	11
4-acetyl-amino 2-hydroxy phenyl arsinic acid . . .	10.0	0.1	100
5-acetyl-amino 2-hydroxy phenyl arsinic acid . . .	10.0	0.1	100
3-acetyl-amino 4-methoxy phenyl arsinic acid (O-methyl stovarsol)	2.0	0.8	2.5
N-phenyl glycineamide 4-arsinic acid (tryparsamide) . . .	14.0	0.6	23
*3-hydroxy 1 : 4-benzisoxazine 6- arsinic acid . . .	3.00	0.12	25
*3-hydroxy 1 : 4-benzisoxazine 8- amino 6-arsinic acid . . .	14.0	>7	<2.0
*3-hydroxy 1 : 4-benzisoxazine 8- acetyl-amino 6-arsinic acid . . .	16.0	0.2	80
*3-hydroxy 1 : 4-benzisoxazine 8- glycinamido 6-arsinic acid . . .	16.0	>8.0	<2.0
*3-glycinamido 4-methoxy phenyl arsinic acid . . .	12.0	>5.0	<2.4
Benzoxazolone 4-arsinic acid . . .	1.0	0.1	10
*Benzoxazolone 6-acetyl-amino 4- arsinic acid . . .	14.0	0.6	23
*3-acetyl-amino 4-hydroxy 5-amino- phenyl arsinic acid . . .	7.0	>5.0	<1.4
3 : 5-diacetyl-amino 4-hydroxy phenyl arsinic acid . . .	10.0	>8.0	<1.2
*2-hydroxy 5-diglycinamide phenyl arsinic acid . . .	12.0	>8.0	<1.5

Table III. comprises compounds containing tervalent arsenic of the type: $\text{As}=\text{O}$, $\text{As}=\text{Cl}_2$, etc. The curative dose of these compounds when administered orally is of the same order as that of the arseno derivatives.

It will be seen from Table I. that in the arseno series of compounds the best ratios are given by the mono- and di-acetyl derivatives of salvarsan, and of the isomeric base 2 : 2' dihydroxy 5 : 5' diamino arsenobenzene. The

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ratio obtained increases with the increase of acetyl groups present in the molecule even although, as is definitely the case in the derivatives of 2:2' dihydroxy 5:5' diamino arsenobenzene, the minimum curative dose remains unchanged.

A study of the rates of elimination of these bodies will be of very considerable interest, and it is intended to undertake this work shortly.

The introduction of an acetyl amino group into one benzene ring of the salvarsan molecule leads to the diminution both of the ratio and the curative action of the drug. The introduction of a similar group into the other benzene ring, thus rendering the body again symmetrical, reduces the ratio to a minimum, the product being as toxic as salvarsan but possessing no curative action on the toxic dose.

TABLE III

Base.	M.T.D. mgs. per gm.	M.C.D. mgs. per gm.	Ratio M.T.D. M.C.D.
3-amino 4-hydroxy phenyl dichlorarsine	0.40	0.025	16
3-amino 4-hydroxy phenyl diiodoarsine	0.50	0.05	10
*3-acetyl amino 4-hydroxy phenyl dichlorarsine	0.40	0.06	7
*3-acetyl amino 4-hydroxy phenyl arsenious oxide	0.50	0.055	9.0

In the series of arsinic acids (Table II.) it will be noticed that the second and third compounds are isomers of stovarsol, and the results obtained indicate that these are more active curatively and less toxic than stovarsol itself. It therefore appears desirable that they should be tried in those directions in which stovarsol has been found to be useful. There are, in all, ten possible isomers of stovarsol, and complete investigation of these is desirable.* A number have already been prepared and investigated by Fourneau,⁹ but not, so far as we are aware, from the point of view of oral administration.

The substitution of methoxy for the hydroxy group of

* The number of possible isomers of stovarsol has recently been completed by Fourneau.¹¹

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stovarsol was investigated, but no indication was obtained that the compound possessed permanently curative action such as has been described by Hewitt and King¹⁰ in the case of certain other methoxy compounds.

The benzisoxazine arsenic acids form an interesting series of compounds. They are heterocyclic compounds, which bear on the one hand some resemblance in chemical structure to stovarsol, and on the other to tryparsamide. 3-hydroxy 1:4 benzisoxazine 6-arsinic acid has the constitution shown by the formula II., while stovarsol and tryparsamide may be represented by I. and III. respectively.

(I.)

(II.)

(III.)

TABLE IV

Substance.	Method of Administration.	M.T.D. mgs. per gm.	M.C.D. mgs. per gm.	Ratio M.T.D. M.C.D.
Tryparsamide . . .	Intravenous injection .	2.5	0.7	3.6
	Subcutaneous injection .	1.8	0.6	3.0
	<i>Per os</i> . . .	14.0	0.6	23.3
3-acetylamino 4-hydroxy phenylarsinic acid (stovarsol).	Intravenous injection .	1.3	1.0	1.3
	Subcutaneous injection .	2.0	1.1	1.8
	<i>Per os</i> . . .	4.5	0.4	11.3
3-hydroxy 1:4 benzisoxazine 6-arsinic acid.	Intravenous injection .	0.2	0.15	1.3
	Subcutaneous injection .	0.2	0.15	1.3
	<i>Per os</i> . . .	3.0	0.12	25.0
3-hydroxy 1:4-benzisoxazine 8-acetylamino 6-arsinic acid.	Intravenous injection .	1.2	0.3	4.0
	Subcutaneous injection .	1.2	0.3	4.0
	<i>Per os</i> . . .	16.0	0.2	80

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The resemblance of the compounds to tryparsamide is borne out by the results obtained when these bodies are tested both orally and by injection. This is shown in Table IV.:

It will be noted that in each instance the curative dose is the same whether the drug is administered by the mouth or by injection. In this respect the compounds differ from those of the arseno series of compounds, and from numerous other arsinic acids where, in general, a much larger dose is required *per os* than by injection to produce a curative effect as shown in the following table :—

TABLE V

Substance.	Method of Administration.	M.T.D. mgs. per gm.	M.C.D. mgs. per gm.	Ratio $\frac{\text{M.T.D.}}{\text{M.C.D.}}$
3:3' diamino 4:4' dihydroxy arseno-benzene (salvarsan).	Intravenous injection.	0.125	0.010	12.5
	Subcutaneous injection .			
	<i>Per os</i> . . .	0.90'	0.03	30
3-amino 3'-acetyl-amino 4:4' dihydroxy arseno-benzene (acetyl salvarsan).	Intravenous injection .	0.20	0.02	10
	Subcutaneous injection .	0.30	0.03	10
	<i>Per os</i> . . .	4.0	0.05	80
3:3' diacetyl-amino 4:4' dihydroxy arseno benzene (di-acetyl salvarsan).	Intravenous injection .	0.175	0.03	6
	Subcutaneous injection.	0.40	0.05	8
	<i>Per os</i> . . .	8.0	0.08	100
5:5' diacetyl-amino 2:2' dihydroxy arsenobenzene.	Intravenous injection .	0.15	0.008	19
	Subcutaneous injection .	0.20	0.007	29
	<i>Per os</i> . . .	7.0	0.02	350
3:3' diamino 4:4' dihydroxy 5-acetyl-amino arseno-benzene.	Intravenous injection .	0.15	0.025	6
	Subcutaneous injection.			
	<i>Per os</i> . . .	1.5	0.06	25

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TABLE V—*continued*

Substance.	Method of Administration.	M.T.D. mgs. per gm.	M.C.D. mgs. per gm.	Ratio $\frac{\text{M.T.D.}}{\text{M.C.D.}}$
3 : 3' diamino 4 : 4' dihydroxy 5 : 5' diacetyl-amino arseno-benzene.	Intravenous injection .	0.2	0.15	1.3
	Subcutaneous injection.			
	<i>Per os</i> . . .	1.2	> 1.0	< 1.2
3-hydroxy 1 : 4-benzisoxazine 8-glycin-amido 6-arsinic acid.	Intravenous injection .	1.5	> 1.0	< 1.5
	Subcutaneous injection .	1.5	> 1.0	< 1.5
	<i>Per os</i> . . .	16.0	> 8.0	< 2
3-hydroxy 1 : 4-benzisoxazine 8-amino 6-arsinic acid.	Intravenous injection .	0.7	> 0.5	1.4
	Subcutaneous injection .	0.7	> 0.5	< 1.4
	<i>Per os</i> . . .	14.0	> 7.0	2

The two benzisoxazine derivatives referred to in Table IV. are seen to be more active than tryparsamide under comparable conditions. Further than this, observations made during the course of the animal tests suggest that these products, especially in the case of the acetyl-amino derivatives, are likely to be less productive of nervous symptoms. Further studies in this series in which numerous isomeric compounds are again possible are being continued.

REFERENCES

- (1) RAVAUT. *La Presse Médicale*, April, 1926, No. 32, p. 497.
- (2) MARCHOUX. *Bull. de la Soc. de Path. Exot.*, 1923, xvi., p. 79.
- (3) *Jour. Roy. Army Corps*, 1926, xlvii., pp. 300 and 399.
- (4) MANSON BAHR. *Lancet*, September 12th, 1925, p. 545.
- (5) LEVADITI. *Lancet*, September 19th, 1925, p. 593.
- (6) FOURNIER, LEVADITI, NAVARRO-MARTIN et SCHWARTZ. *Ann. de l'Institut. Pasteur*, 1922, xxxvi., p. 729.
- (7) BROWN and PEARCE. *J. Exp. Medicine*, 1919, xxx., p. 421.
- (8) LEVADITI NICOLAU et GALLOWAY. *Bull. de la Soc. de Path. Exot.*, 1926, xix., 737.
- (9) FOURNEAU, NAVARRO-MARTIN et M. et MME. TREFOUEL. *Ann. de l'Institut. Pasteur*, 1923, xxxvii., p. 551.
- (10) HEWITT and KING. *Jour. Chem. Soc.*, 1926, **129**, 817.
- (11) FOURNEAU, M. et MME. TRÉFOUEL, et MME. DE LESTRANGE-TREVISE. *Ann. de l'Institut Pasteur*, 1926, xl., 933.