'MELARSEN' AND 'MELARSEN OXIDE'

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Activity Against Trypansamide-Resistant Trypanosomes

THE melaminyl arsonate, 'Melarsen', that is, the di-sodium salt of 4-melaminylphenylarsonic acid [I], and the corresponding arsenoxide, 'Melarsen oxide' [II], have been suggested for the therapy of *T. gambiense* sleeping sickness^{1,2,3}. The most recent published statement on field trials of the latter compound is by Van Hoof⁴, who mentions that it is effective against cases of trypanamide-resistant sleeping sickness. There seems, however, to have been no publication, as yet, of any trials in laboratory infections which might give solid support to the expectation of efficacy in such cases. We have therefore tested the point by comparing the action of 'Melarsen oxide' on our laboratory strain of *T. rhodesiense* and on its trypanamide-resistant offshoot. (*T. rhodesiense* is not normally sensitive to trypanamide and other arsenicals, but in the course of many years of mouse-passage our strain has acquired a high degree of sensitivity, and tests against laboratory infections of this strain have proved to be a reliable screening method for the selection of compounds for use against *T. gambiense* infections in man.)

Mice were treated on the second day after intra-peritoneal inoculation of 2×10^4 trypanosomes, when the blood contained about 1 to 10 parasites per microscope field ($\frac{1}{4}$ th obj.). 'Mapharside', 3-amino-4-hydroxyphenylarsenoxide, was used instead of trypanamide to confirm that the trypanamide-resistant offshoot had retained its resistance unimpaired since this character was acquired eighteen years ago⁵.

TABLE 1. EFFECTS OF 'MAPHARSIDE' AND 'MELARSEN OXIDE' ON INFECTIONS OF THE PARENT STRAIN AND TRYPARSAMIDE-RESISTANT OFF-SHOOT OF *T. rhodesiense* IN MICE

Drug	Trypanosome strain	Dose (intraperitoneal), mgm./20 gm.				
		0.2	0.1	0.025	0.015	0.01
'Mapharside'	Parent	4 4C	5 5C	10 1C 9R	33 1C 29R 3N	15 8R 7N
	Resistant	8 8N	5 5N			
'Melarsen oxide'	Parent	4 4C	4 3C 1R	4 4C	17 8C 9R	19 1C 15R 3N
	Resistant	4 4C	4 4C	4 3C 1R	4 3C 1R	4 2R 2N

Results shown as follows:

4 mice treated; 1 cured (blood permanently cleared of trypanosomes), 1 relapsed (blood temporarily cleared), and 2 not affected (blood not cleared).

Table 1 shows that (a) the trypanamide-resistant offshoot has retained a full measure of resistance, and (b) 'Melarsen oxide' is equally as efficacious against the resistant as against the parent strain.

'Melarsen' also was shown, by similar tests, to be equally effective against both strains.

Interference by 'Surfen C'

The commonly used term 'arsenic-resistance', in reference to trypanosomes resistant to aromatic arsenicals such as trypanamide, 'Atoxyl', 'Mapharside', etc., has long been recognized as a misnomer, since it is the non-arsenical substituted phenyl radical rather than the mere presence of arsenic which may determine whether or not a compound is active against so-called 'arsenic-resistant' trypanosomes. Thus, it has been known since the time of Ehrlich that 'Atoxyl'-resistant trypanosomes are sensitive to arsenophenylglycine and to other aromatic arsenicals containing an acetic acid or carboxyl substituent^{6,7}. This feature has again been given prominence by Eagle's introduction of γ -(*p*-arsenosphenyl)butyric acid, active against trypanamide-resistant trypanosomes⁸. King and Strangeways⁹ suggested that such carboxyl-substituted arsenoxides differ from others in their mode of fixation on, or entry into, the trypanosome cell, while the ultimate lethal mechanism (presumably an inactivation of essential —SH groups) is the same for all types of arsenoxide. We have provided further circumstantial support for this view in arguments depending on the finding that *p*-amino-benzoic acid interferes with the trypanocidal action of Eagle's compound but not with that of 'Mapharside', while glutathione interferes with the action of both⁹.

With the demonstration of 'Melarsen oxide' as a representative of a further class of arsenoxide active against trypanamide-resistant trypanosomes, it became of interest to find out whether suitable interference experiments might provide similar circumstantial evidence for the view that this type of

arsenoxide also differs from others in its mode of fixation on the trypanosome cell, but not in the essential mechanism of its lethal effect.

The obvious type of compound in which to seek for the property of selective interference with 'Melarsen oxide' would be a substance containing the melamine nucleus. Such a compound is 'Surfen C', bis(2-methyl-4-amino-6-quinolyl)melamine [III], which is itself a chemotherapeutic agent of significant activity against *T. congolense* and *T. brucei*¹⁰. It also exercises some action on our strain of *T. rhodesiense*. In the experiments below, mice were infected with the parent strain of *T. rhodesiense* and treated under the standard conditions of timing and degree of infection mentioned above; in these circumstances more than 24 hours elapse before the blood becomes free of trypanosomes after treatment with 'Surfen C' even in maximal doses.

Table 2 gives the results of a number of interference experiments and shows that while *p*-aminobenzoic acid interferes selectively with Eagle's compound, 'Surfen C' interferes similarly with 'Melarsen oxide'.

TABLE 2. SELECTIVE INTERFERENCE: *p*-AMINOBENZOIC ACID AGAINST γ -(*p*-ARSENOSOPHENYL)BUTYRIC ACID (EAGLE'S COMPOUND), AND 'SURFEN C' AGAINST 'MELARSEN OXIDE'

Treated with (intraperitoneally)	Main treatment preceded 5 min. by		
	—	<i>p</i> -Aminobenzoic acid (70 mgm./20 gm.)	'Surfen C' (4 mgm./20 gm.)
'Mapharside' 0.015 mgm./20 gm.*	cl	cl	cl
Eagle's compound 0.015 mgm./20 gm.*	cl	n	cl
'Melarsen oxide' 0.003 mgm./20 gm.*	cl	cl	n

Each cell of the table represents results of treating at least eight mice. *ED₅₀, that is, dose causing temporary disappearance of trypanosomes from the blood in 95 per cent of mice (estimated by probit analysis of numerous trials).

cl = blood cleared of trypanosomes in 24 hours.

n = blood not cleared of trypanosomes in 24 hours.

Other tests showed that glutathione interferes equally with the action of 'Melarsen oxide' and of the other arsenoxides. If, then, glutathione interference be accepted as evidence that the essential mechanism of lethal action is by interference with —SH constituents of the parasite cell¹¹, we may conclude that 'Melarsen oxide' (like Eagle's compound) shares this mode of action with other arsenoxides, but that it differs in its fixation on, or route of entry into, the trypanosome, this fixation or route being selectively blocked by 'Surfen C'.

It is reasonable to suppose that it is the melamine nucleus of 'Surfen C' which determines the selective nature of its interference with 'Melarsen oxide'. If this were so, then no such selective interference should be exercised by 'Surfen' [IV], which differs from 'Surfen C' by having a carbamide instead of a melamine linkage between the two quinoline systems¹⁰. This anticipation was substantiated by interference experiments in respect of 'Surfen' and the three arsenoxides. It was found, however, by examining the blood at hourly intervals within eight hours of treatment, that instead of complete absence of any interference with the three arsenicals (as found in similar tests of 'Surfen C' with 'Mapharside' and Eagle's compound, respectively), 'Surfen' does exercise some degree of interference with each of the three arsenicals. The significance of this non-specific interference will be elaborated elsewhere, but mean-

while it is established that the interference of 'Surfen C' operates selectively against 'Melarsen oxide', and presumably depends on the presence of the melamine system in both compounds*.

The facts, therefore, warrant the view that while the ultimate lethal mechanism of 'Melarsen oxide' is the same as that of other arsenoxides (by inactivation of —SH groups, perhaps of the pyruvate oxidase system¹²), it has a radically different means of fixation on, or entry into, the trypanosome cell, and it is this difference which may underlie the fact that it is active against trypanosomes resistant to the more usual type of arsenical. We have recently reached a similar conclusion in connexion with anti-malarials. Representatives of the earlier pyrimidine prototypes of 'Paludrine', such as 3349¹³, are active against 'Paludrine'-resistant *P. gallinaceum*, and we have connected this finding with the conception that the pyrimidine precursors of 'Paludrine' share with the latter a common (but unknown) ultimate mode of lethal action on the malaria parasite, while differing in their mechanisms of attachment to, or entry into, the parasite cell¹⁴.

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* Note added in proof. This supposition has been further supported by finding that melamine itself interferes with the therapeutic action of 'Melarsen oxide', but not of 'Mapharside' or Eagle's compound.

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INTERNATIONAL STUDY OF RHEOLOGY

International Joint Committee on Rheology

IN December 1945 the International Council of Scientific Unions instituted a Joint Committee on Rheology, with representatives of the International Unions for Physics, Chemistry, Biological Sciences, and Geodesy and Geophysics, to which afterwards was added the International Union for Theoretical and Applied Mechanics. The International Union of Physics will act as 'mother union' for this Committee.

The Committee held its first meeting during September 8–9, 1947, in London. It was decided that its terms of reference should be to co-ordinate research and to investigate problems concerning the deformation and the flow of matter, and the following were nominated as officers for the year 1947–48: *Chairman*, Dr. Th. von Karman, Pasadena, Cal.; *Vice-chairman*, Mme. A. Dobry-Duclaux, Paris; *Secretary*,