THE BIOLOGICAL ACTIVITY OF ARSENOSOBENZENES IN RELATION TO THEIR STRUCTURE*

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I. GENERAL CONSIDERATIONS

Three types of organic compounds containing arsenic linked directly to carbon have been used successfully in the treatment of spirochetal and protozoal diseases: the pentavalent arsonic acids ($RAsO_3H_2$), the arseno compounds (R-As=AsR'), and the arsenoso compounds (RAsO) (or their dithiol derivatives). In these compounds R is usually benzenoid, although similar compounds with a heterocyclic ring, *e.g.*, pyridine, are known to be actively trypanocidal (15). Polycyclic, aliphatic and alicyclic arsenicals have also been tested, but are generally either only weakly active or inert (19, 87).

An important therapeutic consideration is whether the compounds are active as such or must first be modified in the animal host. The classic studies of Ehr-

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lich (53, 54) showed the arsonic acids to be highly active therapeutically, but inactive *in vitro*. He concluded (55) that they were reduced to an active trivalent form by the body tissues; the arseno and arsenoso compounds were in fact found to be directly active *in vitro*. The arsenoso compounds were discarded because of their high toxicity. The arseno compounds, notably arsphenamine and neoarsphenamine, found wide therapeutic application.

There has, however, been some difference of opinion as to whether the arseno compounds are active as such. Voegtlin and Smith (122) demonstrated in 1920 that after the injection of arsonic acids or arseno compounds there was a latent period before trypanosomes disappeared from the blood of infected animals, while arsenoso compounds produced an immediate and striking decrease. These results were interpreted to mean that the arsenoso compounds were directly trypanocidal, while the arsonic acids had to be reduced and the arseno compounds oxidized to the arsenoso form in the animal body before becoming active. Other workers, however, found that the arsphenamines as such, and without further modification in the animal body, were active against trypanosomes and spirochetes (101, 102, 129). The discrepancy was resolved by later *in vitro* studies with *Treponema pallidum* (32). Although the arsphenamines were apparently quite active *in vitro* against this organism when tested by the usual anaerobic technic, when both the dissolution and testing of the drugs were carried out under an atmosphere of nitrogen, their activity was strikingly reduced. The arsphenamines as such are therefore not directly treponemicidal, but in the course of dissolution and dilution prior to testing there is sufficient oxidation to the arsenoso compound to make the solution active. In general, the arseno compounds are amorphous powders which are almost impossible to obtain in a state of chemical purity, and exist in several states of molecular aggregation. Both factors have been shown to influence their parasiticidal activity and toxicity (93, 128).

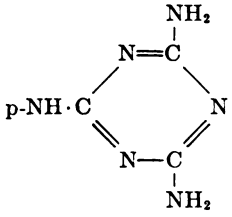
The fact that the arsonic acids and arseno compounds must be modified in the animal body before they exert their therapeutic action prevents the correlation of their chemical structure with biological activity unless all the compounds are modified *in vivo* to the same degree. This assumption is certainly not justified in the case of the arsonic acids which are excreted in large part unchanged. Cohen, King and Strangeways (23) studied the rate of oxidation of a number of arsenoso compounds, on the assumption that this might be a measure of the rate of reduction of the corresponding arsonic acids *in vivo*, and thus of their toxicity. No such correlation was found. Similarly, in later studies from our laboratory (47) there was no regular correlation between the toxicity (or trypanocidal activity) of arsonic acids and that of the corresponding arsenosobenzenes (Table 1).

The arsenoso compounds therefore appear to be the drugs of choice in an attempted correlation of chemical structure and biological activity. Unfortunately for that purpose, most pharmacological studies on the aromatic arsenicals have dealt with arsonic acids and arseno compounds, on the assumption that the arsenoso compounds were too toxic for therapeutic use. This assumption was

questioned by Tatum and Cooper (114) who demonstrated that m-amino-p-hydroxy-phenylarsenoxide, which they named "mapharsen" (oxophenarsine hydrochloride) possessed a higher therapeutic index against *Treponema pallidum* than the arsphenamines. Prior to the advent of penicillin, this compound was widely regarded as the drug of choice for the treatment of syphilis. A large series of arsenoso compounds were later found to possess a higher therapeutic index than the corresponding arsonic acids against *T. equiperdum* (47), although

TABLE 1

The poor correlation between (A) the toxicities of arsonic acid and the corresponding arsenoso compounds, and (B) the trypanocidal activities in vivo *of arsonic acids and the corresponding arsenoso compounds. (Summarized from 40, 41, 46, 47)*

SUBSTITUENT GROUP	A. TOXICITY IN MICE			B. TRYPANOCIDAL ACTIVITY IN MICE (*T. equiperdum*)		
	LD_{50}		Ratio of LD_{50}	CD_{50}		Ratio of CD_{50}
	As^{V}	As^{III}		As^{V}	As^{III}	
	*mg./kg.**			*mg./kg.**		
p-$CONHCH_2CONH_2$	1750	15	115	380	1.4	270
p-$NHCH_2CONH_2$	950	19	50			
p-$NHCONH_2$	1025	11	93	305	1.16	267
p-OCH_2CONH_2	745	9.5	78	80	0.82	98
3-$NHCOCH_3$-4-OH	765	6	127	565	1.91	296
3-NH_2-4-OH	775	17	46	85	0.62	137
p-SO_2NH_2	595	18	33	170	1.0	85
p-OH	550	2	275			
"Melarsen" 	260	12	22	30	0.048	625
3-NH_2-4-$CONH_2$	290	15	19			
3-NH_2-4-$(CH_2)_3COOH$	140	12	12	>190	1.26	>150
p-NH_2	165	1.5	110			

* mg./kg. of As

Gough and King had previously reported no essential difference between the two classes of compounds in a smaller series (69).

The present paper will consider the toxicity and parasiticidal activity of arsenosobenzenes in relation to the substituent groups on the benzene ring, preliminary to a discussion of their mode of action. Although attention has focussed largely on the mono-substituted compounds (28–31), a number of di-substituted derivatives of arsenosobenzene have also been prepared (22) (30, 86). In addition to the derivatives of arsenosobenzene, a limited number of arsenoso compounds with naphthalene, biphenyl, pyridine and similar ring structures have

been studied. Finally, a number of thioarsenites, compounds formed by the condensation of arsenosobenzenes with various thiol compounds, have been tested for toxicity and parasiticidal activity in a number of protozoal infections.

Toxicity has usually been determined as the 50 per cent lethal dose on single intraperitoneal injection in white mice (34), but has in some studies been determined also in rats, rabbits (47) and dogs. In general, the relative toxicity of the compounds in mice closely paralleled their relative toxicity in rabbits (Fig. 1). Parasiticidal activity has been studied with *Treponema pallidum* (34) and *Trypanosoma equiperdum* (47), both *in vitro* and *in vivo;* a few compounds have further been tested against *Trypanosoma cruzi* (38, 39), *Wuchereria bancrofti* (82, 116), *Litomosoides carinii* (88, 89), *Endamoeba histolytica* (2–6), and *Leishmania donovani* (39).

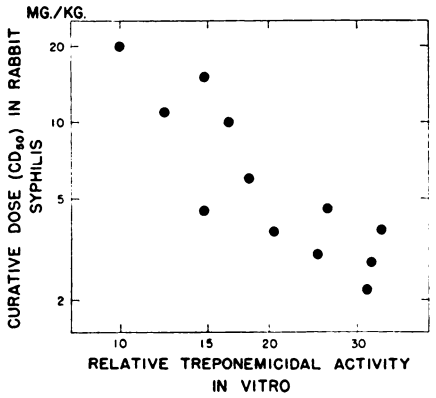


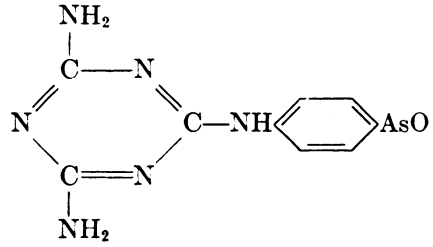
FIG. 1. Correlation between activity of arsenosobenzenes on *Treponema pallidum in vivo* and *in vitro*. (After 34, 40, 41, 46.)

Relative treponemicidal activity *in vitro* determined by direct immobilization; the unsubstituted arsenosobenzene is used as the reference compound (100 per cent activity).

In some infections (*cf.* Fig. 1 in re syphilitic infection, and *cf.* (47) for data in experimental trypanosomiasis) there was a reasonably satisfactory correlation between the relative parasiticidal action of a series of compounds *in vitro* and their therapeutic action *in vivo*. With other organisms, however, there was no correlation whatever between the direct parasiticidal action of arsenosobenzenes *in vitro* and their therapeutic action *in vivo*. With both *L. donovani* and *Trypanosoma cruzi*, a number of compounds which were highly active when added directly to the cultured flagellate proved wholly ineffective in the treatment of hamster infections (38, 39). The failure of the drug in these two instances may be related in part to the fact that in both infections the parasite in the infected host is in a different stage of its life cycle and differs morphologically and perhaps metabolically from the cultured organisms. Further, in both infections the organisms localize inside host cells, within which the drug may not attain the same concentrations as in extracellular fluids. Another example of the importance of the permeability factor *in vivo* is the relative ineffective-

ness of the arsenobenzenes in the treatment of central nervous system syphilis or trypanosomiasis. Even compounds which are highly effective in the treatment of the early stages of the disease may be relatively ineffective in cases with central nervous system involvement.

When a number of arsenosobenzenes were tested for therapeutic activity in both mouse and rabbit trypanosomiasis, there was generally good agreement in their relative activity in the two host species (47). An exception was, however, noted in the case of "melarsen oxide"



(59–63). The trypanocidal activity (*T. equiperdum*) of this compound in mice (CD_{50} = 0.2 mg./kg.) greatly exceeded that in rabbits (CD_{50} = 10 mg./kg.), and was far greater than would have been indicated by its trypanocidal activity *in vitro* (39). This peculiarly enhanced parasiticidal activity in mice deserves further study.

II. THE BIOLOGICAL ACTIVITY OF SUBSTITUTED ARSENOSOBENZENES

A. Activity in relation to structure

Unsubstituted arsenosobenzene is highly toxic for every cell species so far tested, and shows no evidence of specificity in its cytotoxic effect. Since it is as toxic for the mammalian host as for the parasite, the problem of finding therapeutically useful arsenosobenzenes becomes one of finding substituent groups which depress the toxicity of the compound for the host to a greater extent than they depress its parasiticidal action.

The effect of a number of single substituents on the toxicity and direct treponemicidal (*T. pallidum*) activity of arsenosobenzene is shown in Fig. 2 and Table 2. The latter also shows their direct trypanocidal activity (*Trypanosoma equiperdum*). Despite the wide variations encountered between closely related compounds, the data permit some tentative generalizations with respect to the correlation between chemical structure and biological activity in this series of compounds.

1. "*Inert*" *substituents.* There were a number of "inert" substituents (*e.g.*, —Cl, —NO_2, —NH_2, —OH, —CH_3, —F) which did not significantly affect either the toxicity or parasiticidal activity of arsenosobenzene. The activity: toxicity ratios of the resulting compounds were therefore substantially the same as that of the highly active and highly toxic parent arsenosobenzene. Like the latter, these compounds apparently have no selective or specific effect on a particular parasite. The position of such substituents on the benzene ring also had relatively little effect. Thus, the ortho-, meta- and para-arsenosotoluene com-

pounds had relative treponemicidal activities of 84, 97 and 102, respectively (referred to the parent arsenosobenzene as 100), relative toxicities of 88, 100 and 118, and activity:toxicity ratios of 0.95, 0.98 and 0.83.

2. *Acidic substituents.* Most acidic substituents markedly depressed both the treponemicidal and trypanocidal activity of arsenosobenzene, and resulted in compounds with a low level of activity (*cf.* Fig. 2 and Tables 2 and 3). The toxicity of these compounds was also reduced, but usually not to the same degree

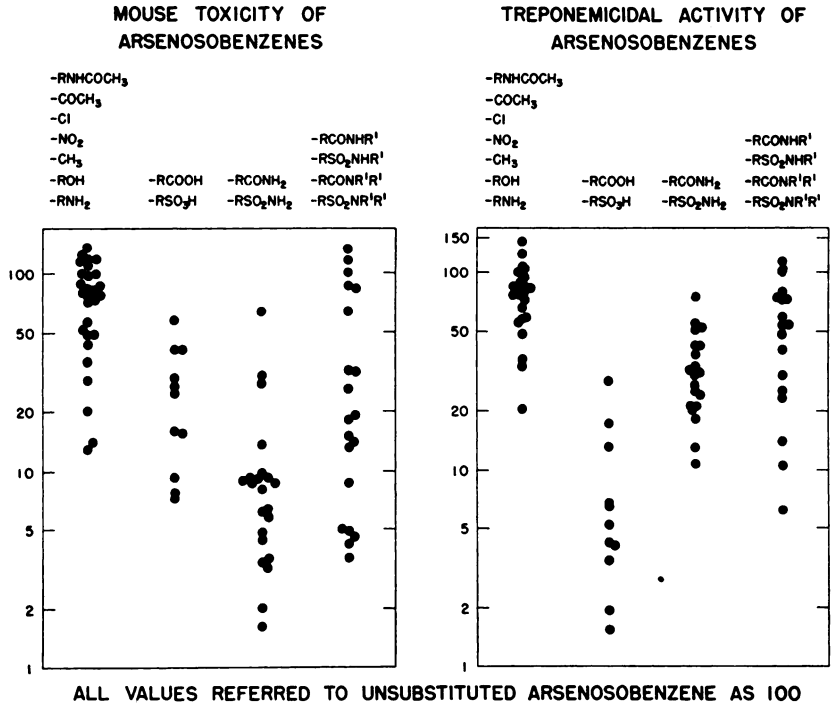


FIG. 2. The effect of various types of single substituents on the toxicity and treponemicidal activity of representative arsenosobenzenes. (After 41, 46, 47.)

Treponemicidal activity determined by direct immobilization *in vitro;* the unsubstituted arsenosobenzene is used as the reference compound.

as the parasiticidal activity. In consequence, the parasiticidal activity:toxicity ratios of the acid-substituted compounds were even less than that of the parent compound. There were, however, a few important exceptions to this generalization, notably the γ-(p-arsenosophenyl)-butyric acid-, δ-(p-arsenosophenyl)-valeric acid-, and ε-(p-arsenosophenyl)-caproic acids (*cf.* Table 3). The first two compounds were actively trypanocidal against *Trypanosoma equiperdum*, both *in vivo* and *in vitro*, although they had no significant activity against *T. pallidum*. Conversely, the last-named compound had considerable treponemicidal activity, but was almost inert against *Trypanosoma equiperdum*. These exceptions emphasize the danger of generalizing even on the basis of a large number of closely

TABLE 2

The effect of various single substituents on the toxicity and the treponemicidal and trypanocidal activity of arsenosobenzene

(All values referred to that of unsubstituted arsenosobenzene as 100.) (Summarized from 40, 41, 46, 47)

SUBSTITUENTS		TOXICITY IN MICE*	TREPONEMICIDAL ACTIVITY *in vitro** (*Trep. pallidum*)	TRYPANOCIDAL ACTIVITY *in vitro** (*T. equiperdum*)	RATIO OF TREPONEMICIDAL ACTIVITY *in vitro*: TOXICITY	RATIO OF TRYPANOCIDAL ACTIVITY *in vitro*: TOXICITY
"Inert"	NO_2, $—CH_3$, —Cl $—NH_2$, —OH, —F	(17) 36–123 85	(17) 36–147 85	(7) 57–102 91	0.33–1.47 1.0	0.80–1.2 0.92
"Slightly active"	$—RNH_2$, —ROH, $—NHCOCH_3$, R—$NHCOCH_3$, $—OCOCH_3$	(12) 6.7–76 23	(12) 21–78 38	(6) 31–71 34	0.38–4.8 1.76	1.2–6.0 2.2
Acidic (*cf.* Table 3)	—COOH, $—SO_3H$, —RCOOH	(15) 5.8–41 16	(14) 0.7–28 4.7	(12) 0.06–54 3.0	0.04–2.7 0.24	0.002–6.1‡ 0.1
Acid amides† (*cf.* Table 5)	$—CONH_2$, $—SO_2NH_2$, $—RCONH_2$	(16) 3.2–14 7.2	(16) 11–52 30	(12) 1.4–73 25	2.3–6.0 4.5	0.4–7.4 3.8

* ( ) = no. of compounds tested.
36–123 = range.
85 = median.

† Substituted amides not included.

‡ Anomalous result; next highest ratio was 0.9; *cf.* Table 3.

related compounds. They emphasize also the specificity of the reaction of some arsenoso compounds with particular parasites (*cf.* page 132).

The para-arsenosophenylbutyric acid compound has proved highly effective in the treatment of early human trypanosomiasis (*Trypanosoma gambiense*) (35, 37). It has proved effective also against so-called arsenic-resistant strains of that species (35, 37, 47, 48, 118) (*cf.* page 136). In the late forms of the infection, however, after the central nervous system has been invaded, it has proved relatively ineffective, presumably because the compound does not there attain

TABLE 3

The relative molar toxicity and parasiticidal activity of acid-substituted phenyl arsenoxides. (After 36, 39, 44, 47).

COMPOUND	pK	TOXICITY*	RELATIVE ACTIVITY IN VITRO AGAINST:			
			Trep. pallidum	*Tryp. equiperdum*	*Trypanosoma cruzi*	*L. donovani*
Unsubstituted phenyl arsenoxide	—	100	100	100	100	100
o-SO_3H	2	5.7	1.5	0.02	0.02	0.02
p-COOH	4.0	41	6.7	0.45	0.04	0.21
p-CH_2COOH	4.35	41	4.2	4.7		
p-$(CH_2)_2COOH$	4.7	7.3	4.1	2.8		
p-$(CH_2)_3COOH$	4.9	8.7	1.9	54		32
p-$(CH_2)_4COOH$	5.23	7.4	—	27	47	30
p-$(CH_2)_5COOH$	5.35±	8.1	22	7.5	12	2.5
p-COOH	4.0	41	6.7	0.45	0.04	0.21
m-COOH	4.25	16	13	—	0.3	1.6
o-COOH	5.55	27	28	3.2		1.5±
3-NO_2-4-COOH	2.6	27	18	17	24	40±
p-OCH_2COOH	3.3	25	4.2	4.5	0.3	6.8
3-NH_2-4-COOH	4.7	15	20	4.0	0.05	0.12
p-$NHCO(CH_2)_2COOH$	4.75	7.7	6.4	0.4±		0.15±
3-NH_2-4-$(CH_2)_3COOH$	5.34	7.2	—	26	62	26

* Single intraperitoneal injection in white mice. All values referred to that of phenyl arsenoxide as 100.

actively trypanocidal concentrations. Its possible use in conjunction with tryparsamide in these late manifestations is currently under study. In loa-loa, although the drug caused a temporary disappearance of micro-filaria from the blood stream, no permanent cure was effected (56). Corresponding to the results obtained in experimental mouse infections with *Trypanosoma cruzi* (cf. page 124), the compound proved ineffective in the treatment of the human infection (113). In surra (*Trypanosoma evansi*), Mace, Ott and Cortez (80) have reported that, although the drug has some measure of trypanocidal activity, cure is effected in only a small proportion of the animals treated at subtoxic levels of

the drug. The results obtained with p-arsenosophenylbutyric acid in a number of experimental trypanosomal infections are summarized in Table 4.

a. *Evidence for modification of acid-substituted arsenoso compounds in vivo.* There is reason to believe that with these acidic compounds the observed toxicity is not that of the compound itself but of a derivative formed in the body. 1) As is discussed in a following section, the toxicity of arsenicals is apparently determined by the degree to which they are bound by the host tissues (73). There was a high degree of correlation between the rate of excretion of an arsenical and its toxicity, the least toxic compounds being excreted at the

TABLE 4

Trypanocidal activity of γ-para-arsenosophenylbutyric acid in vivo

TRYPANOSOMAL SPECIES	HOST ANIMAL	TOTAL CD_{50}	REFERENCES
		mg./kg.	
T. equiperdum	Mouse	3.4*	47
	Rabbit	6.0†	47
T. gambiense	Guinea pig	4	119
	Man	7‡	35, 37
T. rhodesiense	Mice	3	17
		7	
T. congolense	Mice	No effect	17, 26
	Cattle	No effect	39
T. evansi	Mules and horses	7.5±§	24
	Rats and dogs	Recurrence prevented in 50% of animals only at toxic dosages. (10 doses at 3.5–5 mg./kg. each)	80
T. cruzi	Rats	Only temporary disappearance of organisms from blood	39
	Mice		26

* Single intraperitoneal injection.
† 4 daily intravenous injections.
‡ 7 to 14 daily intravenous injections.
§ 6 injections.

fastest rate. Further, the degree to which arsenoso compounds were bound by red cells *in vitro* proved a generally reliable measure of their systemic toxicity. The acid-substituted compounds were bound to only a negligible degree by erythrocytes *in vitro;* correspondingly, after intravenous injection they were at first excreted rapidly. However, after a few hours, the rate of excretion fell off abruptly, which suggested that the arsenical had been modified to a more toxic form which was then retained in the body. 2) After lethal doses of acid-substituted compounds, death was usually delayed as compared with similarly toxic doses of other arsenoso compounds not containing acidic groups (Fig. 3).

These several lines of evidence indicate that the acidic group may be modified in the animal body, possibly by esterification or some other type of conjugation, to form a more toxic arsenical. The ordinary body mechanisms for defense

against organic acids in this particular case apparently increase the reactivity of the compound with the tissues, and increase rather than decrease its toxicity.

b. *The effect of pH on the parasiticidal effect of arsenoso compounds.* There is reason to believe that such slight parasiticidal activity as acid-substituted arsenoso compounds possess is largely a function of the non-ionized molecule, and that the ion is usually relatively inactive. In preliminary studies there was a rough correlation between the pK of the acid and its treponemicidal activity *in vitro* (44). The effect of pH on both treponemicidal and trypanocidal action was therefore studied in a larger series of compounds (36).

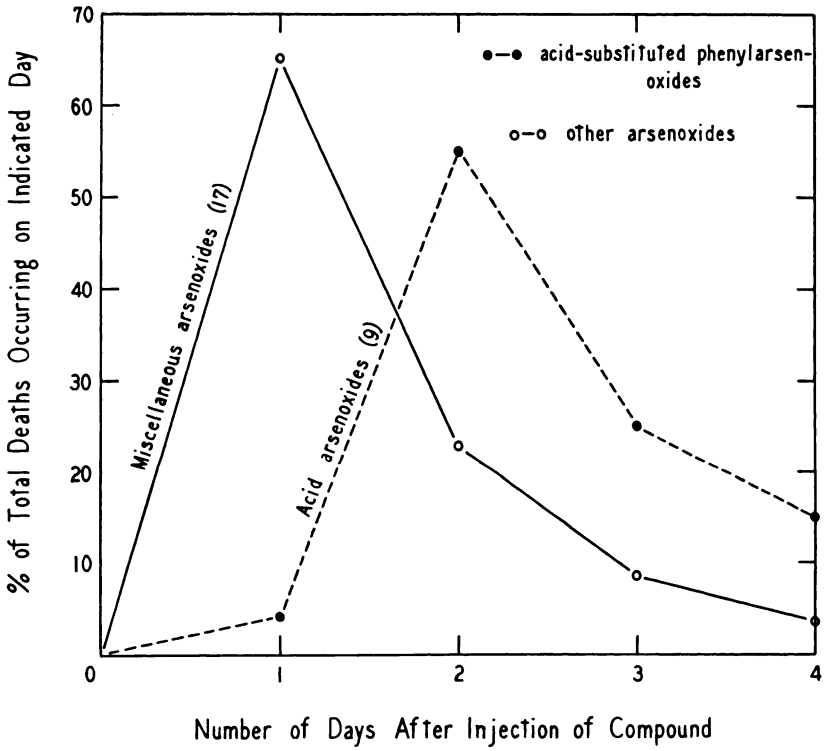


FIG. 3. The characteristic delay in the death of white mice injected intraperitoneally with LD_{40} to LD_{60} of acid-substituted arsenosobenzenes. (After 73.)

The parasiticidal activity of arsenosobenzene and of derivatives containing non-acid substituents was independent of the pH of the testing medium over the total range of viability of the organism. With acid-substituted compounds, however, the activity against both *Trypanosoma equiperdum* and *Treponema pallidum* usually increased strikingly with the hydrogen ion concentration (Fig. 4). This effect of pH was referable to the fact that the ionized salts of the acid-substituted compounds were relatively inactive as compared with the undissociated acid. The activity changed with pH in inverse relation to the degree of ionization, and was indeed predictable from the pK of the acidic group. With strong acids, such as the p-SO_3H compound (pK = 2±), there was no demon-

strable change in activity in the range pH 8.5–5.5, throughout which the compound would be essentially completely ionized. With weak acids, however, the activity varied as much as a hundred-fold over the range pH 8.5–5.5 (*cf.* right hand portion of Fig. 4).

With several of the acids, the ionized form, while less active than the undissociated molecule, nevertheless had a definite parasiticidal action. With such compounds, the total parasiticidal activity at a given pH was therefore a sum of the (slight) activity of the ionized fraction and the activity of the non-dissociated free acid, the proportion of the latter at a given pH depending on the

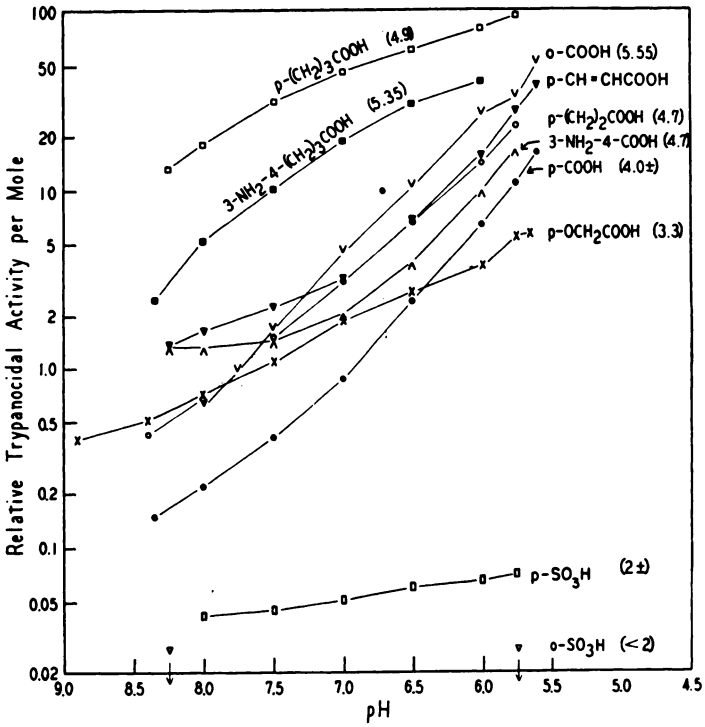


FIG. 4. The effect of pH on the trypanocidal activity of acid-substituted arsenosobenzenes. (After 36.)

Figures given in parentheses are the pK′ values of the various acids.

pK of the compound. Thus, the ions of the p-$(CH_2)_3COOH$ and 3-NO_2-4-COOH arsenosobenzenes had an unusually high trypanocidal activity which contributed significantly to the total activity noted at, *e.g.*, pH 7.0.

One may therefore conclude that in a series of acid-substituted arsenoso compounds, the most effective against a given organism would be that compound with the highest pK (*i.e.*, with the largest proportion in undissociated form at body pH), the ion of which was also moderately active against the parasite. In a series of compounds with substituent groups R—COOH or R—SO_3H, some interest therefore attaches to the effect of the radical R on the dissociation constant of the terminal acidic group (*cf.* Table 3). In the series —$(CH_2)_nCOOH$,

there was a progressive increase of 0.25–0.35 units in the pK of the compound with each additional methylene group; an extension of the series might disclose compounds of therapeutic interest.

The high activity *in vitro* of some of these acid-substituted compounds against cultures of *Trypanosoma cruzi* and *Leishmania donovani* is particularly to be noted. They were, however, not effective in the treatment of the experimental infections, perhaps because the intracellular localization of the parasites in the infected animal rendered them inaccessible to the drug. In addition, preliminary data (39) indicate that the susceptibility of the intracellular parasites to arsenoso compounds may be less than that of the cultured flagellate.

The mechanism of action of arsenoso compounds in general, and of these acid compounds in particular, and the explanation for the extraordinary difference between the parasiticidal activities of the free acid and its ion will be discussed in a following section (page 133).

3. *Acid-amide and ester substituents.* A third group of substituents concerning which it was possible to make some generalizations with respect to biological activity were the acid amides. Compounds so substituted were remarkably uniform in their toxicity and parasiticidal activity. As shown in Table 5, their direct trypanocidal and treponemicidal activities were intermediate between those of the generally inactive acids and the highly active arsenosobenzene. However, the most important effect of the amide substituents was a regular and marked decrease in toxicity, so that the amide-substituted compounds had activity:toxicity ratios as much as six times higher than that of the parent arsenosobenzene (45, 46). A similar effect had been noted by Gough and King in studying amide-substituted arsonic acids in mice infected with *Trypanosoma equiperdum* (69).

The favorable therapeutic effect of the amide groups was essentially the same with both sulfonamides and benzamides. The position of the groups on the benzene ring was also unimportant. However, with both the p-$CONH_2$ and p-SO_2NH_2 compounds, the integrity of the terminal amide grouping was usually essential for the favorable effect on toxicity. Substitution of the amide hydrogens with methyl or ethyl groups successively increased the toxicity at a greater rate than the activity, so that the therapeutic index of these substituted amides was considerably less than that of the amides. The p-SO_2NH_2, p-SO_2NHCH_3 and p-$SO_2N(CH_3)_2$ arsenoso compounds had treponemicidal activities *in vitro* of 29, 72 and 112, relative toxicities of 4.8, 18 and 93, and activity:toxicity ratios of 6.1, 4.0 and 2.3, respectively. Similar effects were obtained on substituting amide hydrogens in the p-$CONH_2$ compound.

In contrast to the $CONHCH_3$ or —$SO_2NHC_2H_5$ compounds, when the terminal grouping was one which was itself "eutherapeutic," *i.e.*, depressed toxicity more than activity, the resulting compound was just as useful as the parent amide. Thus, the $CONHC_2H_4OH$, $CONHC_6H_4NHCOCH_3$ and $SO_2NHC_2H_4OH$ compounds compared favorably with the parent amides with respect to toxicity and parasiticidal activity.

The favorable effect of amide-substitution in depressing the toxicity of phenyl-

arsenoxide to a much greater extent than its parasiticidal activity has been noted in a number of other infections. In cotton rats infected with *Litomosoides carinii* and in dogs infected with *Dirofilaria immitis*, Otto and Maren (88, 89) found a number of trivalent amide-substituted arsenicals to be effective against the adult forms at less than the toxic dosages. Fulton and Yorke (64) had previously commented on the efficacy of reduced tryparsamide (the p-$NHCH_2CONH_2$ compound) in *Trypanosoma rhodesiense* infections in mice. The reduced form

TABLE 5

The relative molar toxicity and parasiticidal activity of amide-substituted arsenosobenzenes (45–48)

(All values referred to that of the unsubstituted arsenosobenzene as 100)

COMPOUND	RELATIVE TOXICITY IN WHITE MICE	RELATIVE ACTIVITY IN VITRO AGAINST:				
		T. pallidum	*T. equiperdum*	Arsenic-resistant *T. equiperdum*	*T. cruzi*	*L. donovani*
m-$CONH_2$	9.8	41	39	1.75	0.25	0.53
p-$CONH_2$	9.6	45	45	0.34	0.07	0.08
p-$CH{=}CHCONH_2$	9.7	42	73	—	—	0.98
p-CH_2CONH_2	8.6	20	31	—	—	—
p-$(CH_2)_2CONH_2$	13.5	33	60	8.15	8.3	4.5
p-$CONHCONH_2$	6.4	34	34	2.97	0.16	0.08
p-$CONHCH_2CONH_2$	3.86	24	15.0	0.68	<0.04	0.09
p-$CONHCH_2CH_2CONH_2$	3.2	13	—	—	—	—
p-$CH_2CONHCH_2CONH_2$	3.4	11	1.5	—	<0.08	5.2
p-OCH_2CONH_2	9.0	52	26	2.02	0.18	4.3
p-$NHCONH_2$	8.1	38	29	0.57	0.2	0.07
p-$NHCH_2CONH_2$	4.5	22	—	—	—	—
p-$NHCO(CH_2)_2CONH_2$	9.0	25	—	—	—	—
m-SO_2NH_2	6.1	21	7.8	—	—	—
p-SO_2NH_2	4.8	29	24	0.16	0.03	0.05
p-$SO_2NHCH_2CONH_2$	3.5	17	1.4	—	—	0.22
p-$CONHC_2H_4OH$	4.8	22	39	0.23	0.07	0.05
p-$SO_2NHC_2H_4OH$	4.2	23	9.0	0.18	—	0.13
p-$CONHCH_2CN$	4.5	27.0	19.5	0.42	0.08±	0.15
3-NH_2-4-$CONH_2$	5.6	28	52	2.76	0.06	0.15
3-OH-4-$CONH_2$	23	45	48	—	—	—

of carbarsone (p-$NHCONH_2$ arsenosobenzene) has been found to be more actively amebicidal both *in vitro* and *in vivo* than the corresponding arsonic acid, and effective doses were reasonably well tolerated by rabbits, monkeys and man (2, 4).

(Unlike amide-substitution, the esterification of acid compounds greatly increased both their parasiticidal activity and their toxicity. In consequence, such ester-containing compounds, when stable, were not better therapeutically than the parent arsenosobenzene. Many hydrolyzed in aqueous solution, in which case their biological activity was the same as that of the acid-substituted compound.)

4. *Miscellaneous substituents* (*—RNH_2, —NHR, —RNHR'*). Highly variable results have been obtained with arsenosobenzenes containing terminal or substituted amino- or hydroxy-groups in a ring substituent. The treponemicidal activity *in vitro* of a number of such compounds referred to that of arsenosobenzene as 100 varied between 3 and 78; their toxicity varied between 6.7 and 76; and their activity:toxicity ratio varied between 0.38 and 2.6 times that of the parent arsenosobenzene. In no instance was a compound obtained which was sufficiently parasiticidal and non-toxic to suggest the possibility of its therapeutic use for the particular infections tested.

An important exception, however, is provided by "melarsen oxide" (p-(2,4-diamino-S-triazinyl-6)-aminoarsenosobenzene) (59–63). This compound is a highly active trypanocidal agent (*cf.* page 111) and has proved effective in the treatment of early cases of the human disease (*T. gambiense*). Earlier reports indicated that like para-arsenosophenylbutyric acid it was ineffective in the late manifestations of the disease (85). A more recent report by Friedheim, however, suggests that it may have an activity in late cases as well (61). The anilinopyrimidine analagous to this anilino-triazine compound was less actively trypanocidal (7).

5. *The importance of the terminal grouping in a substituent.* Single substituents have thus been shown to have widely varying effects on the toxicity and parasiticidal activity of arsenosobenzene. In a number of the compounds, the substituent was a side chain of varying length. In general, and regardless of the length or nature of the side chain, the activity and toxicity of the compound were usually determined by the nature of the terminal functional groupings. Thus, the —CH_3 group had been found to be "inert," while the —$CONH_2$ and —SO_2NH_2 groups had a highly favorable effect on toxicity. The p-CH_2CONH_2, $(CH_2)_3CONH_2$ arsenosobenzenes all behaved as amides (Table 4); while in the CO_2NHCH_3 and the $CO_2N(CH_3)_2$ compounds the favorable effect of the amide grouping was diminished or abolished by the substitution of methyl groups for amide hydrogens (*cf.* page 118). Similarly, when an amide hydrogen was replaced by a group carrying a terminal acidic group (*e.g.*, the $CONHCH_2COOH$), the pharmacologic and parasiticidal properties of the compounds were determined by the terminal acidic group and not by the amide.

6. *Multiple substituents* (*41*). The effects of multiple substitution on the toxicity and parasiticidal activity of arsenosobenzene are difficult to interpret. Only a relatively small number of compounds have been tested; in no case could the effect of double substitution be anticipated from the effect of each group alone. Thus, six of the ten possible arsenosoaminophenols were prepared and tested for activity on *T. pallidum in vitro* (Table 6). The —NH_2 and —OH substituents separately, whether o-, m-, or -p- to the arsenoso group, had resulted in a uniform series of compounds, with treponemicidal activities varying only from 72 to 98, toxicities from 49 to 85, and activity:toxicity ratios from 1.0 to 1.46. The treponemicidal activity of the six aminophenol compounds was also remarkably uniform, varying only between 39 and 57; but their toxicities varied between 6.94 and 78.9, so that the activity:toxicity ratios varied ten-

fold, from 0.54 to 5.5. The combination 3-NH_2-4-OH which is the well-known compound oxophenarsine ("mapharsen"), was the most favorable combination in the entire series. At the other extreme, the combination 3-NH_2 2-OH gave a less favorable therapeutic index than arsenosobenzene itself. Any change in the 3-NH_2-4-OH combination, either by the introduction of a third substituent, extending either group on a side chain, or substitution of the hydrogen in either group, abolished its highly favorable properties.

Similarly variable and unpredictable results were obtained with the arsenosoaminobenzoic acids. Nine of the ten possible isomers were tested for direct trypanocidal activity. Unlike the example of the aminophenols just cited, the trypanocidal activities of these compounds varied 40-fold, from 0.6 to 23, with the 3-NH_2-2-COOH compound showing the highest activity (36, 47).

While no combination of two wholly inert substituents (—CH_3, —NO_2, —Cl, etc.) resulted in compounds with a highly favorable activity:toxicity ratio, one

TABLE 6

The treponemicidal activity and toxicity of aminophenol-substituted arsenosobenzenes. (After 41)

SUBSTITUENT GROUPS	MOLAR TOXICITY	MOLAR TREPONEMICIDAL ACTIVITY	RATIO OF ACTIVITY: TOXICITY
3-NH_2-4-OH	6.9	42	6.1
3-OH-4-NH_2	10	41	3.9
2-NH_2-3-OH	10	34	3.3
2-OH-5-NH_2	22	39	1.8
3-OH-5-NH_2	74	57	0.78
2-OH-3-NH_2	79	43	0.54

cannot say unequivocally that further search would not reveal such a combination.

Since the favorable therapeutic activity of oxophenarsine in the treatment of syphilis rests primarily on its strikingly reduced toxicity (6.9 per cent of that of the unsubstituted arsenosobenzene in mice and 9.4 per cent in rabbits), it was to be anticipated that it would also be useful in a number of other infections in which it had a direct parasiticidal activity. This has proved to be the case. It was just as effective in the treatment of yaws as in the treatment of syphilis. Although it is definitely parasiticidal against *Borrelia recurrentis* and causes the rapid disappearance of the organisms in mouse infections, its therapeutic activity in the human infection in terms of cure and reduced mortality are questionable. Other workers (116) have reported initially favorable results in the treatment of human filariasis with mapharsen. Although Anderson and Chuan (3) found it to be amebacidal *in vitro*, its therapeutic value in this disease has not been thoroughly studied.

7. *Thioarsenites.* The chemistry and pharmacology of the thioarsenites have been studied by Barber (8), Cohen, King and Strangeways (21), and Strangeways (111), who prepared a number of such compounds and determined their

efficacy in experimental trypanosomiasis in white mice. Strangeways (111) concluded that their trypanocidal activity, whether *in vitro* or *in vivo*, depended on their hydrolysis to the corresponding arsenoso compound, the reverse of the reaction shown on page 125. Murgatroyd (85) tested two of these compounds (the di-glutathionyl- of 4-$NHCOCH_3$-2-OH-arsenosobenzene (K350) and the di-cysteinyl of 4-$CONH_2$-arsenosobenzene (K352)) in the treatment of *T. gambiense* infections in man. In maximum tolerated doses, they had no effect on central nervous system infections, and relatively small doses were toxic. Eckler and Shonle (51) studied the therapeutic activity of the disodium salt of p-[bis (p-sulfophenylthio)arsino]-acetanilid in rat trypanosomiasis and rabbit syphilis, with results which compared favorably with those obtained with arsphenamine. In the treatment of experimental and human filariasis, Otto and Maren (88, 89) and their coworkers (116) found the dithioglycolate of the 4-$CONH_2$ compound to kill the adult forms of *Dirofilaria immitis* in dogs and of *Litomosoides carinii* in cotton rats, and the microfilaria of *Wuchereria bancrofti* in man, and to have essentially the same activity as the parent arsenosobenzene. In experimental amebiasis, Anderson and his coworkers (2–6) found both the dithioglycolate and dithiosalicylate of reduced carbarsone (p-$NHCONH_2$-arsenosobenzene) to be effective in the treatment of monkeys infected with *Endameba histolytica*. In human cases, Anderson and his coworkers (6) were able to cure 33 of 39 and 41 of 44 cases treated with the dithioglycolate and dithiosalicylate, respectively, at total dosages which varied from seven grams given in a period of 24 hours, to three grams given over a period of 10 days. In human trypanosomiasis (*T. gambiense*) Friedheim (62) has reported initially favorable results with an "alkyl mercapto" derivative of melarsen oxide.

A number of thioarsenites prepared and tested in this laboratory are listed in Table 7. As there shown, they were regularly less toxic but also less actively parasiticidal *in vitro* than the parent arsenosobenzenes. *In vivo* also, whether in the treatment of rabbit or mouse trypanosomiasis, their therapeutic index, expressed in Table 7 as the ratio of $\frac{LD_{50}}{CD_{50}}$, was generally no more favorable and in several instances less favorable than that of the corresponding arsenosobenzene.

Depending on the particular thiol compound used for conjugation, the thioarsenites often have a clear advantage over the corresponding arsenosobenzene in their greater solubility. It has not been proved, however, that they have a more favorable therapeutic index in man than the parent arsenosobenzene, *i.e.*, that their toxicity has been reduced to a greater extent than their parasiticidal activity. In at least some experimental infections, that has not been found to be the case (*cf.* Table 7).

It is an open question whether the activity and toxicity of thioarsenites is determined solely by the degree to which they hydrolyze to the corresponding arsenosobenzene, or whether they may act directly. It is true that an excess of thiol abolishes the parasiticidal activity of an arsenoso compound, both *in vitro* and *in vivo* (*cf.* page 127 et seq.). These data would suggest that in accordance with the suggestion of Gough and King ((69);*cf.* also (21)) the hydrolysis of

TABLE 7

The relative toxicity and therapeutic activity of thioarsenites and the corresponding arsenoso compounds (39)*

—AsO = arsenosobenzene; —As(SR)$_2$ = corresponding dithioarsenite

	TREPONEMICIDAL ACTIVITY *in vitro*		TOXICITY IN WHITE MICE		THERAPEUTIC INDEX $\left(\frac{LD_{50}}{CD_{50}}\right)$			
					Mouse trypanosomiasis†		Rabbit syphilis	
	—AsO	—As(SR)$_2$	—AsO	—As(SR)$_2$	—AsO	—As(SR)$_2$	—AsO	—As(SR)$_2$‡
o-CH_3	84	109	88	90	—	—	—	—
p-OCH_2CONH_2	52	32	9.0	7.3	$\frac{33}{3.1} = 10.7$	$\frac{73}{7} = 10.4$	$\frac{10.5}{3.8} = 2.8$	$\frac{15\text{–}20}{4\text{–}6} = 3\text{–}4$
p-$CONH_2$	45	39 23a 21b	9.6	4.6 6.1 5.1	$\frac{27.5}{3.5} = 7.9$	$\frac{108}{6.9} = 15.7$	$\frac{9.1}{2.8} = 3.3$	—
p-$NHCONH_2$	38	21	8.1	2.9	$\frac{35}{3.8} = 9.2$	$\frac{180}{16} = 11.2$	$\frac{11.7}{4.6} = 2.5$	$\frac{35}{10} = 3.5$
p-$CONHCH_2CONH_2$	32	17	5.7	1.6	$\frac{79.5}{5} = 15.9$	$\frac{352}{16} = 22$	—	—
m-SO_2NH_2	21	22	6.1	3.3	—	—	—	—
p-SO_2NH_2	29	24	4.8	3.3	$\frac{63}{7.1} = 8.9$	$\frac{165}{21} = 7.9$	$\frac{16.1}{6} = 2.7$	$\frac{30\pm}{15\pm} = 2\pm$
3-NH_2-4-OH	38	29 0.86c	6.9	4.1 1.4	—	—	$\frac{13}{3} = 4.3$	$\frac{30}{8} = 3.8$
m-COOH	13	11	16	15	—	—	—	—
p-SO_3H	3.4	3.6	29	24	—	—	—	—

* All were dicysteinyl compounds, with the exception of the compounds indicated as a, b, and c, which were the dithiosalicylate, dithioglycolate, and dimercaptopropanol, respectively.

† *Trypanosoma equiperdum.*

‡ Approximations only.

the thioarsenite to the free arsenoso compound may be essential for parasiticidal activity. It is conceivable, however, that the thioarsenite reacts directly with a tissue thiol according to the equation

$$\underset{\text{Thioarsenite}}{RAs(SR')_2} + \underset{\substack{\text{Cellular}\\\text{thiol}}}{2R''SH} \rightarrow \underset{\substack{\text{Cell}\\\text{thioarsenite}}}{RAs(SR'')_2} + \underset{\substack{\text{Free}\\\text{thiol}}}{2R'SH}$$

This reaction would involve the transfer of arsenic from one thiol directly to another. In such case an excess of thiol would inhibit parasiticidal activity, not by preventing hydrolysis but by competing with the cellular thiols for the thioarsenite (*cf.* page 130).

The finding of Peters and Stocken (91) that the condensation of "mapharsen" with BAL (2,3-dimercaptopropanol) results in a compound which in rats is four times more toxic than mapharsen itself has not been confirmed. A number of workers (63, 96, 100) have found that this thioarsenite also is approximately one fourth as toxic as the parent arseno compound. In mice the LD_{50} of the addition compound prepared in our own laboratory was 230 mg./kg. and 60 mM/kg. as compared with 43 mg./kg. and 12.4 mM/kg. for mapharsen.

B. The selective parasiticidal action of the arsenosobenzenes

The parent arsenosobenzene and those arsenoso compounds with "inert" substituents are highly toxic to the animal host and uniformly parasiticidal *in vitro* for all the organisms which have been tested. However, when substituents were introduced which modified the biological activity of the compound, they often developed a high degree of specificity. Thus, the amide-substituted compounds were relatively non-toxic for the mouse or rabbit host, uniformly active against *T. pallidum*, highly variable in their action on *Trypanosoma equiperdum*, and only negligibly active against *L. donovani* or *Trypanosoma cruzi* (Table 4). The acid-substituted compounds were generally only slightly parasiticidal. A few were highly active, however, against *Trypanosoma equiperdum*, *Trypanosoma cruzi* and *L. donovani*, and others were active against *T. pallidum* (*cf.* Table 3).

It is apparent that the relative activity of a series of compounds in the treatment of, *e.g.*, mouse trypanosomiasis cannot serve as a guide to their possible activity in the treatment of syphilis (93); nor do results obtained in the treatment of dog filariasis (*Dirofilaria immitis*) or cotton rat filariasis (*Litomosoides carinii*) necessarily apply to the treatment of the human infection with *Wuchereria bancrofti*. Similarly, results in the treatment of *Trypanosoma gambiense* infections in man do not necessarily carry over to the treatment of infections with *Trypanosoma rhodesiense* or *Trypanosoma cruzi*.

Possible reasons for this selective action are discussed in a following section.

C. Time–Dosage Relationship

The rate of the parasiticidal action of arsenosobenzenes *in vitro* increases with their concentration. Thus, in the immobilization of trypanosomes *in vitro*, a

high concentration may have an instantaneous and total effect, while at threshold concentrations it may require four, eight or even 24 hours to immobilize only a fraction of the organisms. It is a reasonable assumption that the high concentrations are more rapidly effective, first, because the binding of the arsenical by the cell is correspondingly more rapid (*cf.* page 135), and second, because larger amounts are bound. These findings suggest that *in vivo* also, a single large dose might be essentially as effective as the same total dose given over a longer period of time in many divided injections. Fulton and Yorke (64) did find in *T. rhodesiense* infections that a low concentration of p-$NHCH_2CONH_2$ arsenosobenzene acting over a long period of time was as effective as a high concentration acting over a short period. In experimental trypanosomiasis (*T. equiperdum*) of rats, however, Swinyard and coworkers (112) found that the therapeutic efficacy of a series of small subcurative doses decreased the longer the time interval allowed between individual injections. In this case, the longer interval permitted rapid remultiplication of the surviving trypanosomes. In rabbit syphilis, Eagle and Hogan (43) found that the total curative dose of mapharsen (CD_{50}) was largely independent of the schedule of administration, whether the curative dose was given in one or 16 injections, or whether treatment was completed within one day or extended over six weeks. The disparity between these results and those of Swinyard is probably related to the fact that the generation time for *T. pallidum in vivo* has been estimated to be of the order of 30 hours (25, 81). An interval of even several days during which the arsenic was no longer at treponemicidal levels would therefore not permit remultiplication to an important degree.

III. MODE OF ACTION OF ARSENOSO COMPOUNDS

A. The reactivity of arsenicals with thiols

Ehrlich originally suggested that drugs were effective only to the degree to which they were bound by "chemoreceptor" groups in the parasite, and laid down the thesis that "corpora non agunt nisi fixantur" (55). He further suggested that thiol groups might determine that fixation, although no definite chemical reaction was postulated (52).

The reaction between arsenosobenzenes and thiol compounds to form thioarsenites has been briefly discussed in a preceding section. In 1923 Voegtlin, Dyer and Leonard (120) demonstrated that trypanosomes contained —SH groups, that organic compounds such as cysteine and glutathione which contained —SH groups inhibited the trypanocidal action of trivalent arsenicals *in vitro*, and that the injection of such compounds immediately before an otherwise fatal dose of an arsenoso compound prolonged the life of the animal. They concluded that the effect of the arsenoso compounds *in vivo* was based on the reaction

$$RAsO + 2R'SH \rightarrow RAs(SR')_2 + H_2O,$$

and that the specific sulfhydryl compound which acted as the "chemoreceptor" in determining toxicity was primarily glutathione (121). The possibility that

other sulfhydryl-containing compounds might be involved was considered by a number of workers (97, 98, 104). The inhibiting effect of sulfhydryl compounds on arsenicals was extended to *T. pallidum* with the demonstration (33) that cysteine, glutathione and thioglycolic acid in excess abolished the treponemicidal action of arsenicals *in vitro*, as well as that of mercury and bismuth compounds. *In vivo*, thiols and dithiols in appropriate dosage decreased or abolished both the toxic and parasiticidal effects of arsenoso compounds even when administered hours after otherwise lethal (or curative) doses ((38, 50, 92); *cf.* Fig. 5).

The antagonistic effect of thiols on arsenosobenzenes as outlined in the foregoing paragraphs involves two quite different mechanisms. Thiol compounds in excess react with arsenicals to form thioarsenites, inhibit the hydrolysis of

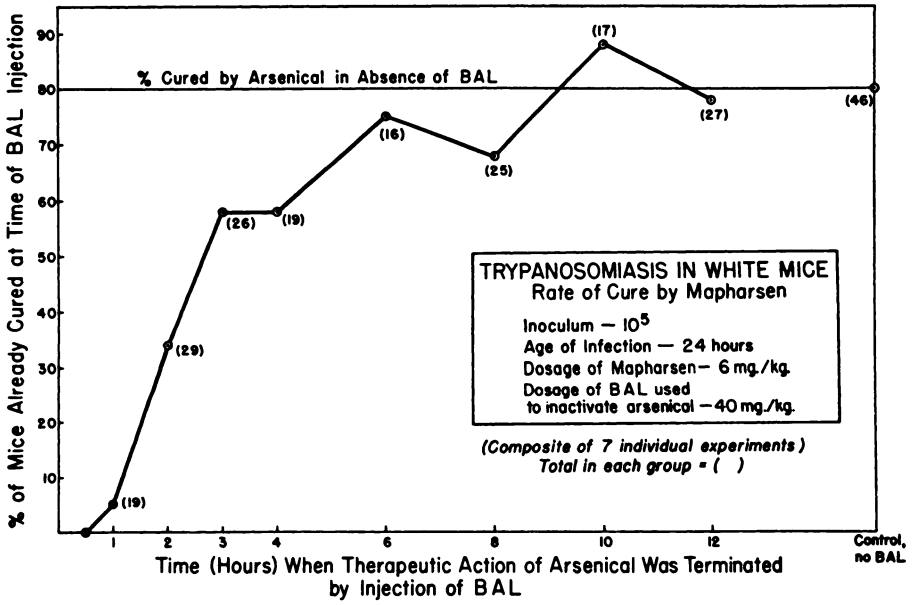


FIG. 5. The inhibition of the trypanocidal action of oxophenarsine in white mice by 2,3-dithiopropanol (BAL). (After 38.)

the formed thioarsenites to the free arsenoso compounds, and thus prevent the interaction of the latter with the host tissues or with the parasites. In addition, however, even after arsenosobenzenes have combined with the tissue cells or parasites, thiol compounds may reverse that combination, and remove the arsenical from its combination with the cells (34, 38, 50). When cysteine was added to a suspension of *Treponema pallidum* which had previously been incubated with arsenicals and in which the larger proportion of the organisms had already been immobilized, not only was the treponemicidal action of the arsenical immediately halted but a large proportion of the already immobilized organisms were revived (34). The same phenomenon has been even more strikingly demonstrated with trypanosomes "killed" by arsenical and then exposed to BAL (2,3-dithiopropanol) (50). *In vivo* also, it is apparent from Fig. 5 that the parasiticidal action of arsenoso compounds may be successfully reversed by

thiol compounds given hours after the arsenical had been injected, and after the organisms had been given ample opportunity to combine with the injected arsenical. Similar results have been reported in rats by Pfeiffer, Jenney and Ross (92). In experimental syphilis also, BAL given up to six hours after an other wise curative dose of oxophenarsine reversed the therapeutic action in a significant proportion of the animals (39). The same compound decreased the lethal action of a number of arsenoso compounds in rabbits (50). The ability of thiol compounds to abstract arsenicals from the cell after they have already entered into combination with cell components is manifested not only in the revival of microorganisms or animals already seriously damaged by the arsenicals, but may be demonstrated by the direct chemical analysis of the cells affected (50). The arsenical presumably leaves the cell in the form of the thioarsenite. In the animal host also, certain thiols have been shown to counteract strikingly the toxic effects of arsenosobenzenes, and that effect likewise is associated with the removal of arsenic from the host cells and with its accelerated excretion (*cf.* page 128).

B. The reactivity of arsenicals with —SH groups in enzymes

The fact that arsenicals react strongly with thiol compounds to form thioarsenites, and also the fact that thiol compounds are able to remove arsenic after it has already entered into combination with cellular constituents do not, however, prove that a similar combination with cell thiols is responsible for the toxic action of arsenicals. More recent work has indicated that this may actually be the case, and that the reactivity of arsenicals with —SH groups in protoplasm is the factor which determines their cytotoxic effects. It now seems unlikely that glutathione as such is necessarily or even primarily the cellular grouping affected.

In 1933 several workers reported on the role of thiol groups in the reversible deactivation of enzymes. Hellerman, Perkins and Clark (72) studied the inactivation of crystalline urease with mercurials such as phenylmercuric hydroxide, and Bersin and Logermann (14) noted that the activity of papain was destroyed by many oxidizing agents. In both instances, the enzymatic activity was restored by the addition of either hydrogen sulfide or potassium cyanide. Maschmann (83) had suggested that such reactivation of papain or cathepsin by H_2S involved reductions of disulfide linkages in the enzyme to active thiol groupings essential for activity.

Bersin (13) then correlated the work of Cohen, King and Strangeways (21) on the reaction between arsenicals and thiol compounds *in vitro* and the several previous indications as to the importance of thiol groups in the activity of many enzymes, and systemically studied the effects of a number of arsenic compounds on papain. He showed that papain was inhibited by both arsenoso compounds and arsonic acids, and that this inhibition was reversed by glutathione. In explanation, he proposed the following alternative reactions:

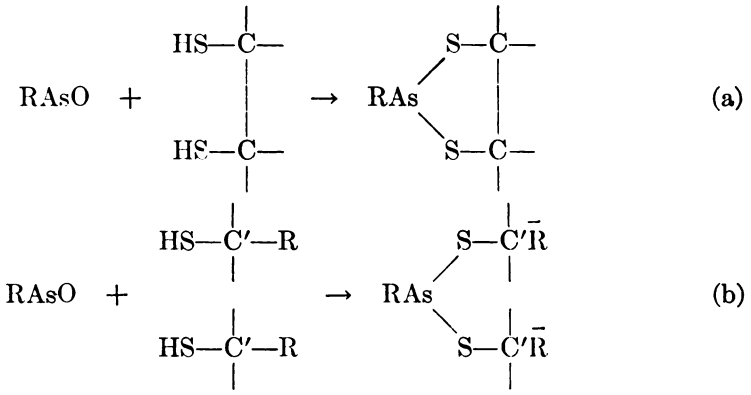
(1) $4\ \text{Enz—SH} + \text{RAsO}_3\text{H}_2 \rightleftarrows \text{RAs(S—Enz)}_2 + \text{Enz—S—S—Enz} + 3\text{H}_2\text{O}$

(2) $2\ \text{Enz—SH} + \text{RAs(OH)}_2 \rightleftarrows \text{RAs(S—Enz)}_2 + 2\ \text{H}_2\text{O}$

A marked difference in the inhibitory actions of the p-acetamido- and p-aminophenylarsonic acids was explained on the basis of a difference in the equilibrium constant in reaction (1) above.

Barron and his coworkers (9–12) have studied the inhibiting effect of arsenicals on a number of enzyme systems, including enzymes involved in nitrogen, fat and carbohydrate metabolism. Many were reversibly inhibited by arsenoso compounds and by the war gas "Lewisite" (ClCH = CH As Cl_2); in such cases, —SH groups in the enzyme protein were believed to be essential to their enzymatic activity. Similarly, Gordon and Quastel (67, 68) found that arsenoso compounds reversibly combined with and inactivated such thiol enzymes as urease, pyruvic oxidase, succinic dehydrogenase and choline esterase, while enzymes in which —SH groups were presumably not part of the active centers, such as invertase, catalase, lactic dehydrogenase and cytochrome oxidase, were unaffected. The inactivating action of the arsenicals on the susceptible enzymes was reduced or prevented by the addition of free thiols or of other proteins capable of combining with the arsenical. Chen (19a) also found mapharsen to inhibit hexokinase, adenosine triphosphatase and 3-phosphoglyceraldehyde in preparations of lysed *T. equiperdum*. Conversely, dithiols have been found to inhibit certain enzymes in which the protein moiety has a heavy metal prosthetic group (10, 123).

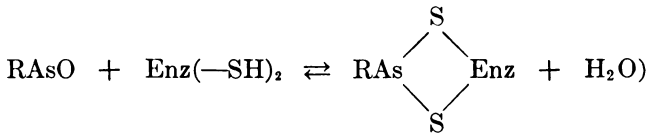
In England, Peters and his coworkers (90, 91, 107–110, 124) have extensively investigated the reaction of enzymes and trivalent arsenicals. They considered the primary site of attack of the arsenicals in the animal body to be on the pyruvate-oxidase system, which is particularly sensitive to trivalent arsenicals. Stocken and Thompson (107) reacted sodium arsenite and Lewisite with kerateine in buffered solution and showed that from 73 to 92 per cent of the bound arsenic had combined with protein thiol groups in the molecular ratio 1:2. Further, the protein sulfur was now resistant to oxidation, while the thiol groups in kerateine are normally highly susceptible. They therefore postulated that the arsenic had combined with two neighboring thiol groups on the protein molecule, *i.e.*, that the protein contains a dithiol which is the primary reactive site for arsenicals. On physicochemical grounds the ring compound formed by the reaction of a dithiol with an arsenical (a) was believed to be more stable than the straight chain thioarsenite formed with 2 molecules of a monothiol (b):



There may be several gaps in this argument. No dithiol such as that postulated by Stocken and Peters has yet been identified as a constituent of kerateine or any other protein in the amounts implied by their experimental data. It seems unlikely also that arsenicals would react with —S—S— groups in proteins, or that the major portion of the free monothiol groups in a protein molecule would be arranged in closely adjacent pairs. The fact that the arsenic:sulfur ratio of the compound formed between thiols and kerateine in solution approaches 1:2 is not conclusive evidence for the presence of a dithiol, since the arsenic molecule could be bridging two molecules of kerateine. Further, in the absence of resonance there seems to be no necessary basis for assuming that a bond between two atoms in a ring is necessarily stronger than the same bond in a straight chain compound. Whittaker (124) did find that those dithiols which he believed to react with arsenic to produce strained rings were less effective in counteracting the toxic effects of arsenic than dithiols which formed a stable ring structure. This does not, however, prove that the latter are necessarily more stable than a straight chain compound. Slater (106) has furnished strong evidence that the reaction between arsenicals and enzyme proteins may be more complex than that suggested by Peters and his coworkers. He has pointed out that while the reaction is supposed to be:

$$RAsO + 2HS—Enz \rightleftarrows RAs(S—Enz)_2 + H_2O$$

(or, on the basis of the Stocken-Peters concept,



there may be no reactivation of the enzyme when the arsenical is removed or the reaction mixture is greatly diluted.

However, whether the reasoning of Stocken and Peters is valid in its entirety, it has led to a significant advance in the treatment of heavy-metal poisoning. Following up the previously cited data, Stocken and Thompson (108) found that dithiols, and particularly BAL (British Anti-Lewisite; 2,3-dimercaptopropanol; $HSCH_2CH(SH)—CH_2OH$) were more effective than monothiols in protecting the pyruvate-oxidase system in pigeon brain against arsenicals. In animals suffering Lewisite burns or injected with toxic arsenicals, the dithiols greatly increased the rate of excretion of arsenic (18, 49, 96, 107–110) and strikingly counteracted the toxic effects of a number of trivalent arsenicals in experimental animals. As an outcome of these studies, BAL has found extensive use in the local and systemic treatment of arsenic poisoning (18, 49, 50, 78) and poisoning due to such heavy metals as mercury (79), antimony (16, 42) and gold (20, 94). It is ineffectual against cadmium poisoning (66) and of questionable value in lead poisoning (65, 99).

Summarizing the above evidence, one may conclude that (a) arsenoso compounds combine with sulfhydryl compounds to form reasonably stable thio-

arsenites; (b) many enzyme groups contain sulfhydryl groups which are necessary in the intact state for the action of the enzyme, and such enzymes are inactivated by arsenoso compounds and other heavy metal compounds capable of reacting with —SH groups; (c) this inactivation can be reversed by thiols, and in particular by certain dithiols; (d) thiol compounds, and particularly dithiols, protect animals and microorganisms against the toxic effects of arsenicals, can on occasion reverse the toxic action of arsenicals after they have already become manifest, and in such cases actually reverse the combination of the arsenical with the cell or tissue.

On the basis of these facts it is a generally-accepted working hypothesis that the cytotoxic action of the arsenosobenzenes rests on their ability to combine with thiol groups in essential enzyme systems, both in the animal host tissue (toxicity) and in the invading parasitic cell (therapeutic activity). This may be expressed schematically as

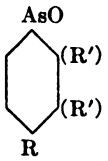
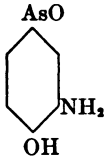
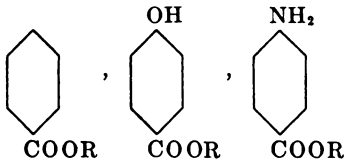
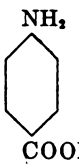
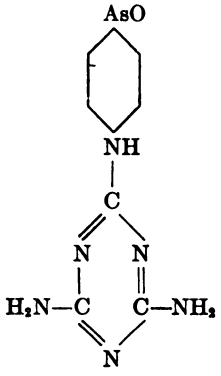
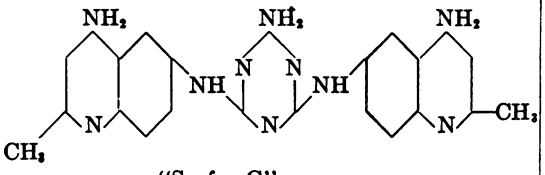
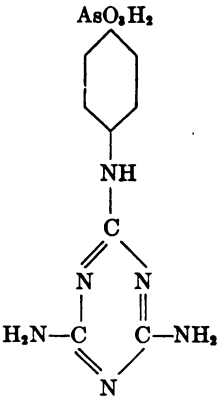
$$\underset{\text{arsenoso compound}}{RAsO} + \underset{\text{enzyme in host tissue or in parasitic cell}}{2R'SH} \rightleftarrows \underset{\text{tissue or cell thioarsenite}}{RAs(SR')_2} + H_2O$$

C. Other arsenic-detoxifying mechanisms

A number of compounds other than thiols have been shown to prevent or to reverse the cytotoxic action of arsenicals in varying degree. In no case are the results as striking or as complete as those obtained with the thiols; nevertheless, the mode of action of the arsenicals cannot be considered to have been adequately elucidated until the mechanism of these detoxifying effects has been established. Thus, ascorbic acid has been found to interfere with the action of mapharsen (58); a number of quinoid dyes constituting reversible redox systems in a narrow potential range similarly interfered with the trypanocidal effect of 3-NH_2-4-OH arsenosobenzene (74); para-aminobenzoic acid was found to interfere with the trypanocidal action of "butarsen" (para-arsenosophenylbutyric acid) (125) but not that of melarsen oxide or "mapharsen" (126) on *T. rhodesiense*. The trypanocidal action of the latter compound was, however, antagonized by a number of benzoic acid esters and amides (103). Finally, Surfen C (bis-(2-methyl-)4-amino-6-quinolyl-melamine) interfered selectively with the action of "melarsen oxide," as did also the pentavalent analog of the latter compound, "melarsen" (126). There is no obvious common denominator for the antagonistic action of these widely disparate compounds on arsenosobenzenes (Table 8). Further, these inhibitory effects are not necessarily associated with an inhibition of toxicity (103). In several cases the antagonistic effect may conceivably be related to a similarity in chemical structure. It seems unlikely that these inhibitors would compete with the arsenoso compound for SH groups in an enzyme protein. The latter reaction is determined by the —AsO grouping, and there is no evidence that compounds which are sterically similar but which lack the —AsO group would have a similar reactivity. Williamson and Lourie

TABLE 8

The inhibition of the cytotoxic action of arsenosobenzenes by a variety of agents

ARSENICAL TESTED	PARASITICIDAL ACTION INHIBITED BY:	TEST ORGANISM	REFERENCES
Arsenosobenzenes in general 	Thiols generally (—RSH)	*T. pallidum; Tryp. equiperdum*, etc.	(*cf.* pages (125–127))
 Oxophenarsine ("mapharsen")	Quinoid dyes Ascorbic acid  and corresponding substituted amides (—CONHR) (but *not* by p-aminobenzoic acid)	*Tryp. brucei*	74 58 103
 γ-p-Arsenosophenylbutyric acid	 p-aminobenzoic acid (but *not* by benzoic acid, p-OH-benzoic acid, or Surfen C)		125
 "Melarsen oxide"	 "Surfen C"	*T. rhodesiense*	126
	 "Melarsen" (but *not* by p-aminobenzoic acid)		

(126) have suggested that the site of the competitive inhibition is not the specific cell component inactivated by the arsenical, but perhaps a surface configuration on the cell which constitutes the portal of entry for the drug (*cf.* page 137).

D. Possible explanations for the varying reactivity of different arsenicals against the same organism, and of the same arsenical against different organisms

How does the theory of reactivity of arsenicals with enzyme —SH groups explain the experimental observations, cited in the preceding sections, 1) that a series of chemically closely related arsenoso compounds may vary 100-fold in toxicity, and vary just as widely in their parasiticidal action on a given microorganism, and 2) that the same arsenical may vary widely in its effect on different organisms? The striking effect of γ-(p-arsenosophenyl)-butyric acid against certain trypanosomal species and its inactivity against *T. pallidum* is but one example of the parasite-specificity of many arsenic compounds (*cf.* Tables 1–4).

1. *The possibly varying reactivity of different arsenicals and thiols as the basis of their parasite specificity.* The reaction indicated on page 125 is freely reversible, being shifted to the left in alkaline solution and to the right in acid solution (21). It is therefore conceivable that the variation in toxicity or activity of a series of arsenoso compounds might be a function of the hydrolysis constant of the thioarsenite formed. It is also possible that the rate of the above reaction is the determining factor. In either case, the usual relationships between chemical structure and reactivity should apply. In this particular case it should be possible to predict both the relative rate of the reaction and the hydrolysis constant by means of Hammett's equation (70):

$$\text{Log } k - \text{Log } k^0 = \rho\sigma$$

where k is the rate or hydrolysis constant for the substituted aryl groups, k^0 is the corresponding constant for the unsubstituted group, ρ is a constant for the particular chemical reaction studied and which is independent of the groups, and σ is a constant for each substituent group and is independent of the reaction (70). If either toxicity or parasiticidal activity were quantitatively dependent closely on the reaction above, that activity should be a function of the σ values. Actually, no correlation whatever was found between the Hammett σ values and either the toxicity or parasiticidal activity of a large series of arsenoso compounds (27).

Again on the basis of the Hammett equation, if the parasiticidal action of arsenicals and their toxicity to the animal host were both quantitatively dependent upon their reactivity with thiol groups, then in a given infection involving the same parasite and the same host, the therapeutic index of a series of arsenoso compounds should be reasonably uniform. The reactivity of an arsenoso compound with thiols in the parasite and thiols in the host cell should vary concomitantly, and their ratio should be independent of the particular substituent on the benzene ring. Instead, that ratio varied as much as 60-fold in a relatively limited series (*cf.* Tables 1–4). Even when the same compound

was tested against the same organism (*Trypanosoma rhodesiense*) but in different animal hosts, Tatum, Pfeiffer and Kuhs (115) obtained therapeutic indices of 20–25, 4–5 and 5 in rats, rabbits and dogs, respectively.

It seems clear that possible differences in the rate or equilibrium constants of the reaction between arsenoso compounds and thiols to form thioarsenites are inadequate to explain the known variations in the parasiticidal action or toxicity of these compounds.

The studies of Barron and Singer (11, 12) on the relative inhibitory action of various arsenicals against a number of enzymes *in vitro* are pertinent in this connection, although the quantitative aspects of their data are partially obscured by the varying amounts of tissue impurities in the enzyme preparations which were capable of combining with and inactivating the arsenicals. The susceptibility of the different enzymes to the same arsenical was generally of the same order of magnitude (11). Using eighteen different enzyme systems, they found that under the particular experimental conditions, most of the enzymes were almost completely inhibited (by 80 to 100 per cent), but that pyruvate dismutation was inhibited by only 35 per cent and pyruvate condensation by 56 per cent. The degree of inhibition obtained with five different arsenoso compounds acting on the same enzyme, succinoxidase, were also not significantly different (12). These findings suggest that factors may supervene in the reaction of arsenicals with formed cells which do not complicate their reactivity with enzyme proteins in solution. However, from the same laboratory it was later shown that *d*-amino acid oxidase and yeast carboxylase were inhibited by p-aminophenyldichlorarsine but not by Lewisite (7). Also, transaminase was almost completely inhibited by p-arsenosobenzoic acid, but only 33 per cent inhibited by the same concentration of Lewisite. The authors suggest that these differences may arise from a different spatial arrangement of the thiol groups on the enzyme protein.

2. *The possibly varying permeability of the cell and a consideration of "arsenic-resistance."* In attempting to explain the widely varying biological activity of arsenosobenzenes and their selective parasiticidal action, consideration must be given the fact that the unsubstituted arsenosobenzene is highly toxic for all cells against which it has been tested, and that substituent groups generally depress that activity. The specificity of arsenosobenzenes is thus not an activity newly bestowed upon the compound by the substituent group, but results rather from the fact that the activity against one cell type has been suppressed to a lesser extent than that against other cells. It follows that the reactive grouping is the arsenoso (—AsO) grouping, common to all the arsenosobenzenes, and not the other substituents. How the latter serve to modify the reactivity of the —AsO grouping is a moot question. No evidence has yet been presented that substituent groups affect either the dissociation constant of the —AsO group or its reactivity with SH compounds. There is no evidence that differently substituted compounds react to a widely varying degree or at widely varying rates with a given SH compound, or that the same arsenosobenzene will react differently with different SH compounds.

As an alternative explanation of the varying biological effects of different

arsenoso compounds, one may consider the possibility that although their reactivity with SH compounds is reasonably uniform, the arsenicals vary widely in their ability to penetrate the cell wall, either in the microorganism or the host, and that it is these differences which largely determine the varying parasiticidal action and toxicity of these compounds. This thesis is strongly supported by the data on the binding of arsenicals by cell suspensions. It was first shown by Thuret (117) that a suspension of red blood cells *in vitro* bound the highly active arsenoso compounds, but not the relatively inactive arsonic acids. This finding was extended by Hogan and Eagle (73), who demonstrated that in a large series of arsenoso compounds the amount of arsenic bound by red blood cells *in vitro* under standard conditions was roughly proportional to the systemic toxicity of the arsenical, strongly suggesting a causal relationship

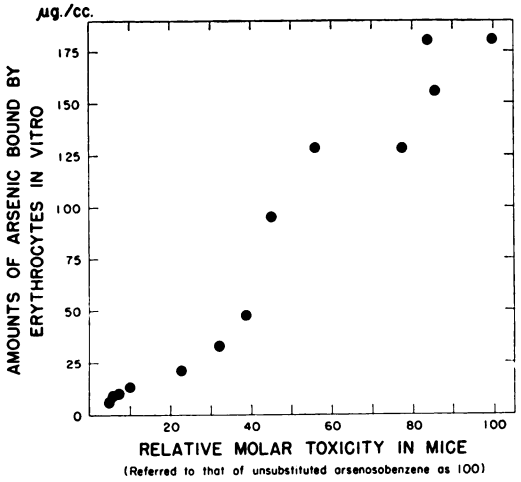


FIG. 6. The correlation between the affinity of arsenosobenzenes for erythrocytes *in vitro* and their systemic toxicity in mice. (After 61.)

(Fig. 6). The corollary of this finding was also demonstrated: when compounds of widely varying toxicity were injected at dosages which produced equivalent toxic effects *in vivo*, essentially similar amounts of arsenic were bound by the tissues of the host.

(The binding of arsenicals by erythrocytes was also studied by Fink and Wright (57). With oxophenarsine, these workers found an approximate linear relationship between the logarithm of the amount in the cells and the logarithm of the amount in the plasma, and found also that from 60 to 90 per cent of the bound arsenic could be released from the red blood cells by simple resuspension in fresh plasma. They therefore suggested that the binding of arsenicals was largely physical in nature. These important findings are difficult to reconcile with the thesis that arsenoso compounds react with —SH groups in the cells as previously discussed, and further study seems indicated.)

Eagle and Magnuson (48) subsequently showed that, just as the degree to

which arsenicals were bound by red blood cells was related to the systemic toxicity of the compounds, so the binding of arsenicals by trypanosomes was a measure of their parasiticidal activity (Table 9). The correlation between the amount of the arsenical bound under standard conditions and its trypanocidal activity was again so regular as to suggest a causal relationship. This quantitative study confirmed and extended the previous qualitative findings of Yorke, Murgatroyd and Hawking (130), Hawking (71) and Reiner, Leonard and Chao (95) that actively trypanocidal compounds were bound by the organisms, while inactive compounds were not.

The wide differences in the amounts of arsenical bound by a trypanosomal or erythrocyte suspension could perhaps be due to corresponding differences in the reactivity of the several arsenicals with a given cellular enzyme system. Dif-

TABLE 9

The relationship between the trypanocidal activity of arsenosobenzenes and their combining affinity with the organisms. (After 48)

Original As concentration = 0.166 mg. %; no. of trypanosomes = 250 × 10^6 cc.; vol. of 10^9 sedimented organisms = 0.48 cc.

SUBSTITUTED ARSENOSOBENZENE R—AsO	RELATIVE TRYPANOCIDAL ACTIVITY (REFERRED TO ARSENOSOBENZENE AS 100)	CONCN. OF As IN TRYPANOSOMES MG. %	AVERAGE RATIO OF $\frac{[As]\ TRYPANOSOMES}{[As]\ SUPERNATANT}$
p-SO_3H	0.06	0.41	2.4
p-$SO_2NHCH_2CONH_2$	1.4	0.68	5.2
p-OCH_2COOH	4.5	1.5	11
p-$CONHCH_2CONH_2$	15	5.7	53
3-NH_2-4-OH	27	6.5	87
p-$SO_2N(C_2H_5)_2$	35	7.4	93
p-$CONH_2$	45	7.7	113
m-OH	66	8.2	119
o-CH_3	91	9.5	186
Unsubstituted arsenosobenzene	100	9.7	224

ferences of this order of magnitude have, however, not been demonstrated in the reaction of arsenoso compounds with thiols *in vitro;* it seems unlikely that the hydrolysis constant of a series of thioarsenites involving the same thiol and different arsenoso compounds would differ by a factor of, *e.g.*, 10^2–10^4 (*cf.* pages 111–120). However, the results are consistent with the thesis that different arsenicals vary in the ease with which they can pass through the cell wall, and that it is this selective permeability which determines the varying activity of a series of closely related compounds against the same cell.

The results obtained with "arsenic-resistant" strains of trypanosomes offer additional strong evidence that the varying amounts of arsenical bound by the organisms reflect the varying permeability of the cell wall to the several compounds, rather than their varying reactivity with a cell thiol or thiols. Hawking (71) showed that unsubstituted arsenosobenzene was as actively trypanocidal against an "arsenic-resistant" strain of *T. equiperdum* as against a normal

strain; but that arsenoso compounds containing certain substituents, notably the —$NHCH_2CONH_2$ group, were not effective against the resistant organism and were not bound by it. Similar results were obtained by Yorke, Murgatroyd and Hawking (130), using an arsenic-resistant strain of *T. rhodesiense*. On the basis of their studies with an arsenic-resistant strain of *T. rhodesiense*, King and Strangeways (75, 76) concluded that arsenicals may act on these organisms in at least three different ways. Arsenosobenzene, arsenosoxylene, and similar compounds which fall into the group of compounds with "inert" substituents as defined on page 111 were equally active against both the normal and "arsenic-resistant" strains. The resistant organisms were resistant to several amide- and acetamido-substituted compounds which were highly active against the normal strain, while several acid-substituted compounds had an equally low measure of activity against both the normal and resistant variants. They concluded that while the actual intracellular process which determines the death of the organism is the same in each instance, the several types of compound entered the trypanosome by different mechanisms. Eagle and Magnuson (48), working with a strain of *T. equiperdum* which had spontaneously become "arsenic-resistant," found that arsenosobenzene, many of its simple derivatives (chloro-, methyl-, and nitro-, etc.), and arsenosobenzenes with substituents carrying a terminal acidic group were just as active against this resistant variant as against the parent normal strain and were bound just as strongly by it. On the other hand, arsenoso compounds containing terminal amide, amine or acetamide substituents were $\frac{1}{5}$th to $\frac{1}{200}$th as active against the resistant strain as against the normal strain, and the amount of arsenical bound was correspondingly reduced. In human trypanosomiasis, "arsenic-fast" strains of *T. gambiense* which pose so serious a therapeutic problem in large areas of Central Africa have regularly proved resistant to amide-substituted arsenicals (*e.g.*, tryparsamide), but are normally susceptible to γ-(p-arsenosophenyl)-butyric acid and to melarsen oxide (37, 61, 119).

It is thus well established that so-called "arsenic-resistant" trypanosomes are normally susceptible to certain types of arsenicals and that this susceptibility is associated with the ability of these arsenicals to combine with the cell. The only simple explanation is a selective permeability of the cell for certain arsenicals. Otherwise, one must assume that arsenicals with contrasting effects on "resistant" strains (*e.g.*, acid- and amide-substituted compounds) combine selectively only with certain thiols in the cell and, further, that normal and resistant trypanosomes differ qualitatively with respect to their thiol content. The former assumption in particular seems improbable.

The importance of cell permeability is further indicated by the fact that, with acid substituted arsenoso compounds, the activity against both *Tryp. equiperdum* and *T. pallidum* decreased strikingly as the pH of the testing solution was increased (*cf.* page 116), and the degree to which the compounds were bound by the organisms at varying pH decreased concomitantly. It thus appears that the ion is relatively inactive because it is bound by the organism to only a limited degree, while the undissociated acid is highly active because it is strongly bound. These differences can hardly be related to the varying reactivity of the

salt and of the free acid with thiols; the salts of acid-substituted arsenoso compounds react as readily with cysteine and other thiols as do arsenoso compounds with non-ionic substituents. However, the results are consistent with the thesis that the ionized form passes into the interior of the cell only to a limited degree as compared with the undissociated molecule. The many analogies with other types of compounds and other cell species have been summarized by a number of workers (1, 36, 105). (The possibility that the arsenicals are bound on the outside wall of the organism, rather than within the cell, has been discussed by Reiner, Leonard and Chao (95) and by Eagle (36). The latter concluded that the quantitative relationships between the amount of arsenical bound and the available surface area make it unlikely that the major portion of the arsenic bound by trypanosomes attaches to the cell membrane.)

According to the permeability theory, the cell wall would be relatively impermeable to certain arsenoso compounds whereas others can penetrate with ease. Having penetrated, the arsenicals are so firmly bound to some of the cellular constituents (thiols) that they no longer figure in the intracellular-extracellular equilibrium. In consequence, the drug continues to diffuse into the cell, and the total intracellular concentration continues to build up, attaining 50, 100 and even 200 times that in the surrounding fluid. The factors determining which compounds can penetrate the cell wall and which cannot are not clear. Unresolved also is the nature of the changes in the "resistant" cell which make it almost wholly impermeable to some compounds which are normally highly active.

A number of drugs have been found to antagonize the cytotoxic effects of arsenicals (*cf.* page 130). Unfortunately, there are no data as to whether these antagonists actually prevent the concentration of the arsenical within the cell. If that proves to be the case, the antagonists may either prevent the passage of the arsenical across the cell wall or, by blocking the union of the arsenical with an intracellular compound, prevent its rapid accumulation and parasiticidal action. Work and Work (127), commenting on the striking antagonism of p-aminobenzoic acid for γ-(p-arsenosophenyl)-butyric acid, concluded that "the only explanation for this action is to assume that the drug acts ultimately in the same manner as other trypanocidal arsenicals, but that PABA may be preventing or limiting its admission into the trypanosome cell."

IV. SUMMARY

1. Of the three types of arsenicals used in the treatment of spirochetal and protozoal diseases, only the arsenoso compounds have a direct parasiticidal activity, the arsonic acids and arseno compounds being active by virtue of their conversion to arsenoso compounds in the animal host.

2. Unsubstituted arsenosobenzene was one of the most toxic and most actively parasiticidal compounds in the entire series tested, with no demonstrable selective action among the organisms studied.

a) Substitution with a single $-CH_3$, $-NO_2$, $-Cl$, $-NH_2$, $-OH$, or $-F$ group either did not significantly affect the toxicity or parasiticidal activity of the parent arsenosobenzene, or reduced them both to the same slight degree.

b) Acid-substituents strikingly decreased both the direct parasiticidal and

toxic action of arsenosobenzene. The ionized form was generally inactive, the undissociated acid highly active, and the effect of pH on parasiticidal activity could be related to its effect on dissociation. However, there were important exceptions to the general inactivity of the ionized compounds (*e.g.*, the 3-NO_2-4-COOH and p-$(CH_2)_3COOH$ arsenosobenzenes); several compounds in this series are of distinct therapeutic interest.

c) Amide substituents usually caused a slight decrease in treponemicidal and trypanocidal activity, but a striking decrease in toxicity. As a class, they are therapeutically the most promising group of arsenoso compounds so far studied. Substitution in the amide hydrogens usually diminished its favorable effect on toxicity, exceptions being noted in groups which also reduced the toxicity of arsenosobenzene when substituted directly onto the benzene ring (*e.g.*, $-C_2H_4OH$, $-C_6H_4NHCOCH_3$).

d) Unlike amidification, esterification of acid substituents resulted in compounds approaching arsenosobenzene in their high toxicity and general parasiticidal action. Many of these compounds readily hydrolyzed in aqueous solution, their biological activity then corresponding to that of the free acid.

e) The effect of complex substituents was usually determined by the nature of the terminal group in the substituent (*e.g.*, $(CH_2)_3CONH_2$; $CON(CH_3)_2$).

f) Two substituent groups had an effect which could not be predicted from those of the substituents taken singly. Unlike the case of the single substituents, in these di-substituted compounds the position on the benzene ring profoundly modified the activity of the compound.

g) Both the toxicity and direct parasiticidal activity of thioarsenites were somewhat less than those of the corresponding arsenosobenzene; in the treatment of rabbit syphilis and mouse trypanosomiasis, the therapeutic index was no better than that of the parent compound.

3. There are several instances of arsenoso compounds with a therapeutic index in a particular infection greatly exceeding that of chemically closely related compounds (*e.g.*, "mapharsen," "melarsen oxide," p-arsenosophenylbutyric acid). These exceptions emphasize the unreliability of generalizations with respect to the effect of a given type of substituent on the biological activity of arsenosobenzene.

4. There is considerable evidence to support the view that the toxicity and therapeutic activity of arsenoso compounds are determined by the amounts which enter into combination with cellular components. The widely varying activity of a single arsenoso compound against different organisms, and of a series of such compounds against the same organism, is related to the amount of arsenical bound.

5. It is a reasonable working hypothesis that arsenoso compounds combine reversibly with sulfhydryl groups in essential enzyme proteins, and that their cytotoxic effect is due to that combination. Many essential enzymes contain sulfhydryl groups, and most such enzymes can be inactivated by arsenoso compounds and reactivated by sulfhydryl or other agents which can compete with the enzyme protein for the arsenical.

6. The highly selective action of particular arsenoso compounds against certain cells could be explained on the basis of either (a) the varying affinity of different enzymes for the same arsenical, or of different arsenicals for the same enzyme, or (b) differences in the permeability of a given cell to different arsenicals. While the evidence is not conclusive, the latter assumption appears best to explain the available data.

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