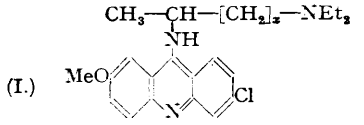
78. *Some Physico-chemical Properties of Acridine Antimalarials, with Reference to their Biological Action. Part III. The Correlation of Physico-chemical Properties with Biological Action.*

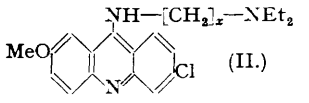
By S. F. Mason.

The degree to which members of two homologous series of acridine antimalarials (I and II) inhibit the enzyme system, diamine-oxidase, has been found to parallel the values of the reduction potentials of these compounds. No definite correlation between their antimalarial activities and their physico-chemical properties has been discovered, but an explanation of the order of their activities has been suggested, in terms of (*a*) the factors that possibly determine the permeability of these drugs through organic material, and (*b*) the equilibrium distribution of these compounds amongst the various organic phases within an organism, namely, their lipoid partition coefficents, surface activities, and relative protein affinities.

In Part I of this series (p. 345) an attempt was made to relate the antimalarial activities of two homologous series of acridine drugs (I and II) with their reduction potentials and basicities, but no relation was observed. This was ascribed to the complexity of antimalarial activity *in vivo*. A given drug must permeate through the cellular tissue of the host and perhaps of the parasite in order to interfere with some enzyme system, or systems, within the parasite, and *en route* may become distributed amongst the various phases, aqueous, lipoid, and protein, of both organisms, so that only a small concentration of the drug is present at the site of action. Thus the " antimalarial activities " of a series of drugs, as determined *in vivo*, may be a combination of their relative permeabilities and distribution factors, as well as of their relative potencies at the site of action.



(I.)



(II.)

It was thought that these two aspects of antimalarial activity might be separated for individual treatment, and in Part II of this series (preceding paper) the physico-chemical properties that might influence the permeability and distribution of these drugs in organic systems, namely, their lipoid partition coefficients, surface activities, and relative protein affinities, were measured for all members of series (II). The other factor contributing to the relative antimalarial activities of such a series of drugs, namely, their relative efficacies at the site of action, cannot be investigated physico-chemically at all fully as insufficient is known at present concerning the mode of action of 5-diaminoacridine antimalarials on the malaria parasite and other biological systems. However the physico-chemical properties chosen for investigation in Part I were selected by considering such knowledge as we have on this subject, the conclusion being reached that the reduction potentials and basicities of the acridine homologues (I and II) might be related to their activities on some simple biological system, such as an enzyme system, which would be free from the complication of the relative permeability and distribution factor.

Use was therefore made of the fact that 5-diaminoacridines inhibit the oxidation of diamines by diamine-oxidase. The concentrations required to inhibit this enzyme system to the extent of 50% were measured in the case of each of the acridine homologues (I and II), the values obtained being listed in Table I below.* This particular enzyme system was chosen because it might possibly be one of the vital activities of the malaria parasite that is suspended by acridine antimalarials analogous to Mepacrine (I; $n = 3$). Compounds of this type, to which all the derivatives used in the present work belong, are diamines, and thus might be expected to inhibit the utilisation of a structurally similar (diamine) metabolite by a biological organism. Furthermore Silverman and Evans (*J. Biol. Chem.*, 1944, **154**, 521) have shown that polyamines restore growth to cultures of bacteria inhibited by Mepacrine, and have demonstrated competitive antagonism between Mepacrine and polyamines in this connection.

This is not to suggest of course that the figures quoted for the inhibition of the diamine-oxidase system by our acridine derivatives provide a measure of their relative efficacies at the

* The author is indebted to members of the Pharmacology Department, Oxford, for these measurements; notably to Dr. Duthie, who studied the four members of the branched-chain homologues (I), and to Dr. Blaschko, who investigated the six members of the straight-chain series (II).

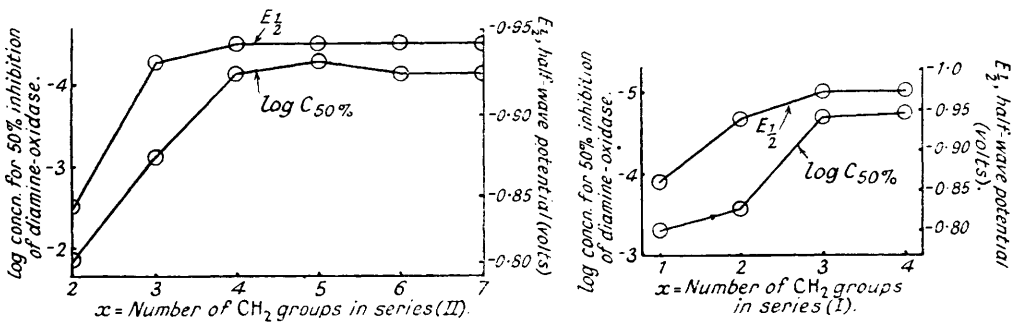
site of action within the malaria parasite, for there is no evidence as yet that the inhibition of diamine-oxidase is even a necessary, much less a sufficient, cause of antimalarial action.

TABLE I.

	x.	$E_{\frac{1}{2}}$, 1st step, v.	Concentration required to produce 50% inhibition of diamine oxidase, M.	$E_{\frac{1}{2}} - \frac{RT}{nF} \cdot \log C_{50\%\ \text{inhib.}}$ $n = 1$, v.	$n = 2$, v.
Series II.	2	−0·84	$1{\cdot}45 \times 10^{-2}$	−0·73	−0·79
	3	−0·93	$7{\cdot}5 \times 10^{-4}$	−0·75	−0·84
	4	−0·94	$6{\cdot}5 \times 10^{-5}$	−0·70	−0·82
	5	−0·94	5×10^{-5}	−0·69	−0·82
	6	−0·94	$6{\cdot}5 \times 10^{-5}$	−0·70	−0·82
	7	−0·94	$6{\cdot}5 \times 10^{-5}$	−0·70	−0·82
Series I.	1	−0·86	5×10^{-4}	−0·67	−0·75
	2	−0·94	3×10^{-4}	−0·74	−0·84
	3	−0·97	2×10^{-5}	−0·70	−0·84
	4	−0·97	$1{\cdot}7 \times 10^{-5}$	−0·70	−0·84

The first half-wave potentials (oxidation–reduction potentials) of these acridine homologues parallel to some degree the logarithms of the concentrations required to bring about 50% inhibition of the diamine-oxidase system (Fig. 1). Some connection between these two

FIG. 1.



properties is perhaps to be expected since the system inhibited is concerned with oxidation, a process in which the oxidation–reduction characteristics of an inhibitory drug might be operative. The potential of an oxidation–reduction system, such as one of these acridine drugs present in both an oxidised and a reduced form, is governed by the equation :

$$E = E_{\frac{1}{2}} - \frac{RT}{nF} \cdot \log \frac{C_{\text{red.}}}{C_{\text{oxid.}}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

which may be rearranged

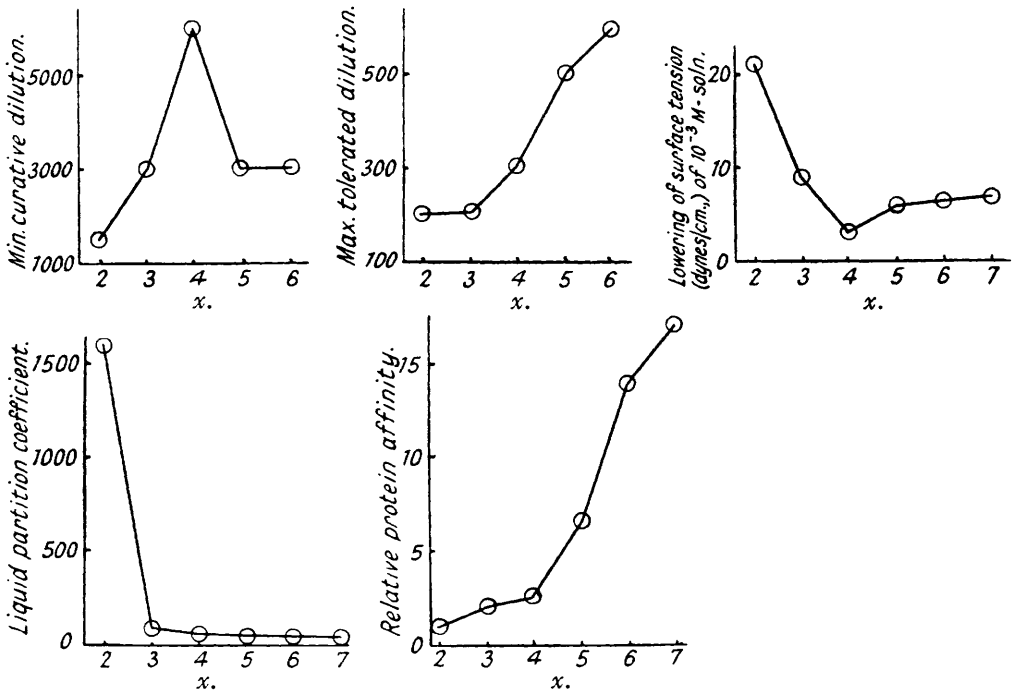
$$E - \frac{RT}{nF} \cdot \log C_{\text{oxid.}} = E_{\frac{1}{2}} - \frac{RT}{nF} \cdot \log C_{\text{red.}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

It is found that the quantity, $E_{\frac{1}{2}} - \frac{RT}{nF} \cdot \log C_{50\%\ \text{inhib.}}$, has approximately the same value for each compound when $n = 1$, $n = 2$ electrons (Table I), the best constancy being obtained when $n = 1{\cdot}5$. This relation is curious, since it may be taken to imply that most of the drug used in inhibiting the diamine-oxidase system exists in the semi-reduced and fully reduced forms under conditions where the enzyme system is inhibited to the extent of 50%. It would also imply (equation 2) that the quantity, $E - \frac{RT}{nF} \cdot \log C_{\text{oxid.}}$, has approximately the same value for each compound in these conditions. Thus if it be assumed that a small constant quantity of the drug remains in the oxidised form, the determinant of a given degree of diamine-oxidase inhibition with these 5-diaminoacridines might be said to be the setting up of an oxidation–reduction system with a definite potential E, as lower half-wave potentials would be compensated by larger concentrations of the drug added to the enzyme system and passing into the reduced forms.

It is difficult to obtain a quantitative measure of antimalarial activity that is at all definitive, since numerical values obtained vary somewhat with the method of determination. However,

the relative activities of the members of the two series (I and II) can be shown qualitatively, and it has long been accepted that the most active members of both series are those in which four carbon atoms separate the two nitrogen atoms of the side chain. The antimalarial data listed in Table II are taken from Magidson (*Ber.*, 1936, **69**, 396), the minimum curative dilution being a measure of the antimalarial activity of the derivative *in vivo*, and the maximum

FIG. 2.



x = *number of methylene groups in series* (II).

tolerated dilution a measure of its toxicity to the host; the physico-chemical data are taken from Part II of this series.

TABLE II.

Compounds (II).

x.	Maximum tolerated dilution.	Minimum curative dilution.	Partition coeffs. between vegetable oil and buffer of pH 7·3.	Relative distribution factors between albumin and buffer of pH 7·3.	Lowering of the surface tension of buffer of pH 7·3 in dynes/cm.[2] at drug concn. 10^{-3} M.
2	200	1500	1600	1·0	21·0
3	200	3000	82	2·1	9·0
4	300	6000	54	2·7	3·0
5	500	3000	43	6·8	5·5
6	600	3000	31	14	6·0
7	*	—	15	17	6·6
5-(3-Diethylaminopropyl amino)acridine	†	—	22	15	6·4

* Tested through the courtesy of I.C.I. (Pharmaceuticals) Ltd. and found inactive at a dose of 4 mg. per 50 g. of body weight of host.

† Slightly active (Mietzsch, *Angew. Chem.*, 1934, **47**, 416).

Table II and the curves on Fig. 2 show that the antimalarial activities of the acridine homologues *in vivo* do not vary in the same way as their activities against the enzyme, diamine-oxidase, indicating that factors determining transfer and distribution in biological systems may be significant in determining their overall antimalarial action *in vivo*. It would appear (Table II) that the effectiveness of the 5-diaminoacridine type of antimalarial drug is enhanced

by those properties that ensure a comparatively high equilibrium concentration of the drug in the aqueous phases of the host–parasite system. Homologues with high lipoid partition coefficients, large protein affinities, and, less significantly, high surface activities at air–water interfaces, are those which are less active against the malaria parasite. Thus the variation of these properties with the length of side chain may go some way towards explaining the variation of antimalarial activity from homologue to homologue, though a knowledge of their relative activities at the site of action within the parasite would be required to assess how important these physico-chemical properties are in determining the overall activity of the drugs *in vivo*. Like the diamine side-chains, the nuclear chloro- and methoxy-substituents of the drugs may play specific biochemical or stereochemical roles in transit and distribution throughout the host–parasite system, and in attacking the parasite, but their effects on the physico-chemical properties of the drugs are similar to variations produced by shortening the side chain, as can be seen by comparing 5-(3-diethylaminopropylamino)acridine with the corresponding 2-chloro-7-methoxy-derivative. The partition coefficient, surface activity, and protein affinity of the unsubstituted compound are quantitatively intermediate between those of the active ($x = 6$) and the inactive compound ($x = 7$), which is of interest in view of the fact that this unsubstituted compound shows slight antimalarial activity.

Correlation also exists between the toxicity of the compound to the host (the maximum tolerated dilution) and its relative protein affinity within the homologous series. This may perhaps be related to the vulnerability of complex functional proteins, such as the enzyme proteins. The protein affinities of these homologues probably vary from protein to protein, but it is possible that their relative affinities remain in the same order, unless specific stereochemical factors intervene. It is for this reason that the correlation is considered to be of possible significance.

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