work. The success of this is shown by the fact that from 1931 until the outbreak of the present war about 180 postgraduate students registered each session. They were divided almost equally between the Faculties of Arts and Science.

As a physical chemist Senter made a number of important contributions to the science, and though he was Principal of Birkbeck College he for many years remained Head of the chemistry department and directed the work of a group of research students. His earlier work was concerned with the development of biological applications of the new science of physical chemistry and included some work on enzyme reactions. His major work was, however, the application of methods of chemical kinetics in the problem of the Walden inversion. This was pioneering work undertaken at a time when little was known about the complexity of such reactions. In a series of papers published in the *Journal* of the Chemical Society between 1914 and 1925 Senter and his students shed a good deal of light on the problem of replacement reactions in solution. After 1925 his duties as Principal compelled him to give less and less time to this work. More recently Ingold and his collaborators have made important advances in this field and have paid tribute to the valuable work which came from Senter's laboratory. Senter's contributions to the Faraday Society's discussions must also be mentioned; in particular his introductory paper to the discussion on "Passivity" is a model of clearness as well as a summary of valuable information. Of his other publications his "Outlines of Physical Chemistry" has already been mentioned; his "Textbook of Inorganic Chemistry" has also been of great help to students. Both books are characterised by extreme clearness of expression and a common-sense attitude towards theory which are especially helpful to beginners.

It would be tedious to recite all the University appointments which Senter held. Suffice it to say that he served on the Senate from 1912 to 1914, 1922 to 1923 and from 1928 to 1939. The last period he was ex-officio a member of the Senate in virtue of his position as Principal of the College. This brought Birkbeck into line with other large colleges whose heads are automatically Senators. In 1934 to 1935 he was Deputy Vice-chancellor. He served on many committees and boards; of these chemists will remember his helpfulness on the Board of Studies in Chemistry, of which he was Chairman for thirteen years.

S. SUGDEN.

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94. *The Reduction Potentials of Acridines, with Reference to their Antiseptic Activity.*

By B. BREYER, G. S. BUCHANAN, and H. DUEWELL.

The behaviour at the dropping-mercury electrode of acridine and of a number of its derivatives has been studied. It was found that the acridines are reduced in two stages, the first leading to the formation of a monohydroacridine radical, the second to dihydroacridine. Diffusion currents and concentrations of most acridines were found to be mutually proportional in solutions from 10^{-3} to 10^{-5} M. The reduction potentials were measured through the pH range from 2 to 12, and, as a result, this further physical property has been correlated with the known relationship between degree of dissociation and biological activity of acridines. It was found that those members having a more negative reduction potential than — 0·400 v. (against the normal hydrogen electrode) at pH 7·3 are most active biologically.

IN the endeavour to correlate physical and biological properties of acridines, Albert, Goldacre, and Rubbo have investigated the basicities and partition coefficients of a series of aminoacridines (*Nature*, 1941, **147**, 332, 709), and shown that the members most active against bacteria are at the same time the most basic and the most hydrophilic.

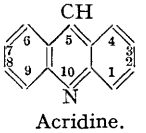
In order to throw further light on the mode of action of acridine drugs, it seemed desirable to measure their reduction potentials and try to correlate the results with the antiseptic activity, especially as McIlwain has recently shown (*Biochem. J.*, 1941, **35**, 1311) that the antiseptic action of aminoacridines can be prevented by adding a hydrogen carrier to the broth in which the bacteria are growing. The effective hydrogen carriers were riboflavin, methylene-blue, and pyocyanine, substances lying within the range E_h° (standard potential at a given pH) from + 11 down to — 275 millivolts at pH 7. This inhibition can itself be "inhibited" by adding more proflavine, and so on. McIlwain concludes that proflavine acts (*inter alia*) by competing with a biological chemical, to wit a hydrogen carrier, within the bacteria for a place in an enzyme system vital to its growth.

It seemed therefore important to know the reduction potential values $E^{\circ\prime}$ of the aminoacridines to find out: (1) whether the active members are as negative in $E^{\circ\prime}$ as would be required if the above theory is true; and (2) whether the ratio of the $E^{\circ\prime}$ values of the active to the biologically inactive aminoacridines is such as to suggest that the value of the redox potential can be used as a measure of their activity, thus eliminating lengthy bacteriological work.

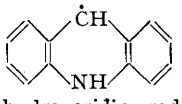
The reduction potentials of the acridine series now reported have been obtained with the help of the dropping-mercury electrode, *i.e.*, by polarography. The application of this electrode for measurements of this kind seems justified, in spite of objections that have sometimes been raised, because it has been shown beyond doubt that the polarographic half-wave potential of organic substances, from an electrochemical point of view, is identical with the $E^{\circ\prime}$ obtained by any other electrode arrangement (cf., *e.g.*, Müller and Baumberger, *Trans. Electrochem. Soc.*, 1937, **71**, 181), provided of course that the electrode reaction be reversible, a limitation which applies equally to the "classical" way of measuring redox potentials. Furthermore, polarography is superior to the

usual method of estimating redox values when the number of electrons involved in the electrochemical reaction (or, for that matter, the number of hydrogen atoms consumed in the reduction of organic molecules) is to be established. The commonly used method relies for this kind of measurement fundamentally on the Nernst equation, which, however, is valid only when the electrochemical process is perfectly reversible. Polarographically the number of electrons (or hydrogen atoms) taken up in the reduction is estimated with the help of Ilkovič's equation (*Coll. Czech. Chem. Comm.*, 1934, **6**, 498), which, being independent of the condition of electrochemical equilibrium, gives most satisfactory results.

At the dropping-mercury electrode, acridine and its derivatives are reduced in two steps of equal height. By applying Ilkovič's equation, *viz.*, $I_d = 605nD^{\frac{1}{2}}Cm^{2/3}t^{1/6}$ [where I_d is the average diffusion current in microamp. (μa.) during the life of a mercury drop, n the number of faradays necessary to reduce one mol. of the substance, C the concentration of the substance in millimols./l., m the rate of flow of mercury from the capillary in mg./sec., D the diffusion coefficient in cm.²/sec., and t the drop time in seconds], it was found that only one hydrogen atom is taken up with each step, meaning that the first product of reduction is a free radical. For this and the other measurements, a very slow-dropping capillary has been used, for it has been found that the results so obtained are more accurate. Some experimental details, by way of example, are given below :



Acridine.



Monohydroacridine radical.

Acridine in borate–HCl buffer, pH 9; temp. 0°.

First step : $I_d = 0{\cdot}130$; $D_0 = 4{\cdot}63 \times 10^{-6}$; $m = 0{\cdot}577$; $t = 8{\cdot}87$; $C = 0{\cdot}1$. Therefore $n = 1{\cdot}02$.

Second step : $I_d = 0{\cdot}132$; $D_0 = 4{\cdot}63 \times 10^{-6}$; $m = 0{\cdot}660$; $t = 7{\cdot}52$; $C = 0{\cdot}1$. Therefore $n = 0{\cdot}96$.

The diffusion coefficients at infinite dilution (D_0) were calculated from Nernst's equation, $D_0 = \boldsymbol{R}T\Lambda_0/nF^2$. The value for Λ_0 (equivalent conductivity at infinite dilution) was obtained by Albert and Goldacre (J., 1943, 454) on 2-amino-10-methylacridinium bromide at 0°, and found to be 19.

The diffusion current for 10^{-4} M solutions of acridines, calculated from Ilkovič's equation, is 0·135 μa. at 0°. The values actually found for the different members of the series vary within a range of ± 5% from the figure theoretically required. The diffusion current varies by 1·12% for 1°. The proportionality between step height and concentration is very good within the range of 10^{-3}—10^{-5} M, except for acridine and 4-aminoacridine, which show proportionality only within the range 10^{-4}—10^{-5} M. In these two instances, at concentrations higher than 10^{-4} M, the whole shape of the polarographic curve is altered as well. Three steps appear instead of the usual two. In the case of acridine the height of the first step remains unchanged within the whole range from 10^{-3} to 10^{-4} M. At the same time the $E_{\frac{1}{2}h}$ (potential of the dropping mercury electrode at the midpoint of the step, referred to the normal hydrogen electrode) is shifted from − 0·042 v. to − 0·099 v. at pH 2, and from − 0·054 v. to − 0·124 v. at pH 4. The height of the second step diminishes with dilution, but there is no proportionality between dilution and step height. At a concentration of 10^{-4} M this second step disappears altogether. The potential shift with increasing dilution is considerable : at pH 2 from − 0·329 v. at 10^{-3} M to − 0·473 v. at $1{\cdot}25 \times 10^{-4}$ M, and at pH 4 from − 0·314 v. to − 0·454 v., respectively. The third step consists of a slow, drawn-out rise, and starts approximately at − 0·410 v. (pH 2) and − 390 v. (pH 4). It disappears at a dilution of $2{\cdot}5 \times 10^{-4}$ M. For 4-aminoacridine, the height of the first step diminishes with dilution, but considerably less than is required theoretically. The potential is shifted from − 0·143 v. at 10^{-3} M to − 0·166 v. at 10^{-4} M at pH 2, and from − 0·174 v. to − 0·200 v. at pH 4. The height of the second step, as in the case of acridine, diminishes slowly with dilution, to disappear at a concentration of 10^{-4} M. The potential shifts from − 0·313 v. at 10^{-3} M to − 0·348 v. at $1{\cdot}25 \times 10^{-4}$ M at pH 2, and from − 0·485 v. to − 0·536 v. at pH 4. The third step diminishes with dilution fairly closely to proportionality. The potential shifts from − 0·701 v. at 10^{-3} M to − 0·635 v. at 10^{-4} M (pH 2), and from − 0·761 v. to − 0·729 v. (pH 4).

The most likely interpretation for this peculiar behaviour of acridine and 4-aminoacridine is that at concentrations higher than 10^{-4} M, an intermediate compound between the acridine and the free radical, produced during the first reduction step, is formed. These results are in agreement with those obtained by Lingane *et al.* (*J. Amer. Chem. Soc.*, 1943, **65**, 1348) with 5-phenylacridine, but they interpret the double wave to mean " the formation of a slightly soluble dimer of phenyl- and dihydrophenyl-acridines." It seems doubtful whether this interpretation is correct, for no dihydroacridine is formed at as low a potential as that at which the first wave occurs.

It was interesting to find that the limit of concentration at which acridine will exist as a monomeric substance is 10^{-4} M. This was deduced from the fact that no increase in height of the first step occurred as the concentration was increased up to 10^{-3} M.

The curve analysis of the acridine steps, by plotting $\log i/(i_d - i)$ (i_d = diffusion current, i = current at chosen successive points of the step) against applied E.M.F., yields a straight line as far as the first steps are concerned; but the slope of the curve only exceptionally (*e.g.*, in 1-aminoacridine) approaches the theoretically required value of 59 mv. The values found range from 28 to 82 mv. The curve analysis of the second steps only exceptionally shows a straight line (*e.g.*, in 2 : 8-diaminoacridine) and the slope values vary from 53 to 104 mv., an indication of the irreversibility of the process.

It is obvious that the two reduction processes take place on the nitrogen atom and the C_5 atom, respectively, but it had to be established if the first step in the reduction concerns the former and the second step the latter atom, or *vice versa*. From theoretical considerations it is to be expected that the reduction takes place first on the nitrogen atom. This is confirmed by experiment: plotting of the half-wave potentials ($E_{\frac{1}{2}}$) of the first steps against pH yields curves the bends of which correspond to the pK values found by Albert and Goldacre (*loc. cit.*) in the same series of acridines. It is interesting to note in this connection that in the case of acridone, which contains an imino-group, only one step is obtained at the dropping mercury electrode. This wave closely resembles the second reduction step of the other acridine derivatives, as can be seen from the $E_{\frac{1}{2}}$–pH curve: the shift of $E_{\frac{1}{2}}$ with increasing pH follows closely the type of the second steps of the acridines, *i.e.*, the curve shows first a steep slope and then flattens out after the pK bend has been reached.

Table I contains the half-wave potentials of the acridine derivatives studied. With few exceptions (usually due to either maxima formation or extreme closeness of the two steps) the values are reproducible to approximately ± 10 mv. The maxima are difficult to suppress (only 0·05% of gelatine and in some cases methyl cellulose proved effective), and no $E_{\frac{1}{2}}$ values obtained with the help of suppressors are reported because it was found that their addition affects the $E_{\frac{1}{2}}$ potentials in a completely erratic way.

TABLE I.

(All potentials are negative.)

pH:	2.		4.		5·5.		7·3.		9.		11·8.	
Substance.	1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.
Acridine	0·099	(*a*)	0·124	(*a*)	0·157	1·063	0·313	1·057	0·472	1·069	0·731	1·065
1-Aminoacridine	0·081	0·521	0·176	0·614	0·260	0·735	0·394	0·979	0·570	1·030	0·842	1·041
2- ,,	0·338	0·672	0·396	0·781	0·418	0·875	0·468	1·009	0·549	1·119	0·720	1·134
3- ,,	0·124	0·566	0·199	0·674	0·235	0·749	0·369	0·896	0·489	1·014	0·753	1·050
4- ,,	0·166	0·635	0·200	0·729	0·209	0·847	0·301	0·954	0·423	1·005	0·672	1·006
5- ,,	0·658	(*a*)	0·732	(*a*)	0·834	(*b*)	0·916	(*b*)	0·941	(*b*)	1·073	(*b*)
2 : 8-Diaminoacridine	0·445	0·597	0·604	0·774	0·690	0·929	0·731	1·059	0·706	1·177	0·830	1·225
2 : 8-Diamino-10-methylacridinium bromide	0·422	0·588	0·560	0·728	0·634	0·889	0·650	1·055	0·639	1·153	0·627	1·218
2-Chloro-8-aminoacridine	0·264	0·646	0·302	0·767	0·378	0·868	0·402	(*b*)	0·473	1·077	(*c*)	(*c*)
3-Hydroxyacridine	0·162	0·257	0·202	0·318	0·229	1·029	0·369	1·030	0·506	1·050	0·884	1·046
Acridine-3-sulphonic acid	0·095	(*a*)	0·099	0·932	0·155	0·925	0·281	0·952	0·422	0·962	0·691	0·960
Acridone	(*c*)	(*c*)	—	0·325	—	0·847	—	1·191	—	1·265	—	1·391

(*a*) Hydrogen step overlaps. (*b*) Maximum formation. (*c*) Insufficiently soluble.

The buffers used were: pH 2 and 4, citrate–hydrochloric acid; pH 5·5 and 7·3, phosphate; pH 9, borate–hydrochloric acid; pH 11·8, borate–sodium hydroxide, all after Sörensen. All solutions were 10^{-4} M with regard to the acridine derivative. A saturated calomel electrode served as an anode, but the potentials reported refer to the normal hydrogen electrode, as customary in biological work. The measurements were carried out in a thermostat at 25°.

The reduction potentials for 2-amino-, 5-amino-, and 2 : 8-diamino-acridine are noticeably higher than for the other compounds of the series, a fact exhibiting a parallelism with the pK values of these compounds as found by Albert and Goldacre (*loc. cit.*), who showed convincingly that these variations are due to ionic resonance phenomena. This parallelism is particularly evident at lower pH values, whereas the differences are less pronounced at a pH of 9 and over.

The results of this investigation indicate that there is a definite relationship between reduction potentials and chemotherapeutic activity of the acridines, in the sense that those members which have a more negative $E_{\frac{1}{2}}$ value for the first step than — 0·400 v. at pH 7·3, are biologically the most active (see Table II).

TABLE II.

Substance.	$E_{\frac{1}{2}}$, v. (pH 7·3).	Averaged bacteriostatic index.*
5-Aminoacridine	−0·916	4·6
2 : 8-Diaminoacridine	−0·731	4·6
2-Aminoacridine	−0·468	4·2
1-Aminoacridine	−0·394	0·8
3-Aminoacridine	−0·369	1·6
Acridine	−0·313	1·2
4-Aminoacridine	−0·301	1·8

* Values from Albert and Goldacre (*loc. cit.*): they are expressed in powers of 2.

In the case of the less active members of the series the order of $E_{\frac{1}{2}}$ values does not run parallel with the bacteriostatic index, which is easily understood since the biological activity of acridines must be governed by other factors in addition to the reduction potential. For instance, it has been shown by Albert, Goldacre, and Rubbo (*loc. cit.*) and by Rubbo, Albert, and Maxwell (*Brit. J. Exp. Path.*, 1942, **23**, 69) that the degree of dissociation and the partition coefficient have a direct bearing on the bacteriostatic index of these compounds. There seems to be little doubt, therefore, that the biological activity of the acridines is not connected with a

single physical or chemical property, but is the result of the sum total of such properties. It is evident from this investigation that the active members of the series have reduction potentials so negative that they cannot be reduced under the conditions prevailing in the living organism. The highly negative reduction potential of the biologically most active acridines suggests that they react, by means of their basic groups, with a respiratory enzyme system, the activity of which is inhibited by the combination with the difficultly reducible acridine derivative. Such substances as thionine or methylene-blue, on the other hand, although their chemical structure and basicity are similar to those of the active acridines, display a negligible antiseptic value, most probably because of the fact that their redox potential is very near to that of the living cell. Therefore, their combination with the enzyme will not only not impair but, on the contrary, even effectively take the place of the respiratory system of the cell where this has been damaged by the previous administration of toxic substances (cf. *e.g.*, McIlwain, *loc. cit.*, and Jancso and Jancso, *Z. Immunitäts.*, 1936, **88**, 275).

We are greatly indebted to Dr. A. Albert, not only for the compounds used in this investigation, but also for very helpful discussion during the progress of the work. We also wish to thank Mr. J. B. Willis for preliminary work done in connection with this investigation. One of us (B. B.) is indebted to the University of Sydney for a Commonwealth Research Grant which made the work possible.

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