probably peptides which gave rise to the small quantities of aspartic acid, glutamic acid, glycine, and alanine which were detected in most of the samples after acid hydrolysis. The quantities of glutamic acid were greater than those of the other amino acids in the hydrolyzates.

These results indicate clearly that the ability to maintain the relatively high content of intracellular amino acids of the adult frog must appear at some time after the last stage of development examined in the present study.

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The Interaction of Antimalarials with Nucleic Acids[1]

I. Acridines

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II. Quinolines

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From studies of the physicochemical factors which are of possible importance in the therapeutic activity of antimalarials, we have secured data on the ionization exponents of quinoline and acridine derivatives (*8–10*), and have reported briefly on the interaction of these compounds with plasma proteins (*11*). The present preliminary report on the interaction of these agents with nucleic acids is of interest not only from the standpoint of the possible relationship to antimalarial activity, but also because such interactions provide a plausible basis for explaining the effect of ribonucleic acid in reversing the inhibition by acridines of the growth of certain bacteria (*12, 13*), yeast (*14*), and bacterial viruses (*5, 6*). In addition, these studies provide procedures which may be useful in investigating the structure of nucleic acids and the action of the nucleases.

Yeast ribonucleic acid from the Schwarz Laboratories was purified by the procedure of Fletcher *et al.* (*7*) and Vischer and Chargaff (*19*), particular attention being paid to removal of inorganic salts by dialysis because of the large effect of ionic strength upon the interaction. Microanalysis of a sample dried at 110° C gave: N (Dumas) 16.1%; P (Pregl-Lieb) 8.8%.

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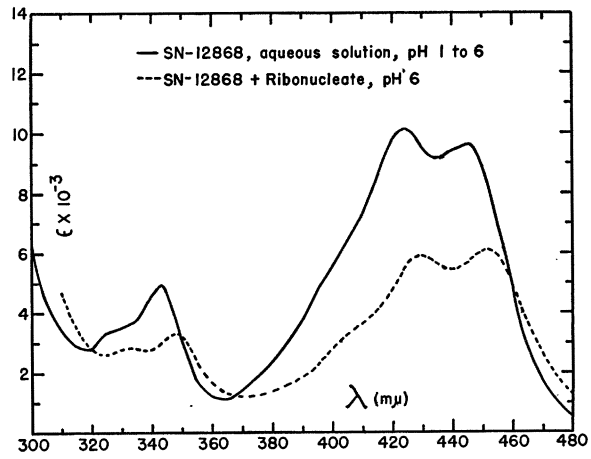

FIG. 1. Absorption spectra for SN-12868.

In Fig. 1, absorption spectra are presented for 2-methoxy-6-chloro-9-(1′methyl-8′-diethylamino-octylamino)-acridine (SN-12868)[2] in aqueous solutions buffered with phosphate at pH 6.0 ($\Gamma/2 = 0.1$) in the absence of and in the presence of sodium ribonucleate at a concentration (0.5 g/100 ml) at which the change in absorption is complete. Half-maximum transformation at this pH and ionic strength is attained when the concentration of ribonucleate (calculated as the anhydrous acid) is 0.013 g/100 ml. The interaction is stronger at lower ionic strength, and is somewhat stronger at pH 6.4 than at pH 6.0. Similar changes in spectrophotometric absorption in the presence of nucleates are obtained with 7-chloro-4-(1′-methyl-4′-diethylaminobutylamino)-quinoline (SN-7618). That the change in absorption is an indication of some type of bond between the nucleate anion and the antimalarial has been verified in the case of the acridine by determining the effect of sodium ribonucleate on the distribution of the acridine between organic solvents and buffered aqueous solutions.

In order to evaluate the interaction more exactly, we have used an adaptation of the mathematical treatment of the ionization of polyvalent acids proposed by Simms (*18*) and by von Muralt (*16*). A maximum number, m, of quinoline or acridine ligands can combine with one molecule of nucleic acid, the nucleic acid being present in a total molar concentration, T^*. For the special case in which the interacting groups in the polyvalent molecule are identical, and electrostatic interference between successively bound ligands is negligible, the binding reaction can be treated as if it consisted of the combination of one molecule of ligand per molecule of monovalent nucleic acid of total molar concentration, T, with $T = mT^*$. The process is represented as

$$N + L \rightleftharpoons NL \qquad (1)$$

An association constant, k', is defined by the equation:

$$k' = \frac{[NL]}{[N][L]} \qquad (2)$$

[2] These are the code numbers employed in the publication, *A survey of antimalarial drugs, 1941–1945*, E. Y. Wiselogle, Ann Arbor, 1947.

in which brackets are used to represent molar concentrations at equilibrium, N is the free monovalent nucleic acid, L is the free ligand, and NL is the complex consisting of one monomeric molecule of ligand combined with one reacting center on the nucleic acid, this center being considered as if it were a separate monovalent molecule. The constant, k', is the intrinsic constant for the special case defined above. This constant can be evaluated spectrophotometrically essentially as described by Clark *et al.* (*1, 17*) for the interaction of nitrogenous bases with metalloporphyrins. The equation is:

$$-\log [N] = -\log \left[T - \frac{S(D_1 - D)}{(D_1 - D_2)} \right] = \log k' + \log \frac{D - D_2}{D_1 - D} \quad (3)$$

in which S is the molar concentration of total ligand which is kept constant during the series of measurements; D_1 is the optical density of the solution when nucleic acid is absent, D_2 is the optical density when the concentration of nucleic acid is sufficiently large to cause complete binding of the ligand, and D is the optical density at some intermediate point at which both species L and NL are present.

Inasmuch as Michaelis and Granick (*15*) have suggested that the polymerized forms rather than the monomeric species of certain basic dyes, such as methylene blue, interact with polyvalent anions of the chondroitin sulfate type (but presumably not with nucleate anions according to these authors), it was desirable to formulate the possible combination of N with L_2, the dimeric species of ligand. The processes would be:

$$L_2 \rightleftharpoons 2L; \; N + 2L \rightleftharpoons NL_2 \quad (4)$$

An association constant for the latter process can be expressed as:

$$k' = \frac{[NL_2]}{[N][L]^2} \quad (5)$$

$$-\log [N] = -\log \left[T - \frac{S}{2}\left(\frac{D_1 - D}{D_1 - D_2}\right) \right] = \log k' + \log \frac{2S(D - D_2)^2}{(D_1 - D)(D_1 - D_2)} \quad (6)$$

In order to compare the data for SN-12868 with that for SN-7618 on the same graph, it is convenient to define a degree of dissociation, $\alpha \equiv [L]/S$. Equations (3) and (6) then take the form of (7) and (8), respectively:

$$-\log [N] = -\log [T - S(1-\alpha)] = \log k' + \log \frac{\alpha}{1-\alpha} \quad (7)$$

$$-\log [N] = -\log \left[T - \frac{S}{2}(1-\alpha) \right] = \log k' + \log \frac{2S\alpha^2}{1-\alpha} \quad (8)$$

The values of T can be calculated for each equilibrium position provided that the molecular weight of the nucleic acid (from which T^* is calculated) and the value of m are known since $T = mT^*$. The molecular weight of the preparation which we have used is unknown. Nevertheless, valuable preliminary tests of the relationships given above can be made by expressing the total concentration of nucleic acid in terms of the gram-atoms of nucleic acid phosphorus per liter of solution (P). The values of T^* and T are related to P by the equation:

$$T = mT^* = mP/p \quad (9)$$

in which p is the number of phosphorus atoms per molecule of nucleic acid. Although absolute values of m and p cannot be calculated when the molecular weight is unknown, the ratio, m/p, can be determined from the spectrophotometric data and the percentage of phosphorus in the nucleic acid.

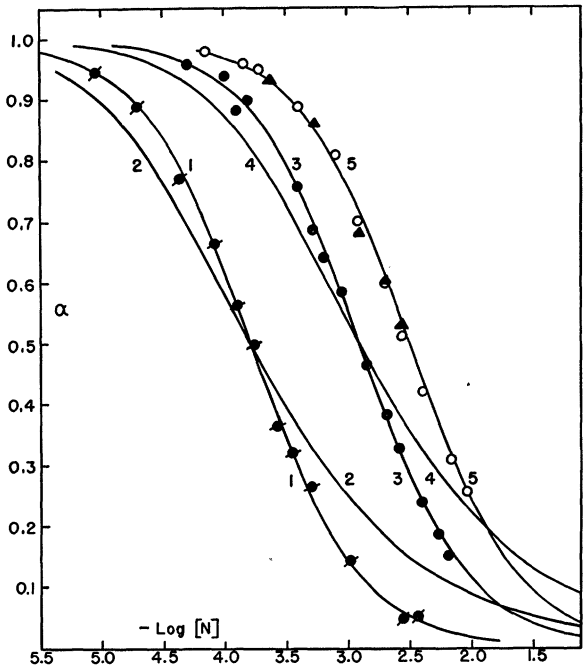

FIG. 2. Interaction of antimalarials with sodium ribonucleate at pH 5.85. -●- SN-12868: $\Gamma/2 = 0.1$; ○ SN-12868: $\Gamma/2 = 1.05$ with sodium chloride; ▲ SN-12868: $\Gamma/2 = 1.06$ with magnesium sulfate; ● SN-7618: $\Gamma/2 = 0.1$. Theoretical curves drawn according to equation (7): curve 1, log $k' = 3.79$; curve 3, log $k' = 2.91$; curve 5, log $k' = 2.50$. Theoretical curves drawn according to equation (8): curve 2, log $k' = 8.19$, $S = 4 \times 10^{-5}$ M; curve 4, log $k' = 7.21$, $S = 5 \times 10^{-5}$ M.

In Fig. 2, data for the interaction of SN-12868 and SN-7618 with yeast ribonucleate are compared with theoretical curves drawn according to equations (7) and (8); the values of the constants are given in the legend of this figure. The values for these constants are tentative pending further work with nucleic acid preparations of proven homogeneity and known molecular weight. However, the present data permit comparison between the relative strengths of interaction of the quinoline and acridine derivatives. In both cases the interactions appear to correspond to the combination of monomeric rather than dimeric ligand with the nucleic acid. The constant for the binding of SN-12868 with the nucleic acid is approximately 7.6 times that of SN-7618 at the same pH and ionic strength.

Increase in ionic strength from 0.1 to 1.05, either by addition of sodium chloride or magnesium sulfate, pro-

duced a 19.5-fold decrease in the binding of SN-12868 by the ribonucleate anion. This suggests that the interactions of cationic acridines and quinolines with polyvalent nucleate anions involve, at least in part, the phenomenon treated theoretically by Danielli (*3*). However, the

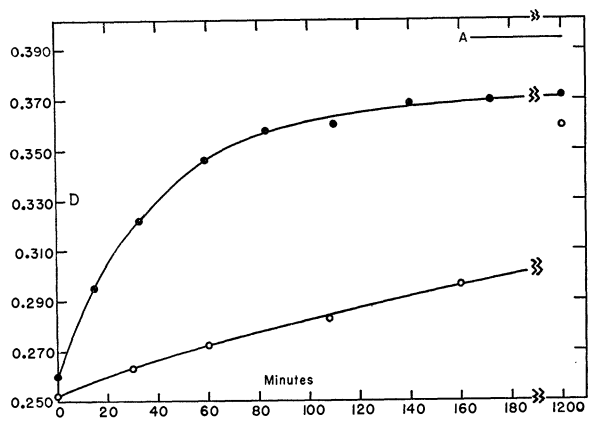

FIG. 3. Change in binding of SN-12868 by ribonucleate during incubation of the latter in 0.5 N NaOH at 24° C (●), and in 0.05 N NaOH at 37° C (○).

difference in binding of SN-12868 and SN-7618 suggests that forces such as hydrogen bonding and van der Waals' forces, are involved in addition to coulombic forces. The interactions reported here should be compared with those between nucleic acids and streptomycin which have been described by Cohen (*2*), and von Euler and Heller (*4*).

That the interaction of these antimalarials with nucleic acids is dependent upon the molecular size of the nucleic acids is suggested by experiments summarized in Fig. 3. Ribonucleic acid was incubated in aqueous solutions of sodium hydroxide. At intervals, aliquots of the solution were transferred to phosphate buffer, HCl equivalent to the alkali in the aliquot was added, SN-12868 was introduced, and the mixed solution was diluted with phosphate buffer in such manner that in every case the solution was 4×10^{-5} M with respect to SN-12868 and contained the equivalent of 0.046 g of ribonucleic acid per 100 ml. Final pH was 5.9 in each case. Optical densities were determined at wavelength 425 mμ. The progressive increase in optical density (Fig. 3) corresponds to progressively diminished binding of SN-12868. The rate of change in 0.5 N NaOH at 24° C was much greater than the rate in 0.05 N NaOH at 37° C, but after 20 hr the optical densities of these two solutions appeared to be approaching an equivalent limiting value, which was less than the optical density (0.394) that would have been observed in the complete absence of binding of SN-12868. The bridging phosphate ester linkages of nucleic acids undergo hydrolytic cleavage in alkaline solution, and these experiments suggest that the binding of acridine derivatives can be used as an indirect measure of such cleavage. Application of these observations in the study of the action of various nucleases is suggested.

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A Rationale for Plasma Therapy in Poliomyelitis

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During the 1948 epidemic of poliomyelitis in Los Angeles, a research program was suggested by one of us (R.M.E.), and initiated by both, in which it was observed that the serum albumin level was frequently lower than normal, and that there seemed to be a correlation between the degree of reduction and the severity of the disease. Because of the importance of serum albumin in maintaining the normal osmotic pressure of blood and of the osmotic pressure in regulating the equilibrium of tissue fluids, and because of the possibility that edema of the cord may contribute to the pathogenesis of the paralysis in poliomyelitis, the suggestion was made that a detailed study be conducted to determine: 1) the alterations in serum proteins which occur during the course of the disease, and 2) the effect on both the serum proteins and the neurologic residual of paralysis as a result of intensive administration of plasma. The results obtained were highly suggestive of a beneficial effect of plasma and will be reported in the near future, with the Los Angeles County Hospital group. It was thought desirable to describe briefly the theory that prompted the suggestion, in the hope that other workers will be stimulated to explore these therapeutic possibilities.