References

1. BELLAMY, D. and KLIMEK, J. W. *J. Bact.*, 1948, **55**, 153.
2. GALE, E. F. *Bull. Johns Hopk. Hosp.*, 1948, **83**, 119.
3. GALE, E. F. and RODWELL, A. W. *J. gen. Microbiol.*, 1949, **3**, 127.
4. MOORE, S. and STEIN, W. H. *J. biol. Chem.*, 1948, **176**, 367.
5. PORTER, J. R. *Bacterial chemistry and physiology.* New York: Wiley, 1946.
6. RANDALL, W. A., PRICE, C. W., and WELCH, H. *Science*, 1945, **101**, 365.
7. SCHWARTZMAN, G. *J. exp. Med.*, 1946, **83**, 65.

A Source of Error in Tracer Experiments with P^{32} [1]

B. A. Rubin

Biology Department, Brookhaven National Laboratory, Upton, Long Island

In dealing with radioactive solutions of high specific activity where total phosphorus is small, the amount of P^{32} adsorbed on laboratory glassware may become significant.

An experiment done in this laboratory to measure this adsorption and to determine the efficacy of silicone-coated glassware illustrates the magnitude of the problem.

A solution of P^{32} containing about 1×10^{-4} mg /ml of total phosphorus was drawn up once into each of four 2-ml pipettes, all new and identical except that two had been coated with silicone. Each of the pipettes was then washed by drawing up 2-ml amounts of distilled water. The wash water from each pipette was collected into two counting sample cups (1 ml each), evaporated, and the number of radioactive disintegrations determined in a Nucleometer.

Average values, in cpm/ml were:

	Coated	Uncoated
Wash 1	390	6,000
Wash 2	110	300
Wash 3	background	110

The count in the first wash of the uncoated pipette is high and variable, depending on both the P^{32} and P^{31} contents, and can cause serious error where it is not suspected.

To avoid this difficulty, we have adopted several techniques. Wherever possible all counting solutions contain a uniform 1 mg/ml of carrier phosphorus at pH 4.5. Where less carrier must be used, care is taken to pipette as rarely as possible and only in solutions containing as much carrier as the procedure permits. When conditions are extremely unfavorable, requiring exceedingly low carrier or high pH values, silicone coating of glassware is necessary.

The coating technique we used was modified from Johannson and Torek (*1*). A 2% solution of "DC 200" was prepared in pure grade CCl_4 (the Dow Company recommends chlorinated organic solvents). The glassware, preferably new, is scrupulously cleaned and heat-dried, completely immersed in the silicone solution, and carefully drained. It is then placed into an oven at 90° C for 2 hr, to evaporate the solvent. The temperature is then raised to 250° C for a period exceeding 4 hr but not longer than overnight. Detailed experiments on the lasting qualities of this type of coating have been done by the Dow Company. This treatment caused no detectable change in calibration.

There is another group of compounds which have been similarly used, called "silanes" (hydrochlorides of unpolymerized silicones). These may be vaporized onto a glass surface, where they react with the film of moisture to form a silicone layer. This process of hydrolysis and polymerization is somewhat difficult to control.

[1] Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

Reference

1. JOHANNSON, O. K. and TOREK, J. J. *Proc. I.R.E.*, 1946. **34**, 296.

Free Amino Acids and Peptides in Frog Embryos

Chao-t'e Li and Eugene Roberts

Department of Anatomy, Washington University School of Medicine, St. Louis

Relatively large quantities of free amino acids have been detected by two-dimensional paper chromatography in tissues of the mouse (*3, 4*), the human, and the frog (*Rana pipiens*). Each of the normal adult tissues examined appeared to have a distribution of free amino acids characteristic for that tissue. Therefore, it was apparent that the ability of these tissues to maintain the characteristic distributions of these constituents must have arisen at some time during embryonic or postembryonic development.

As the first step in the study of the free amino acids of frog tissues at various stages of development, examinations were made of alcohol extracts of material ranging from ovarian eggs to larvae, corresponding to stages 0, 1, 2, 8, 10, 12, 17, and 21 according to Shumway (*5*). In the last stage examined, there was still no intake of food from the environment. Exactly 260 mg of fresh weight of material was placed in ethyl alcohol, the final concentration of alcohol being 70–73%. The material was homogenized in a ground glass homogenizer, centrifuged, and the supernatant fluid decanted and evaporated to dryness. The residue was thoroughly extracted with 3 ml of distilled water. This was then dialyzed against 10 ml of distilled water in a rocking dialyzer (*2*). One half of the dialyzate was evaporated to dryness and placed on paper for chromatography (*1*), and the other half was chromatographed after hydrolysis with 6 N HCl.

In none of the stages studied were there detectable quantities of free amino acids in the extracts. Several ninhydrin-reactive materials were located at spots which disappeared on acid hydrolysis, and which did not correspond in position to any known amino acids. These were

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.

References

1. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1944, **38**, 224.
2. HAMILTON, P. B. and ARCHIBALD, R. M. *Ind. Eng. chem. anal. Ed.*, 1944, **16**, 136.
3. ROBERTS, E. and FRANKEL, S. *Cancer Res.*, in press.
4. ROBERTS, E. and TISHKOFF, G. H. *Science*, 1949, **109**, 14.
5. SHUMWAY, W. *Anat. Rec.*, 1940, **78**, 139.

The Interaction of Antimalarials with Nucleic Acids[1]

I. Acridines

J. Logan Irvin and Elinor Moore Irvin

II. Quinolines

Frank S. Parker

Department of Physiological Chemistry,
The Johns Hopkins University
School of Medicine, Baltimore

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%.

[1] Supported in part by a grant from the Penrose Fund of the American Philosophical Society.

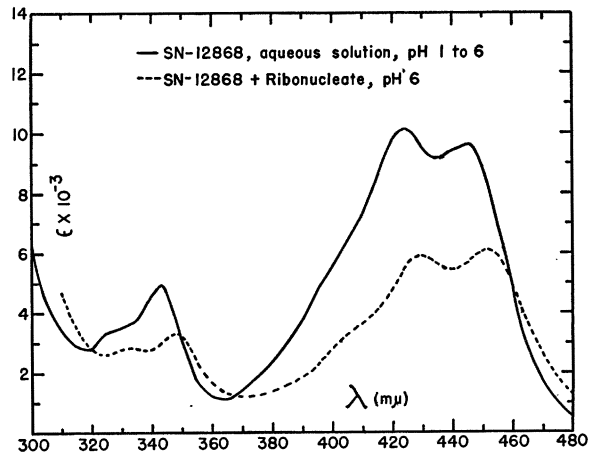

FIG. 1. Absorption spectra for SN-12868.

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.