10. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150,** 279 (1957).
11. W. Z. HASSIL and S. ABRAHAM, *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan). Vol. III, pp. 35–36. Academic Press, New York (1957).
12. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
13. C. R. SHORT and L. E. DAVIS, *J. Pharmac. exp. Ther.* **174,** 185 (1970).

Biochemical Pharmacology, Vol. 20, pp. 3221–3223. Pergamon Press, 1971. Printed in Great Britain

Effect of antimalarial drugs on the efflux of K^+ from *Streptococcus pyogenes*

(*Received* 19 *February* 1971; *accepted* 3 *April* 1971)

ANTIMALARIAL drugs such as primaquine, quinacrine, quinine and chloroquine possess antibacterial properties *in vitro*.[1–5] Hahn *et al.*[6,7] have demonstrated that the antibacterial action of chloroquine against *Bacillus megaterium* is due to the formation of drug–DNA complexes. In other studies, primaquine, quinacrine and quinine were found to alter the permeability of the red cell membrane for K^+.[8] The present study was designed to investigate the effect of the above antimalarial compounds on the efflux of ^{42}K from bacteria.

In preliminary experiments, the antibacterial action of primaquine diphosphate, quinacrine hydrochloride, quinine hydrochloride and chloroquine diphosphate against some common bacteria isolated from hospitalized patients was assessed. One ml of a 1 to 1000 dilution of a 24-hr beef heart broth culture was added to decreasing concentrations of drug and then incubated at 37° for 48 hr. The test tubes were then examined visually for signs of bacterial growth. Primaquine and quinacrine inhibited the growth of two strains of *Streptococcus pyogenes* (group A) at concentrations of 0·1 mM; quinine did the same at concentrations of 0·1 and 0·25 mM, respectively, whereas chloroquine was inactive at 1 mM, the highest concentration tested. The four drugs demonstrated a similar antibacterial effect on two strains of *Diplococcus pneumoniae* and all were inactive against a number of gram-negative enteric organisms at concentrations of 1 mM.

S. pyogenes (group A) was employed in the studies of ^{42}K efflux, since they best withstood the manipulations associated with ^{42}K loading. One ml of a 24-hr culture of *S. pyogenes* was added to 150 ml of beef broth containing 1 mc ^{42}K and the contents were then incubated at 37° for 6–7 hr. The ^{42}K-laden bacteria were washed three times and then resuspended in broth. Aliquots (25 ml) of the well mixed bacterial suspension containing approximately $1·3–1·8 \times 10^7$ organisms were added to glass-stoppered flasks. Four-ml aliquots from each flask were quickly filtered through a 5-ml syringe attached to a Swinnex filter unit containing a pre-filter and 25 mm millipore filter with pores measuring 0·22μ. The bacteria trapped in the pre-filter and filter were washed with 4 ml broth. A 4-ml aliquot of a pH 7·3 phosphate buffer solution of drug was added to each of the flasks, with buffer to the controls. The flasks were agitated at 23° with 4-ml aliquots removed at 5, 10, 15 and 20 min and treated as described above. The bacteria were incubated at 23° rather than 37°, since the lower temperature slowed the efflux of ^{42}K and thus permitted more accurate measurement of this parameter. Bacterial growth during incubation was assessed by the measurement of the optical density of zero-time and 20-min samples using a Klett colorimeter with a red filter. The millipore filter and pre-filter containing the ^{42}K-laden bacteria were placed into plastic test tubes and counted in a plastic well counter.[9] The counting error was less than 2 per cent.

The effect of antimalarial drugs on the efflux of ^{42}K from a strain of *S. pyogenes* (group A) is shown in Fig. 1. With the data plotted on semilogarithmic paper, the efflux of ^{42}K from control cells approximated a first-order process with a half-time of 16 min. In four other similar experiments, the half-time ranged from 16 to 19 min. Primaquine, quinacrine and quinine produced an approximately equivalent increase in ^{42}K efflux over that observed in control cells. In contrast to the control, the efflux of ^{42}K from these cells was not a linear process, but consisted of a rapid initial component with a half-time of about 6 min and a slower one commencing at 5 min with a half-time of about 11 min. The reason for the decrease in drug effect on ^{42}K efflux continued incubation with is unclear. Chloroquine, unlike the above three drugs, did not affect the efflux of ^{42}K. In four other experiments with

other strains of *S. pyogenes*, the action of the four antimalarial drugs on ^{42}K efflux was similar to that shown in Fig. 1.

The efflux of K^+ from *S. pyogenes* is essentially determined by the permeability characteristics of the plasma membrane. Thus, the above data indicate that primaquine, quinacrine and quinine altered the permeability properties of this cellular component. It is of interest that chloroquine, in contrast to the other three compounds, did not influence the movement of bacterial K^+ in the present study and, in a previous study, did not produce a prelytic loss of K^+ from erythrocytes.[8] The explanation for the similar effect of antimalarial drugs on K^+ permeability in cells as unrelated as *S. pyogenes* and the human erythrocyte is unclear. The findings of Steinbuch and Quentin[10] may be germane to this question. They observed that many antimalarial drugs, including those of the present study, were capable of forming molecular complexes with cephalins. Since cephalins are constituents of the plasma membrane of both bacteria and red cells, a common mechanism for the drug-induced alteration in K^+ efflux is suggested. The fact that chloroquine was found to be less reactive with cephalin than either primaquine, quinacrine or quinine may also explain the inability of this compound to influence the efflux of K^+ from *S. pyogenes* and erythrocytes.

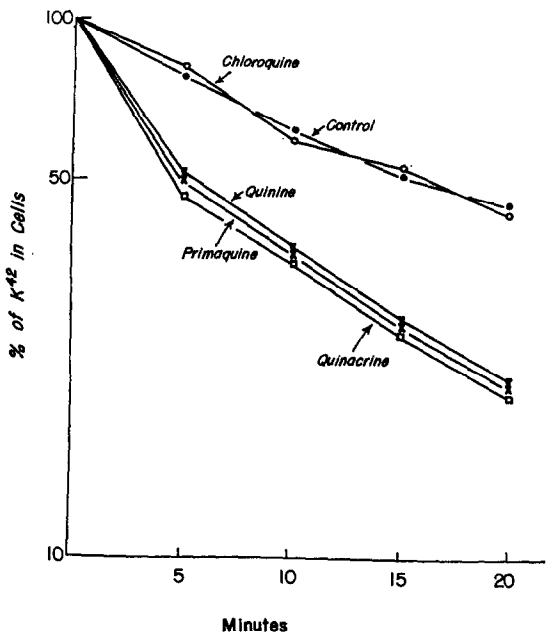

FIG. 1. Effect of antimalarial drugs on the efflux of ^{42}K from *Streptococcus pyogenes* (group A). Organisms were loaded with ^{42}K by cultivating them for 6 hr in beef heart broth containing ^{42}K. The cells were washed three times before the efflux measurements were performed. The concentration of each drug was 0·5 mM.

The optical density, i.e. growth, of the control cells shown in Fig. 1 increased by 19 per cent during incubation. A similar increase in optical density was observed in bacteria exposed to the four antimalarial drugs. In the four other experiments performed, the cells incubated with each of the antimalarial drugs demonstrated similar increments in optical density to those of their respective controls. These findings suggest that the alteration in membrane permeability produced by primaquine, quinacrine and quinine may not be responsible for their inhibitory action on the growth of *S. pyogenes*. Alternately, the changes in membrane permeability may have delayed effects on bacterial growth. This latter possibility is currently under investigation.

Acknowledgements—We would like to thank the Sterling-Winthrop Research Institute for kindly supplying primaquine diphosphate.

Nuclear Medicine and Laboratory Services,
Boston Veterans Administration Hospital and
Boston University School of Medicine,
Boston, Mass., U.S.A.

AARON MILLER
GEORGE NATSIOS
HINTON C. SMITH

REFERENCES

1. M. SILVERMAN and E. A. EVANS, JR., *J. biol. Chem.* **150**, 265 (1943).
2. F. H. JOHNSON and I. LEWIN, *Science, N.Y.* **101**, 281 (1945).
3. F. KRADOLFER and L. NEIPP, *Antibiotics Chemother.* **8**, 297 (1958).
4. L. B. ROBINSON, T. M. BROWN and R. H. WICHELHAUSEN, *Antibiotics Chemother.* **9**, 111 (1959).
5. S. BOLEK, M. SMÉKAL, M. VYKYDAL and Z. ŽIŽKA, *Bratisl. lék. Listy* **45**, 499 (1965).
6. J. CIAK and F. E. HAHN, *Science, N.Y.* **151**, 347 (1966).
7. R. L. O'BRIEN, J. G. OLENICK and F. E. HAHN, *Proc. natn. Acad. Sci. U.S.A.* **55**, 1511 (1966).
8. A. MILLER and H. C. SMITH, *Br. J. Haemat.* **19**, 417 (1970).
9. G. J. HINE, B. A. BURROWS, L. APT, M. POLLYCOVE, J. F. ROSS and L. A. SARKES, *Nucleonics* **13**, 23 (1955).
10. M. STEINBUCH and M. QUENTIN, *Archs int. Pharmacodyn. Thér.* **110**, 10 (1957).

Biochemical Pharmacology, Vol. 20, pp. 3223–3225. Pergamon Press, 1971. Printed in Great Britain

Fluorometric determination of *N*-terminal tryptophan-peptides after formaldehyde condensation

(*Received* 19 *March* 1971; *accepted* 13 *May* 1971)

TRYPTAMINE can be determined fluorometrically after condensation with formaldehyde followed by oxidation with hydrogen peroxide.[1] The method is non-specific in that also *N*-methyltryptamine and tryptophan give formaldehyde-induced fluorophores with spectral properties indistinguishable from those of the tryptamine fluorophore. Tryptamine, *N*-methyltryptamine and tryptophan are known to react with formaldehyde also on silica gel thin layer.[2–5] It was recently reported that formaldehyde is a sensitive chromatographic detection reagent also for tryptophanyl-dipeptides.[6] From this observation it appeared probable that *N*-terminal-tryptophan-peptides could be determined fluorometrically by a method similar to that described for tryptamine. This assumption was confirmed in the present study.

Experimental

Aqueous solutions of tryptamine, tryptophan and various tryptophan-containing dipeptides (see Table 1) were prepared: concentrations of 1–3 μg (free base) per ml. Aliquots of these solutions (usually 0·5 ml) were mixed with 0·1 ml formaldehyde solution (18%) in a total volume of 3 ml, made up with 0·1 N sulphuric acid. After heating the samples in boiling water for 20 min, 0·1 ml 5% hydrogen peroxide was added and heating was continued for another 20 min. The samples were cooled in a refrigerator and then analyzed in an Aminco–Bowman spectrophotofluorometer, equipped with an *x–y* recorder.

Results and comments

All the peptides with an *N*-terminal tryptophan residue gave strong fluorescence after combined treatment with formaldehyde and hydrogen peroxide; no fluorescence resulted if the hydrogen peroxide was omitted. The other tryptophan-containing dipeptides (having the tryptophan amino group engaged in peptide linkage) did not give any fluorescence with the formaldehyde-hydrogen peroxide treatment (Table 1). The spectral properties of the fluorophores of the tryptophanyl-dipeptides were without exception very similar to those of the tryptamine and tryptophan fluorophores (Fig. 1 and Table 1). For tryptophanyl-glycine the fluorescence intensity was proportional to the concentration in the range 0·1 to 3 μg/ml (Fig. 2).

The reaction of tryptamine (and tryptophan) with formaldehyde is believed to result in the formation of 1,2,3,4-tetrahydro-norharman which is subsequently dehydrogenated (by the addition of hydrogen peroxide) to 3,4-dihydro-norharman and/or norharman.[1] It appears likely that the same