of cardiac glycosides in cats or rats exposed to 19·2 ata of helium.* However, other pharmacological aspects of drug action may be influenced by a hyperbaric environment. Indeed, hyperbaric helium (69 at.a.) enhanced the resistance of several strains of *S. aureus* to penicillin.[16] In addition, Small *et al.** found that the chronotropic response to ouabain and digoxin was slightly altered in guinea pigs exposed to 19·2 at.a. of helium. Moreover, prolonged exposure of men to a hyperbaric helium environment may result in enzyme induction or a decrease in the amount of enzyme. Thus, drug metabolism might be altered under such conditions even though acute exposure, as shown in the present *in vitro* experiments, does not alter activity or kinetics of selected enzyme systems. Therefore, additional research is necessary to ascertain that hyperbaric conditions do not affect the actions of drugs that might be used in an underwater habitat.

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REFERENCES

1. J. BEVAN and P. SHARPHOUSE, World Record in Simulated Deep Dive. *Rev. Physiol. Subaquat. Med. Hyperbare*, Suppl. 1, No. 4 (1969).
2. J. MACINNIS, J. G. DICKSON and C. J. LAMBERTSEN, *J. appl. Physiol.* **22,** 694 (1967).
3. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
4. G. K. HANASONO, J. I. HOLLIS and S. L. SCHWARTZ, *Analyt. Biochem.* **34,** 470 (1970).
5. I. NASH, *Biochem. J.* **55,** 416 (1953).
6. H. YOSHIMURA, K. OGURI and H. TSUKAMOTO, *Biochem. Pharmac.* **18,** 279 (1969).
7. D. L. HORWITZ and L. D. HOMER, Project No. MR005.20-0287, Report No. 25, Naval Medical Research Institute, Bethesda, Maryland (1970).
8. D. S. DAVIES, P. L. GIGON and J. R. GILLETTE, *Biochem. Pharmac.* **17,** 1865 (1968).
9. A. L. MISRA, S. J. MULE and L. A. WOODS, *J. Pharmac. exp. Ther.* **132,** 317 (1960).
10. J. R. FOUTS and T. E. GRAM, in *Microsomes and Drug Oxidations* (Eds. J. R. GILLETTE, A. H. CONNEY, G. J. COSMIDES, R. W. ESTABROOK, J. R. FOUTS and G. J. MANNERING), p. 81, Academic Press, New York (1969).
11. S. F. COOK, *J. cell. comp. Physiol.* **36,** 115 (1950).
12. R. E. SOUTH, JR. and S. F. COOK, *J. gen. Physiol.* **36,** 513 (1953).
13. H. R. SCHREINER, Technical Report NR 102-597, Office of Naval Research, Contract Nonr. 4115(00), (July 31, 1966).
14. R. G. BUCHHEIT, H. R. SCHREINER and G. F. DOEBBLER, *J. Bact.* **91,** 622 (1966).
15. A. SMALL, *Toxic. appl. Pharmac.* **17,** 250 (1970).
16. N. A. SCHLAMM, A. L. COYKENDALL and D. E. MEYERS, *J. Bact.* **98,** 327 (1969).

* A SMALL, H. W. MCELROY and R. S. IDE, in preparation.

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Phospholipid metabolism, osmotic stability and reducing potential of human red cells exposed to pentaquine and hydroxy derivatives*

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ACCORDING to the Schönhöfer hypothesis on the therapeutic mechanism of action of primaquine, hydroquinone and quinone derivatives are produced in the conversion of such 6-methoxy-8-aminoquinolines to their active forms.[1,2] In the red cell, these derivatives, presumably functioning

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as redox compounds, have been considered responsible for excessive formation of methemoglobin, depletion of reduced glutathione, and the eventual destruction of those cells which are unable to defend adequately their reducing potential.[3] While the liver is the likely site of quinone formation, George *et al.*[4] suggested that the red cell may be capable of catalyzing this reaction. They demonstrated that the osmotic fragility of normal red cells exposed to primaquine is markedly enhanced by the presence of glucose and postulated that glucose serves to generate the NADPH required for conversion of primaquine to the quinones which act to alter the osmotic fragility of the cells. The investigations of Cohen and Hochstein[5,6] likewise suggest that quinone formation by supporting H_2O_2 generation can be responsible for osmotic instability of red cells coincident with a depletion of reducing potential.

Recently a mechanism for primaquine hemolysis has been proposed which invokes the participation of the red cell membrane phospholipids.[7] On the basis of observations which indicated (1) that primaquine accelerates the conversion of lysolecithin to lecithin in the intact red cell (2) that reduction of red cell lysolecithin content is accompanied by a marked loss in red cell osmotic resistance in the presence of primaquine, and (3) that the osmotic stability of such labilized red cells can be restored by repletion of the lysolecithin, it was postulated that primaquine-induced hemolysis is mediated by an effective reduction in the steady state concentration of membrane lysolecithin.

In view of the prominent role of quinone derivatives in the concepts of primaquine hemolysis related to the Schönhöfer hypothesis, a group of 8-aminoquinoline homologues, including hydroquinone derivatives, were assessed in their capacity to modify the lecithin metabolism of the red cell as compared with their effects on the osmotic stability and reduced glutathione content of the cell. Because of their availability, pentaquine rather than primaquine homologues were used.

Red cell suspensions from clinically normal adults were prepared and rates of long chain fatty acid incorporation into red cell phospholipid were determined by procedures described previously.[7] Erythrocyte osmotic fragility was measured by the procedure of Parpart,[8] and glutathione concentration was determined by the method of Beutler.[9] The 8-aminoquinolines were obtained from the Walter Reed Army Institute of Research through the courtesy of Drs. David P. Jacobus and Robert O. Pick. The compounds were primaquine phosphate, pentaquine, 8(5-isopropylaminoamylamino) quinoline, 5,6-dimethoxy-8(5-isopropylaminoamylamino) quinoline, 6-hydroxy-8(5-isopropylaminoamylamino) quinoline, and 5,6-dihydroxy-8(5-isopropylaminoamylamino) quinoline. Immediately before being used, the compounds were dissolved in Krebs–Ringer buffer and the pH of the solution was brought to 7·4 with NaOH.

TABLE 1. EFFECT OF CH_3O- AND $-OH$ SUBSTITUENTS OF 8-AMINOQUINOLINE ON PHOSPHOLIPID METABOLISM OF INTACT RED CELLS*

No.	R_1	R_2	R_3	[^{14}C]Lecithin nmoles	[^{14}C]Lecithin %Δ
	Control			0·29	
I	H	CH_3O	$CH-(CH_2)_3-NH_2$ CH_3	0·58	+100
II	H	CH_3O	$(CH_2)_5-HN-CH-(CH_3)_2$	0·67	+130
III	H	H	$(CH_2)_5-NH-CH-(CH_3)_2$	0·51	+ 76
IV	CH_3O	CH_3O	$(CH_2)_5-NH-CH-(CH_3)_2$	0·24	− 17
V	H	OH	$(CH_2)_5-NH-CH-(CH_3)_2$	0·36	+ 25
VI	OH	OH	$(CH_2)_5-NH-CH-(CH_2)_2$	0·24	− 17

* Each reaction tube contained 75 nmoles of [1-^{14}C]-oleic acid, 1·35 ml of a suspension of red cells in calcium-free Krebs–Ringer phosphate buffer (pH 7·4), and 2 μmoles of the 8-aminoquinoline as indicated. Final volume was 2·0 ml and the hematocrit was 50%. Incubation was carried out in an agitating water bath at 37° for 90 min. The values given for lecithin formation are the rates of ^{14}C-fatty acid incorporation in 90 min. The data are representative of three similar experiments.

As shown in Table 1, the capacity of the 8-aminoquinoline compounds to stimulate lecithin synthesis was dependent on the substituents at the 5 and 6 positions of the quinoline moiety. Compared with primaquine (I) or pentaquine (II), the demethoxylated derivative (III) retained significant stimulatory capacity, whereas the 5-methoxylated derivative (IV) was completely inactive in this respect. Substitution of a hydroxyl (V) for the methoxyl group at the 6 position reduced the stimulatory capacity to approximately one-fourth of that shown by pentaquine. The dihydroxyl homologue (VI), like the dimethoxyl compound (IV), was totally inactive. Thus, while the hypothetical intermediates in the conversion of pentaquine to its presumed therapeutically active form exhibited minimal or no stimulatory capacity in the conversion of lysolecithin to lecithin, the parent compound, which is used clinically, was most effective. The ability of the demethoxylated pentaquine to stimulate lecithin synthesis suggests that the quinoline nucleus or 8-alkyl amino group contributes to this property of pentaquine and primaquine, whereas the diminished stimulatory capacity of the 5-methoxylated and of the hydroxylated derivatives suggests that additional substituents on the quinoline nucleus have an inactivating effect.

The capacity of primaquine and pentaquine to increase the osmotic fragility of red cells has been well documented.[4, 10] As shown in Fig. 1, the demethoxylated derivative (III) was almost as effective as primaquine and pentaquine in this property. By contrast, the methoxylated pentaquine (IV) and both hydroxylated derivatives (V, VI) failed to alter the osmotic resistance of the red cells. Thus, only those compounds which had the capacity to stimulate markedly the conversion of lysolecithin to lecithin were capable of increasing the osmotic fragility of the cell, and only those compounds which altered the osmotic stability of the cell had this effect on phospholipid metabolism.

As was anticipated from the results obtained with quinonoids by other investigators,[2, 11] the hydroxylated derivatives (V, VI) effected a marked decrease in the reduced glutathione level of the red cells (Fig. 2). This was especially pronounced in the case of the dihydroxylated compound, even at the lowest concentration used. Unexpectedly, 5-methoxy pentaquine (IV) also induced a loss of reduced glutathione which was approximately equivalent to that effected by the monohydroxylated

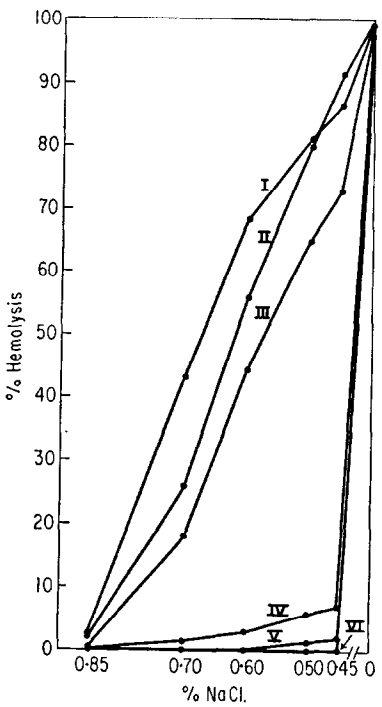

FIG. 1. Osmotic resistance of red cells exposed to 8-aminoquinolines. Suspensions of red cells at a final hematocrit of 37·5% were incubated with agitation in the presence or absence of an 8-aminoquinoline at 37° for 2 hr. Final concentration of the compounds was 10^{-3} M. At the end of the incubation, the osmotic resistance of the cells in NaCl solutions was determined. The Roman numerals refer to the compounds listed in Table 1. The control was identical to VI.

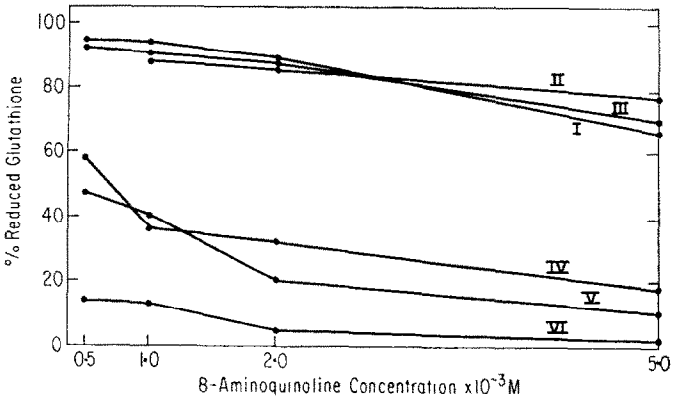

FIG. 2. Effect of 8-aminoquinolines on the level of reduced glutathione in the intact red cell. Suspensions of red cells were incubated as given in Fig. 1. Final concentrations of the 8-aminoquinolines used are indicated on the abscissa. The level of reduced glutathione after 2 hr of exposure to the compounds is expressed as a percentage relative to control incubations containing no 8-aminoquinoline.

compound. Thus, those compounds which induced the greatest loss in reduced glutathione had little or no effect on phospholipid metabolism or osmotic stability of the red cells, whereas those compounds which had the greatest effect on the two membrane-related functions exhibited little or no effect on the oxidant status of the cell.

van Deenen *et al.*[12] analyzed the lipids of red cells from several species and recognized a remarkable correlation between phospholipid compositions of the cells and their permeability behavior for several low molecular weight, water-soluble compounds. The observations reported herein provide further evidence for a remarkable parallelism between phospholipid metabolism and osmotic behavior of the red cell. Additionally, the data indicate that the reduced glutathione content of the red cell may be drastically reduced without jeopardizing the osmotic stability of the cell. Previous studies had also indicated that the stimulatory effect of primaquine on lecithin formation is likewise independent of the reducing potential of the red cell.[13] Finally, analysis of the structure–activity relationship in the series of pentaquine homologues studied indicates that the greatest alteration in phospholipid metabolism and osmotic stability is effected by pentaquine itself and that the hydroxylated derivatives have little or no effect on these aspects of red cell behavior.

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REFERENCES

1. F. SCHÖNHÖFER, *Hoppe-Seyler's Z. physiol. Chem.* **294**, 1 (1942).
2. A. S. ALVING, R. D. POWELL, G. J. BREWER and J. D. ARNOLD, in *Drugs, Parasites, and Hosts* (Eds L. G. GOODWIN and R. H. NIMMO-SMITH), p. 83. Churchill, London (1962).
3. E. BEUTLER, in *The Metabolic Basis of Inherited Disease* (Eds J. B. STANBURY, J. B. WYNGAARDEN and D. S. FREDRICKSON), p. 1060. McGraw-Hill, New York (1966).
4. J. N. GEORGE, R. L. O'BRIEN, S. POLLACK and W. CROSBY, *J. clin. Invest.* **45**, 1280 (1966).
5. G. COHEN and P. HOCHSTEIN, *Biochemistry, N.Y.* **2**, 1420 (1963).
6. G. COHEN and P. HOCHSTEIN, *Biochemistry, N.Y.* **3**, 895 (1964).
7. B. WITTELS, *Biochim. biophys. Acta* **210**, 74 (1970).
8. J. V. DACIE, *The Hemolytic Anaemias*, p. 476. Grune & Stratton, New York (1954).
9. E. BEUTLER, *J. Lab. clin. Med.* **49**, 84 (1957).
10. R. WEED, J. EBER and A. ROTHSTEIN, *J. clin. Invest.* **40**, 130 (1961).
11. I. M. FRASER and E. S. VESELL, *Ann. N.Y. Acad. Sci.* **151**, 777 (1968).
12. L. L. M. VAN DEENEN and J. DE GIER, in *The Red Blood Cell* (Eds C. BISHOP and D. M. SURGENOR), p. 243. Academic Press, New York (1964).
13. B. WITTELS and P. HOCHSTEIN, *Biochim. biophys. Acta* **125**, 594 (1966).