EFFECTS OF PRIMAQUINE ON THE RED BLOOD CELL MEMBRANE. II. K^+ PERMEABILITY IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT ERYTHROCYTES *

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An unexplained anomaly of "primaquine-sensitive" erythrocytes is their ability to resist hemolysis *in vitro,* even when exposed to concentrations of primaquine that are greatly in excess of pharmacologic levels. Beutler, Dern and Alving (1) have studied *in vitro* hemolysis of primaquine-sensitive red cells in detail and demonstrated that it occurs between 1.5 and 4×10^{-3} M primaquine (0.6 to 2.0 mg per ml) when measured in a test system containing saline buffered at pH 7.0. They were unable to demonstrate any difference between normal and abnormal cells with respect to the drug concentration required to produce *in vitro* hemolysis. *In vitro* studies on normal cells, reported in an accompanying paper (2) generally demonstrated no hemolysis with concentrations of less than 5×10^{-3} M primaquine; these observations were made on whole defibrinated blood, and when compared with Beutler's studies of red cells suspended in buffered saline, it appears that serum proteins inhibited hemolysis of red cells exposed to concentrations of primaquine less than 5×10^{-3} M.

In comparing *in vitro* observations on hemolysis produced by primaquine, with the *in vivo* effects of the drug, Beutler and colleagues have (1) suggested that the drug, or one of its metabolic by-products, damages the sensitive erythrocytes, thereby making them susceptible to removal by *in vitro* mechanisms but not to *in vitro* hemolysis. Brodie and Udenfriend (3), who studied the hemolytic effect of primaquine, suggested that a metabolic product of that drug was an agent capable of damaging red cells which might even produce *in vitro* hemolysis.

Previous studies (2) have demonstrated that prelytic increased cation permeability induced in normal erythrocytes by primaquine is a more sensitive indicator of *in vitro* damage to the cells than is hemolysis. This increased permeability, manifested by loss of K^+ from normal erythrocytes is produced by concentrations of primaquine or acetylphenylhydrazine which are 3×10^{-4} M or greater. These *in vitro* concentrations of primaquine appear to have serious deleterious effect on the red cell membrane. Desforges, Kalaw and Gilchrist (4) have demonstrated noncompetitive inhibition of glucose-6-phosphate dehydrogenase by 1×10^{-3} M primaquine. Mohler and Williams (5) have demonstrated ATP instability in blood incubated with 3.4×10^{-3} M phenylhydrazine, and Jandl and Allen (6) have demonstrated oxidative denaturation of hemoglobin in intact red cells following production of methemoglobin *in vitro* by phenylhydrazine. The concentrations of these agents necessary to produce *in vitro* glucose-6-phosphate dehydrogenase inhibition, ATP breakdown or methemoglobin are, however, far in excess of those which will induce loss of K^+ from intact cells (2).

Loss of K^+ in itself is, of course, merely an indication of cell membrane damage but its occurrence serves to emphasize that a variety of deleterious effects is produced *in vitro* by concentrations of drug of 1×10^{-3} M or greater. Since loss of K^+ occurs at lower concentrations of drug than those needed to produce other demonstrated *in vitro* changes, it appeared to be worthwhile to carry out similar studies of the *in vitro* cation permeability of red blood cells from patients with primaquine-sensitive cells. These studies were

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undertaken to investigate the possibility that the loss of K^+ might be a more sensitive indication of an *in vitro* difference between these cells and normal cells.

METHODS

Blood samples were obtained from 7 individuals who were found to have deficient activity of glucose-6-phosphate dehydrogenase in their red cells. Five of these were healthy Negro males with normal hematocrits and no evident disease at the time of the experiments. Blood from one Caucasian female of Sicilian extraction whose glutathione stability test placed her in the "intermediate" group (5) was also tested. In addition, blood was obtained from a sixth Negro male during the course of a Furadantin-induced hemolytic episode. During this episode, the patient's hematocrit dropped from 40 to 29 per cent; at the time when the *in vitro* experiments were conducted, his hematocrit was 35 per cent and he was still receiving the drug, indicating that active *in vivo* destruction of this patient's red blood cells was occurring at the time of the study.

All the blood samples were subjected to the screening test of Motulsky, Kraut, Thieme and Musto (7) for decreased glucose-6-phosphate dehydrogenase activity and found to be abnormal. Glutathione stability tests were conducted as outlined by Beutler (8) and demonstrated abnormally low, postincubation reduced glutathione content of the erythrocytes of all the donors except for the female patient. This patient's reduced glutathione values were 48.5 mg per 100 ml initially and 27.5 mg per 100 ml after incubation with acetylphenylhydrazine.

The conditions of *in vitro* incubation of erythrocytes with primaquine and techniques for measuring loss of K^+, osmotic fragility, reduced glutathione, primaquine and hemolysis are described in an accompanying paper (2). The *in vitro* concentrations of primaquine[1] employed ranged from 1×10^{-6} to 3×10^{-3} M. Loss of K^+ after *in vitro* exposure of red cells to the drug was studied on samples of blood from 3 primaquine-sensitive individuals and 1 individual whose glutathione stability test placed her in the intermediate group.

In an effort to evaluate the role of metabolic products of primaquine, experiments were conducted in which red cells from 2 primaquine-sensitive individuals were incubated with serologically compatible serum from 2 normal individuals who had ingested primaquine diphosphate, 30 mg per day, for 3 to 5 days, the last dose being taken 4 hours prior to donation of the serum. The concentration of primaquine present in these sera ranged from 1 to 3×10^{-6} M. The primaquine-containing sera were incubated with autogenous red cells, and with serologically compatible cells from 2 of the glucose-6-phosphate deficient individuals. Sera obtained from the normal donors prior to ingestion of primaquine were also incubated with the abnormal cells. Incubations without added glucose in the system were extended to 20 hours in the hope of accentuating any differences between normal and abnormal erythrocytes.

[1] The primaquine diphosphate was generously supplied by Dr. George D. Wessinger, Sterling-Winthrop Institute, Division of Sterling Drug, Inc., Rensselaer, N. Y.

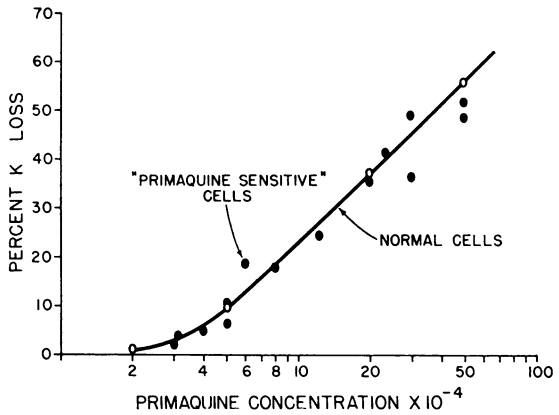

FIG. 1. PERCENTAGE LOSS OF K^+ BY "PRIMAQUINE-SENSITIVE" ERYTHROCYTES INCUBATED WITH VARYING CONCENTRATIONS OF PRIMAQUINE. The mean percentage loss of K^+ by normal red cells in response to varying concentrations of primaquine is indicated by the line connecting the open circles which are representative points taken from the accompanying paper (2). The solid dots represent the experimental results obtained by utilizing primaquine-sensitive red cells.

RESULTS

Incubation of "sensitive" erythrocytes with primaquine. The abnormal red cells were incubated with primaquine in a range of concentrations described above, and in Figure 1 the results of 2-hour incubation studies are compared with similar results obtained from study of normal cells. It is evident, under these circumstances, that the abnormal cells behave no differently from the normal cells and apparently require the same *in vitro* dosage of primaquine to induce prelytic loss of K^+.

Short-term incubation (up to 5 hours) of normal or primaquine-sensitive red cells, either in their own serum or in the serum from normal donors who had taken the drug, produced no significant loss of K^+ or hemolysis. Incubation of these combinations of red cells and serum for 20 hours, in the absence of glucose, yielded the results shown in Table I.

In addition, incubation of a whole blood sample taken from the individual with active hemolytic

TABLE I

*Per cent loss of K^+ induced by 20 hours of in vitro incubation**

	Abnormal red cells	Normal red cells
Autologous serum	28.2, 27	36, 33, 24
Compatible donor serum before ingestion of primaquine	31	38.6, 36
Compatible donor serum after ingestion of primaquine	33.7, 26.2	32.6, 35.6

* Each value is the mean of duplicate determinations.

disease caused by nitrofurantoin (Furadantin), presumably containing Furadantin or its potentially hemolytic metabolites, yielded an 18 hour loss of K^+ of 23.3 per cent with 1 per cent hemolysis.

It is obvious that the abnormal red cells in the presence of primaquine- or Furadantin-containing serum lost no more potassium than did normal red cells, incubated for a like period of time. The normal result obtained on the blood of the patient already undergoing drug-induced *in vivo* hemolysis suggested that destruction of the patient's cells ceased when the blood was removed from the body. All of the values for loss of K^+ from both normal and abnormal red blood cells are consistent with Ponder's observation on loss of K^+ by red cells incubated at 37° C in the absence of glucose (9). Osmotic fragility studies of the abnormal red cells, both initially and after incubation with either added primaquine or primaquine-containing serum, were comparable with those of normal cells treated in similar fashion.

DISCUSSION

The results of these studies on red cell K^+ permeability are entirely consistent with the observations of Beutler, Dern and Alving (1), which point out the similarity of normal and primaquine-sensitive red cells with regard to primaquine-induced *in vitro* hemolysis. They are also consistent with experiments performed by Feldman, Packer, Murphy and Watson (10) who incubated red cells from a sensitive individual with pamaquine-containing plasma from a normal individual and failed to produce hemolysis of the sensitive cells. In spite of the fact that loss of K^+ is a considerably more sensitive parameter of red cell damage than is hemolysis, there is no demonstrable evidence of a difference in this respect between normal and glucose-6-phosphate dehydrogenase deficient erythrocytes exposed to primaquine or to presumed primaquine metabolites in an *in vitro* test system.

These observations emphasize the difficulty in drawing conclusions about primaquine-sensitive erythrocytes from *in vitro* studies and strongly re-emphasize the point made by Beutler, Dern and Alving (1) that *in vivo* mechanisms of red cell destruction are an essential part of the clinical picture of primaquine-induced hemolytic anemia. The need for more investigation in this area is clearly indicated, with emphasis on the relationship between *in vivo* removal mechanisms and the metabolic defect in the cells.

SUMMARY AND CONCLUSIONS

1. Erythrocytes which are deficient in their glucose-6-phosphate dehydrogenase activity manifest prelytic loss of K^+ identical with that of normal cells when exposed to primaquine *in vitro*.

2. Serum from normal individuals taking 30 mg of primaquine daily, when incubated with primaquine-sensitive erythrocytes from the patients, failed to induce a loss of K^+ which differed from that of normal red cells incubated over the same 20 hour period.

3. Altered K^+ permeability in both normal and primaquine-sensitive erythrocytes occurs at concentrations of drug below those which produce various biochemical abnormalities, suggesting that primaquine and other related compounds may sequentially affect the erythrocyte membrane in a critical fashion before altering intracellular biochemical processes.

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