AFTERNOON SESSION—11 MAY 1967

Chairman: Dr. Chapman H. Binford

The Antimalarial and Hemolytic Properties of 4,4′-Diaminodiphenyl Sulfone (DDS)[1, 2]

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The purpose of this report is to summarize the results of recent studies concerning the antimalarial and hemolytic effects of 4,4′-diaminodiphenyl sulfone (DDS; dapsone; diaphenylsulfone). It has long been known that DDS possesses antimalarial activity and that hemolysis represents one of the major undesirable effects of DDS. Because of the superiority of other synthetic antimalarial drugs such as chloroquine, DDS has been of little value as an antimalarial agent; however, recent observations, particularly studies with strains of *Plasmodium falciparum* that are resistant to chloroquine and to other widely used synthetic antimalarial drugs ([19]), have rekindled considerable interest in the potential antimalarial value of DDS. Despite extensive use of DDS for non-antimalarial purposes, many aspects of DDS-induced hemolysis have remained unsettled. Recent investigations have furnished a substantial amount of information concerning the potential value and limitations of DDS as an antimalarial agent and have clarified certain features of DDS-induced hemolysis that are pertinent to the clinical use of DDS for antimalarial or other purposes. These studies have, in addition, provided further insight into the mechanisms involved in, and possible relationships between, the antimalarial and hemolytic effects of DDS.

ANTIMALARIAL EFFECTS OF DDS

Early studies, reviewed by Findlay ([25]) in 1951, indicated, in general but with some exceptions, that sulfones and sulfonamides act mainly on asexual erythrocytic forms of species of plasmodia that cause human malaria and that the activity of these drugs against asexual erythrocytic forms of *P. vivax* is less pronounced than that against asexual erythrocytic forms of *P. falciparum*. In 1941, Coggeshall and co-workers ([16]),

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for example, found that both Promin (glucosulfone sodium; the didextrose sulfonate derivative of DDS) and sulfadiazine displayed activity against asexual erythrocytic forms of both *P. vivax* and *P. falciparum* and that vivax infections appeared less responsive to Promin than did falciparum infections. In 1956, Leiker [30] reported that patients with leprosy being treated with DDS in a highly malarious area of New Guinea were conspicuously free from acute attacks of malaria. Tarabini [45], in 1958, drew further attention to Leiker's observations, reviewed pertinent earlier clinical studies of other workers, and recounted prior experience of his own indicating that the dextrose diglucoside of DDS displayed activity against *P. falciparum*. In 1960, Costa [18] reported additional evidence indicating that DDS exerted effects against *P. falciparum*, and Archibald and Ross [2] reported that among patients with leprosy being treated with DDS in four centers in northern Nigeria, "*P. malariae* parasitemia had disappeared from those taking DDS and that *P. falciparum* trophozoites occur only about one-tenth as often in the DDS group as in the corresponding untreated community." Archibald and Ross [2] observed also that in school children treated with 200 mgm. of DDS, this drug exerted a substantial effect against trophozoites of both *P. malariae* and *P. falciparum*, but that the effect of DDS was not as rapid as that noted when school children were treated with 300 mgm. of chloroquine base. In 1962, Basu and co-workers [4], in India, found that single doses of 200 or 250 mgm. of DDS resulted in clearance of asexual parasites and in termination of fever within 72 hours in patients infected with *P. falciparum* but were inadequate in patients infected with *P. vivax*. Recent studies carried out in Tanzania [17, 29] have furnished additional data indicating that DDS exerts appreciable blood schizontocidal activity against *P. falciparum*.

Studies with young children who had naturally acquired infections and who received 50 mgm. of DDS daily for 10 days and studies with nonimmune adult volunteers who had patent infections with the Chesson strain of *P. vivax* and who received 200 mgm. of DDS daily for from four to seven and one-half days revealed that effects exerted by DDS against asexual erythrocytic forms of *P. vivax* of South West Pacific origin were unimpressive [43]. Investigations with volunteers exposed to mosquito-induced infections with the Chesson strain of *P. vivax* disclosed that relatively high weekly doses of DDS failed to prevent the establishment of or to eliminate tissue schizonts of this strain [43] and that daily administration of 25 mgm. of DDS failed to prevent patency of infections with this strain of *P. vivax* [14].

Studies with nonimmune volunteers revealed that administration of relatively small daily doses of DDS (25 or 50 mgm.), although not invariably effective, exerted a substantial effect in preventing patency of mosquito-induced infections with two strains of chloroquine-resistant *P. falciparum* from South East Asia [19]. Further studies with one of these strains of chloroquine-resistant *P. falciparum*, termed the Malayan (Camp.) strain, indicated that the protective effect of DDS observed reflected a suppressive or clinical prophylactic effect, not a true causal prophylactic effect, and that DDS acted slowly when administered during acute clinical attacks [19].

Limited preliminary studies (Fig. 1) have been carried out with three nonimmune volunteers who received 300 mgm. of DDS *weekly* and who were bitten by mosquitoes infected with the Malayan (Camp.) strain of chloroquine-resistant *P. falciparum*. Two of these three volunteers developed transient patent infections, without marked symptoms of malaria, and one developed a patent infection followed promptly by an acute clinical attack of malaria. Serial determinations of levels of DDS in the blood of these three volunteers (Fig. 1), as well as studies in which volunteers received single oral doses of DDS (Fig. 2), indicated that peak levels of DDS in the blood occurred relatively early during the 24 hour period following ingestion of medication, after which levels of DDS diminished rather rapidly to low or insignificant values. During studies with

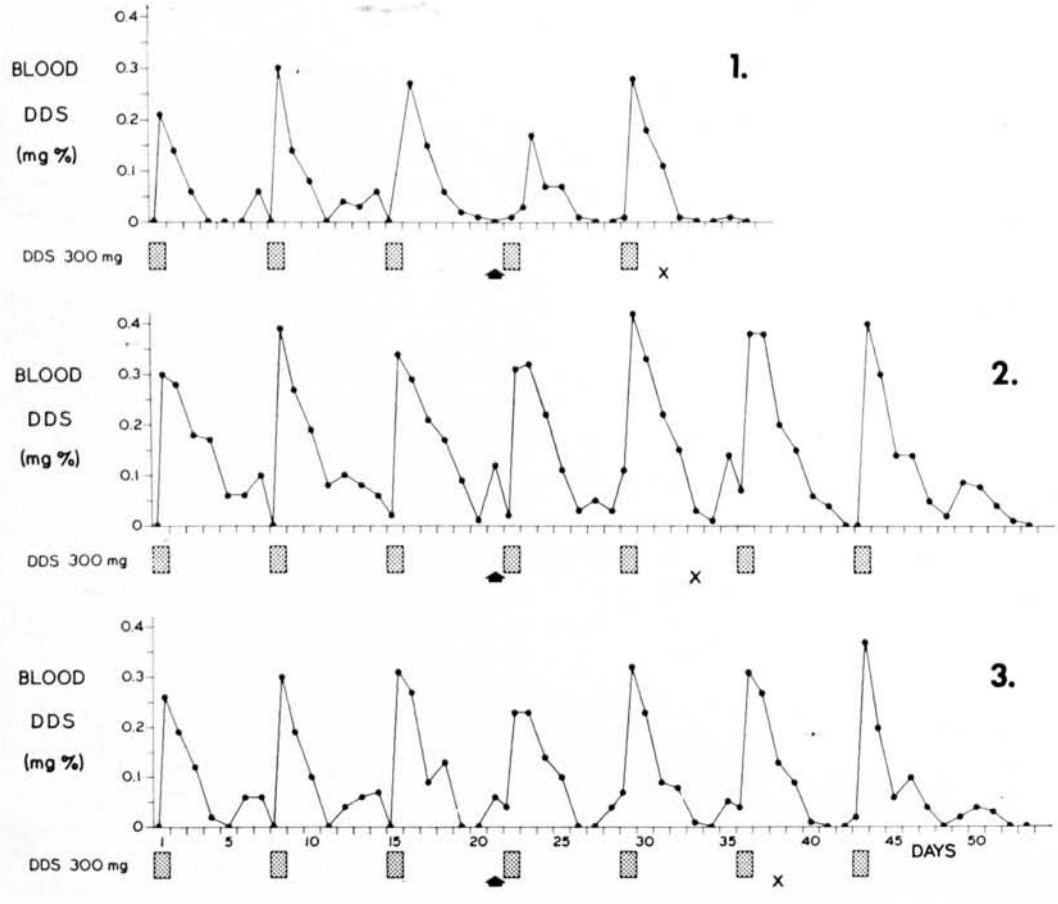

FIG. 1. Studies with individuals receiving DDS weekly. Three healthy, nonimmune, adult, male volunteers each received 300 mgm. of DDS orally once a week as indicated. Levels of DDS in the blood were determined daily. On the day indicated by the arrows. these three men and a fourth man, who received no medication and who served as a control subject, were bitten by the same 10 mosquitoes (*Anopheles stephensi*) infected with the Malayan (Camp.) strain of chloroquine-resistant *P. falciparum*. Examination of the mosquitoes' salivary glands ([19]) indicated that sporozoite inocula were heavy ("infectivity" :40). The control subject developed a patent infection, followed by an acute attack of malaria, after a prepatent period of 11 days. The three individuals receiving DDS weekly developed patent falciparum infections at the times indicated by the x's; Volunteer 1 (top) promptly had an acute clinical attack of malaria, at which point treatment with quinine was instituted and administration of DDS was discontinued, while Volunteers 2 and 3 had only transient patent infections without acute clinical attacks. No recurrence of patent parasitemia was noted in Volunteers 2 and 3 during a follow-up period exceeding 60 days after administration of the last dose of DDS. Conditions and methods of study were as described previously ([19]).

volunteers receiving 300 mgm. of DDS weekly, there was no evidence to suggest cumulative effects with respect to levels of DDS in the blood; just before administration of each dose of medication, DDS was often not detectable in the blood of volunteers receiving DDS weekly (Fig. 1), in contrast to findings noted during studies in which volunteers received DDS daily (Fig. 3). During studies with the three volun-

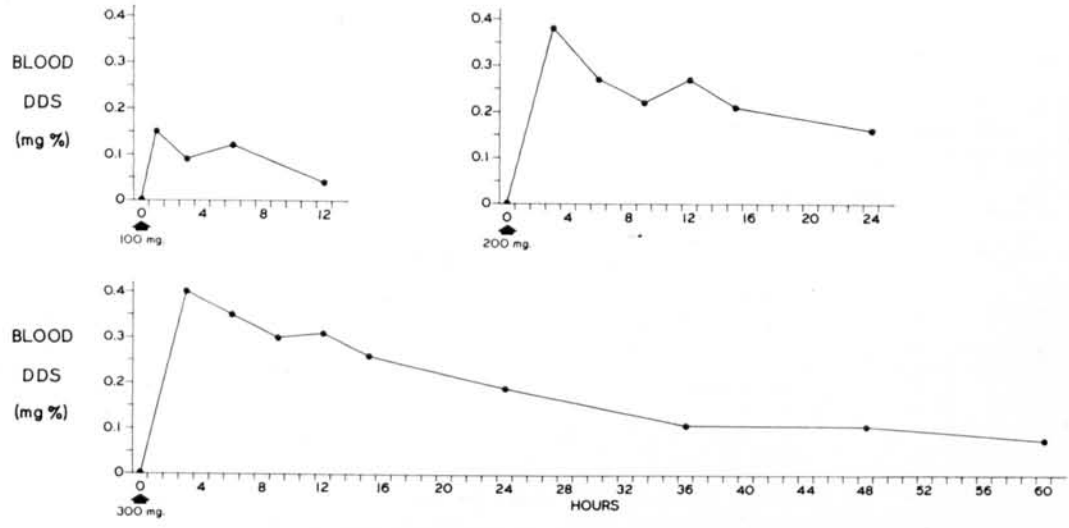

FIG. 2. Levels of DDS in the blood after administration of single doses of DDS. Healthy, adult, male volunteers received single oral doses of 100 mgm., 200 mgm., or 300 mgm. of DDS as indicated by the arrows. Levels of DDS in the blood were determined as described previously ([19]).

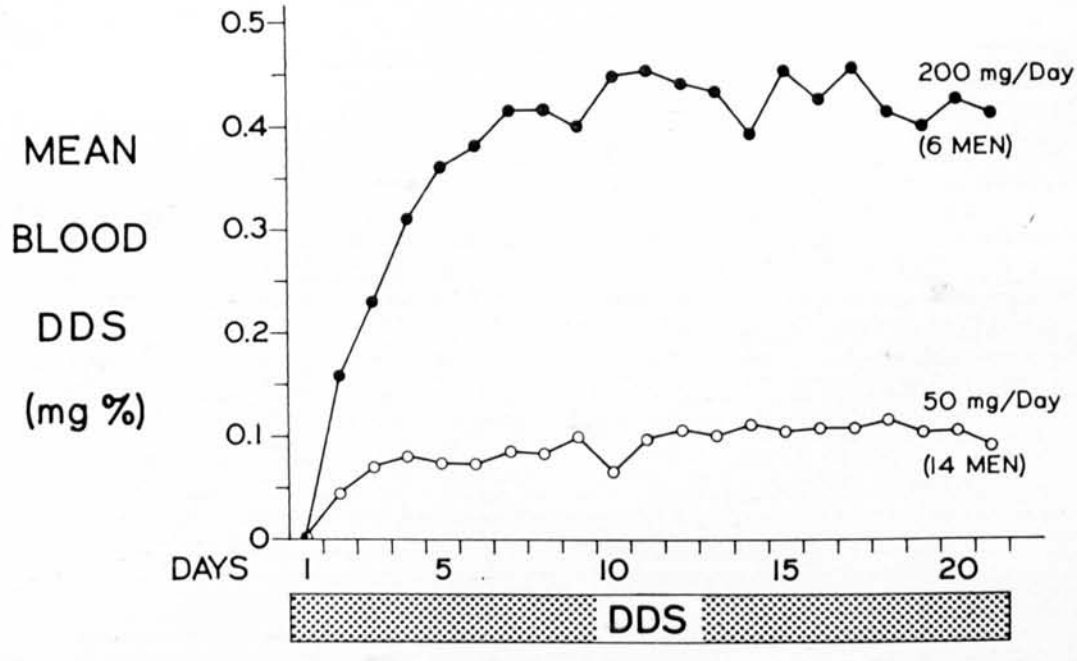

FIG. 3. Mean levels of DDS in the blood of individuals receiving DDS daily. Levels of DDS in the blood of 20 healthy, adult, male volunteers (six receiving 200 mgm. of DDS daily and 14 receiving 50 mgm. of DDS daily) were determined ([19]) daily; samples of blood for determinations of levels of DDS were obtained just prior to administration of each daily dose of DDS.

teers receiving 300 mgm of DDS weekly, one volunteer had transient but moderately severe headaches after administration of each dose of DDS. These findings, including data consistent with previous evidence indicating that DDS is eliminated relatively rapidly from the body, suggested that weekly administration of 300 mgm. of DDS, although partially effective, would not prove as satisfactory as daily administration of lesser doses of DDS in achieving suppression (clinical prophylaxis) of infections with chloroquine-resistant *P. falciparum* in nonimmune persons.

During recent studies in Tanzania, Laing [29] observed that although DDS displayed definite activity when administered to semi-immune patients with acute attacks of falciparum malaria, it could not be considered a reliable drug for treatment of acute malaria. DDS has not been found to destroy mature falciparum gametocytes, and available evidence, although not extensive, indicates that DDS does not display promise as a sporontocidal agent against *P. falciparum.* Thus, DDS has a relatively narrow spectrum of activity in terms of effects exerted against different species or different stages in the life cycle of parasites that cause human malaria, and DDS, employed alone, cannot be considered a satisfactory drug for treatment of acute attacks of malaria.

Resistance to sulfonamides or sulfones, including DDS, has been induced without great difficulty during studies with malaria parasites of lower animals [7]. It appears reasonable to assume that wide and injudicious application of DDS or of other sulfones or sulfonamides as antimalarial agents, especially if used alone, could rather easily or quickly result in selection of malaria parasites resistant to these compounds, with a consequent rapid dissipation of much of the already limited potential antimalarial utility of these agents. To what extent prior use of these agents for nonantimalarial purposes in malarious areas may have already served as a factor favoring selection of malaria parasites resistant to these drugs is uncertain. Clearly, during further exploration of the antimalarial value of DDS or of other sulfones or sulfonamides, consideration should be focused particularly on the potential utility of these drugs administered in combination with other antimalarial agents.

Studies with malaria parasites of lower animals have revealed that, under some circumstances, potentiation of antimalarial effects results when a sulfone or sulfonamide is administered together with chlorguanide or pyrimethamine [42]. In 1958, Hurly [28] presented the results of studies, carried out in the Gambia, indicating that a combination of sulfadiazine and pyrimethamine resulted in potentiation of effects against *P. falciparum.* During their studies in India, reported in 1962, Basu and coworkers [4] found that relatively small doses of DDS administered together with a small dose of pyrimethamine proved adequate for treatment of *both* vivax and falciparum infections, and that the rapidity of subsidence of overt parasitemia and of symptoms in the individuals treated, some of whom were probably semi-immune, was comparable to that achieved with a single dose of 600 mgm. of chloroquine base. In 1963, McGregor and co-workers [34] observed clearance of asexual erythrocytic parasites in a Gambian child infected with *P. falciparum* who received sulfadiazine and pyrimethamine concurrently after prior treatment with pyrimethamine alone had proved ineffective.

In 1964, shortly after making the initial observations indicating that both sulfones and sulfonamides displayed activity against chloroquine-resistant *P. falciparum,* DeGowin and Powell [21] and Powell *et al.* [40] carried out studies in which combinations of drugs involving a sulfone or a sulfonamide were administered to volunteers having patent infections with the Malayan (Camp.) strain of chloroquine-resistant *P. falciparum.* This strain had proved resistant to pyrimethamine or chlorguanide administered alone. These studies revealed that concurrent administration of 660 mgm. of sulfoxone (Diasone) and 300 mgm. of chlorguanide daily for 10 days effected radical cure of infections in two non-immune volunteers studied [40], and that administration of 50 mgm. of pyrimethamine daily for three days together with

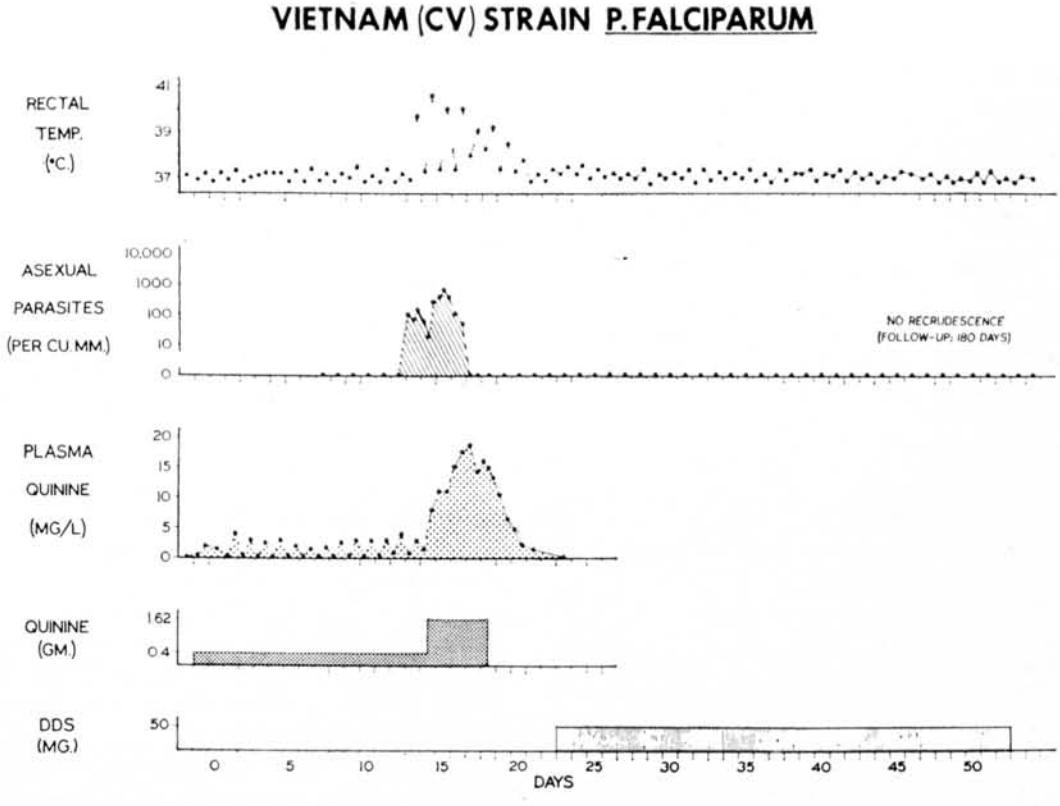

FIG. 4. Administration of DDS following administration of quinine to a volunteer infected with chloroquine-resistant *P. falciparum*. A healthy, nonimmune, adult, male volunteer receiving 7.5 grains of quinine sulfate (405 mgm. of quinine base) daily was bitten by mosquitoes infected with the Vietnam (VC) strain of chloroquine-resistant *P. falciparum*. A patent infection, followed by an acute clinical attack of malaria, occurred, at which point full therapeutic doses of quinine (10 grains of quinine sulfate, equivalent to 540 mgm. of quinine base, every eight hours) were administered for four days. Marked cinchonism with severe abdominal pain and other symptoms, some of which suggested an allergic as well as a toxic reaction to quinine, ensued. These symptoms subsided shortly after administration of quinine was discontinued. The volunteer then received 50 mgm. of DDS daily for 30 days. No recrudescence of falciparum malaria occurred. Other studies with this same strain indicated that it was unlikely that a 4-day course of quinine would effect radical cure of infections with this strain of *P. falciparum* (*Modified from* Eppes *et al.*, Military Med. **131** [1966] 362-371.) (Published with permission of Military Medicine.)

2 gm. of sulfadiazine daily for five days effected radical cure of infections in five or six nonimmune volunteers studied ([21]). But, during studies with both combinations of drugs, clearance of asexual parasites from the blood and subsidence of fever and of other symptoms of malaria occurred relatively slowly following institution of therapy. For control of acute attacks of malaria in nonimmune persons infected with chloroquine-resistant *P. falciparum*, these particular combinations of agents did not appear as satisfactory as quinine.

Studies of other workers have drawn attention to the potential value of combinations consisting of pyrimethamine and certain longer-acting sulfonamides; such combinations, when employed for treatment of patients infected with chloroquine-resistant *P. falciparum*, have displayed considerable

promise ([3, 15]). Whether or not such combinations will prove therapeutically superior to quinine in controlling severe acute attacks of falciparum malaria is uncertain. During our studies with volunteers infected with chloroquine-resistant *P. falciparum*, administration of DDS (Fig. 4) or of a combination of sulfadiazine and pyrimethamine *following* administration of quinine has at times proved of value in achieving radical cure of infections with chloroquine-resistant *P. falciparum* in individuals who have developed adverse reactions to quinine during treatment with this drug and in persons who have had infections that stubbornly recrudesced despite repeated courses of quinine. For initial treatment of nonimmune persons having acute attacks of malaria caused by chloroquine-resistant *P. falciparum*, we have continued to rely chiefly upon quinine. In some groups of persons infected with chloroquine-resistant *P. falciparum* in South East Asia, recrudescences of falciparum malaria after administration of quinine have been observed frequently; recent studies have indicated that the administration of 25 mgm. of DDS daily for 30 days *following* administration of quinine to such individuals exerted a substantial effect in reducing the incidence of recrudescences ([44]).

Investigations have been carried out in which 25 mgm. of DDS was administered *daily* together with *weekly* concurrent administration of 300 mgm. of chloroquine base and 45 mgm. of primaquine base to volunteers who were bitten by mosquitoes infected with chloroquine-resistant *P. falciparum* from South East Asia. This combination of agents consistently prevented patency of infections during treatment and frequently effected radical cure of infections when administration of medication was continued for four or six weeks after exposure to infection ([24]). Field evaluation of the potential prophylactic utility of this particular combination of antimalarial drugs, designed to retain the beneficial effects exerted by chloroquine and primaquine against malaria parasites other than chloroquine-resistant *P. falciparum*, is currently underway. This particular combination of agents is far from ideal from an operational standpoint. There is a need for assessment of the prophylactic value and safety of other possible combinations of agents that involve a sulfone or a sulfonamide and that may be effective in prevention of infections with chloroquine-resistant *P. falciparum*. Repository antimalarial preparations containing diacetyldiaminodiphenyl sulfone have been developed recently ([17]) and are currently being evaluated.

HEMOLYTIC EFFECTS OF DDS

Hemolytic anemia has been recognized as a serious adverse effect of DDS since the first clinical studies with this drug were performed. Some of these initial clinical studies involved administration of what would now be considered very high doses of DDS, such as 1,000 to 2,000 mgm. daily. Adverse hematologic effects and other consequences observed gave rise to the belief that DDS was too toxic for use in human beings ([10]) and led to its temporary but prolonged abandonment. After an interlude of approximately a decade, DDS, in lesser doses, was applied and subsequently used widely for treatment of leprosy. More recently, DDS has been employed in the treatment of a number of other, primarily dermatologic, disorders such as dermatitis herpetiformis ([31, 33, 37]).

Although clinical experience with DDS during the last 10 to 15 years has been extensive, impressions and comments concerning hemolysis or hemolytic anemia caused by DDS have been discordant. Pengelly ([38]) noted that some workers have regarded hemolytic anemia caused by DDS as rare, while others have indicated that hemolytic anemia occurs to a varying degree in almost every patient taking sulfone drugs. He observed shortening of the survival of chromium^{51}-labeled red cells without definite anemia, in one normal person and in four patients with dermatitis herpetiformis receiving 100 to 200 mgm. of DDS daily; he pointed out that these conflicting statements would be compatible had the latter workers used the term "hemolysis" rather than "hemolytic anemia" ([38]).

DeGowin and co-workers ([20]) recently carried out detailed studies of the hemolyt-

ic effects of DDS both in normal persons and in individuals having glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency, an inherited (X-linked) alteration that involves the human red cell and that occurs in more than 100 million people in the world, predisposes to hemolysis induced by the antimalarial drug primaquine and by certain other agents ([11, 12, 13, 46]). DeGowin and co-workers' studies, involving administration of from 25 to 300 mgm. of DDS daily for 21 days to healthy men, demonstrated clearly that G6PD-deficient American Negro men are considerably more susceptible to DDS-induced hemolysis than are normal men. Except in one G6PD-deficient man who developed an intercurrent β-hemolytic streptococcal upper respiratory tract infection, there was a direct relationship between the daily dose of DDS and the extent of hemolysis in both groups studied. Relationships between the dose of DDS and the extent of hemolysis would have been obscure, and hemolysis caused by DDS would have appeared erratic, had results of studies with both groups been considered together without knowledge of the predisposition to drug-inducing hemolysis associated with G6PD deficiency. Hemolysis in normal men receiving 200 mgm. of DDS daily or lesser daily doses of DDS was largely subclinical, manifest mainly by decreased survival of chromium^{51}-labeled red cells, and often associated with little or no frank anemia, in agreement with the studies reported by Pengelly ([38]). Results of DeGowin and co-workers' studies with G6PD-deficient individuals were consistent with previous observations of Dern and co-workers ([22]), who found that transfused "primaquine-sensitive" red cells displayed hypersusceptibility to hemolysis induced by sulfoxone. In the G6PD-deficient man who developed an intercurrent streptococcal infection during DeGowin and co-workers' studies ([20]), hemolysis appeared enhanced, in agreement with previous data ([46]) indicating that certain acute infections or other complications may increase the severity of hemolysis in G6PD-deficient persons. The studies of DeGowin and co-workers underscored the point emphasized by Pengelly and indicated, in addition, that other factors of importance with respect to DDS-induced hemolysis include the dose of DDS, the presence or absence of G6PD deficiency, and the presence or absence of complications such as acute intercurrent infections.

Comparison of the hemolytic effects of DDS with those of primaquine disclosed that, on a mgm. per-kgm. basis, DDS is more hemolytic than primaquine in normal men and less hemolytic than primaquine in G6PD-deficient Negro men ([20]). In one G6PD-deficient man studied by DeGowin and co-workers, for example, hemolysis induced by administration of 100 mgm. of DDS daily proved less than that noted when the same man had been treated with 30 mgm. of primaquine base daily during previous investigations of the hemolytic effects of primaquine. Clinical and laboratory studies, including serial determinations of levels of reduced glutathione (GSH) in red cells, disclosed that the clinical course of hemolysis, including the extent of hemolysis and its self-limited nature, as well as changes in levels of erythrocytic GSH in a G6PD-deficient man receiving 200 mgm. of DDS daily were very similar to the changes that occur in G6PD-deficient Negro men treated with 30 mgm. of primaquine base daily: levels of erythrocytic GSH decreased sharply within two to three days after initiation of administration of DDS, acute hemolysis followed promptly, levels of erythrocytic GSH then increased to values similar to or slightly greater than those noted before treatment, and recovery from anemia, associated with reticulocytosis, ensued, even though administration of 200 mgm. of DDS daily to this G6PD-deficient man was continued during and after the acute hemolytic episode. These studies provided additional evidence supporting previous findings indicating that GSH ([6, 26]) may play an important role in the mechanism of drug-induced hemolysis in G6PD-deficient persons.

During DeGowin and co-workers' studies ([20]), methemoglobinemia exceeding 2 per cent was frequently observed in individuals receiving 100 mgm. or more of DDS daily, but not in persons receiving lesser daily doses of DDS. Considerable variation in the levels of methemoglobinemia was

noted in different individuals receiving the same daily dose of DDS. Levels of methemoglobinemia in G6PD-deficient persons did not appear to differ markedly from levels of methemoglobinemia in normal persons receiving corresponding daily doses of DDS. Sequences of changes in levels of erythrocytic GSH in normal men receiving DDS, however, differed markedly from those noted during studies with G6PD-deficient men (20). Levels of GSH in red cells of normal men receiving DDS increased during the first two weeks of treatment. Increases in levels of erythrocytic GSH preceded the onset of reticulocytosis and were related to the dose of DDS; these increases occurred earlier and were more marked, for example, in men receiving 300 mgm. of DDS daily than in those receiving 100 mgm. of DDS daily. Despite increased levels of erythrocytic GSH, slight hemolysis was evident during the first two weeks of treatment in normal men receiving 200 or 300 mgm. of DDS daily. During the third week after initiation of administration of DDS, levels of GSH in red cells of three of four normal men receiving 300 mgm. of DDS daily decreased and red cell survival studies disclosed a concomitant acceleration of hemolysis in all three men. In a fourth man receiving 300 mgm. of DDS daily and in men receiving lesser daily doses, levels of erythrocytic GSH remained elevated throughout the third week after initiation of treatment and there was no evidence of an acceleration in the rate of hemolysis during this time. These findings indicated that although normal red cells have, in general, a greater capacity to resist DDS-induced hemolysis than do G6PD-deficient red cells, in some normal persons the capacity to resist DDS-induced lysis displayed by many circulating red cells that survive initially during ingestion of DDS can be overcome if administration of a sufficiently high dose of DDS is continued. The diminutions in levels of erythrocytic GSH and concomitant acceleration of hemolysis observed during the third week of administration of 300 mgm. of DDS daily to some normal men were similar to, but less marked than, changes observed initially during studies with the G6PD-deficient man who received 200 mgm. of DDS daily. This resemblance suggests that mechanisms responsible for hemolysis under these two different circumstances may share certain fundamental similarities.

G6PD catalyzes the initial oxidative step in the pentose phosphate pathway of glucose metabolism. This pathway constitutes the only source for generation or regeneration of reduced triphosphopyridine nucleotide (TPNH) in mature cells of human beings. TPNH is important in many reductive processes, including the formation of GSH from oxidized glutathione, mediated by glutathione reductase. GSH may serve to protect hemoglobin and certain other constituents of the red cell against oxidative damage. G6PD-deficient red cells have a lower-than-normal content of GSH (6). A relative inability of G6PD-deficient red cells to generate TPNH and GSH may render these cells unusually vulnerable to destruction by certain agents such as primaquine and DDS. The greater capacity of normal red cells, compared with G6PD-deficient red cells, to resist lysis induced by DDS or certain other agents may reflect the greater ability of normal red cells to accelerate formation of TPNH and GSH.

The activity of G6PD in red cells of normal persons and in red cells of G6PD-deficient American Negro men decreases markedly as the cells age *in vivo* (32, 36, 39). Older circulating red cells of G6PD-deficient American Negro men are considerably more susceptible to lysis induced by primaquine than are younger circulating red cells (5). Red cells of G6PD-deficient Negro men have a slightly shorter-than-normal life span even in the absence of exposure to exogenous hemolytic agents (8). Studies with hemolysates have indicated that the activity of G6PD in a small fraction of probably very old circulating red cells of normal persons is similar to that in red cells of G6PD-deficient American Negro men (29). Administration of primaquine has been found to cause slight shortening of the life span of normal red cells (9). Desforges and co-workers (23) have reported that sulfoxone preferentially destroyed older circulating red cells of a patient with leprosy. Normal red cells, similar to G6PD-deficient red cells, may have an age-related gradient susceptiblity

to lysis induced by certain agents such as primaquine or DDS, but with only a few very old circulating normal red cells having a marked susceptibility to drug-induced lysis comparable to that displayed by many of the circulating red cells in G6PD-deficient Negro men.

In red cells of normal persons and in red cells of G6PD-deficient Negro men, the capacity to form TPNH and GSH may decrease, as activity of G6PD declines, during aging of the cells *in vivo*. During DeGowin and co-workers' studies ([20]) with normal men receiving 200 or 300 mgm. of DDS daily, slight hemolysis detected during the first two weeks of administration of DDS may have reflected chiefly lysis of very old circulating red cells; in some normal men receiving 300 mgm. of DDS daily, many moderately old circulating red cells, although able to survive initially, may have been unable to withstand the cumulative or sustained detrimental effects of DDS, perhaps because of a relatively limited capacity to increase formation of TPNH and GSH, and may have been the ones destroyed when an acceleration of hemolysis was detected, in association with a decrease in levels of erythrocytic GSH, during the third week after initiation of administration of DDS.

We have consistently observed that, after cessation of administration of 200 or 300 mgm. of DDS daily for two to three weeks to normal men, levels of erythrocytic GSH decrease to values well below those noted before treatment ([20, 35]). Long-term follow-up studies with a normal man treated with 300 mgm. of DDS daily for 14 days (Fig. 5) revealed that levels of erythrocytic GSH returned to levels similar to those noted before treatment only over many weeks, apparently as circulating red cells having a decreased average content of GSH were gradually replaced by new red cells having a normal content of GSH that were formed in and released from the marrow following, and in the absence of, exposure to DDS. Levels of DDS in the blood of men so treated decreased to non-detectable values within three to four days after cessation of treatment; yet levels of erythrocytic GSH continue to decrease, and remain decreased, well after DDS is no longer detectable in the blood. The reasons for these protracted effects of DDS upon levels of erythrocytic GSH are not clear; they may reflect delayed or persisting effects of DDS upon circulating red cells, effects of DDS upon young red cells formed in the marrow during and immediately after administration of DDS, or both. Flanagan and co-workers ([26]) observed a similar long-lasting diminution in levels of erythrocytic GSH during studies in which acetylphenylhydrazine was administered to normal persons. Although perhaps not absolutely essential for viability of the red cell, erythrocytic GSH appears important for survival of the cell both in the absence and in the presence of hemolytic agents ([13, 20, 41]). Red cells with a decreased content of GSH that are present in the circulation following cessation of administration of 200 or 300 mgm. of DDS daily for two to three weeks to normal men may have less than normal viability.

During our studies with normal men receiving 200 or 300 mgm. of DDS daily for two to three weeks, levels of the hematocrit have often decreased gradually not only during treatment but also for several days after cessation of treatment, subsequently returning to previous values only slowly over several weeks (Fig. 5). Our studies do not provide insight into circumstances that may obtain if administration of relatively high doses of DDS, such as 200 or 300 mgm., is continued daily for longer than two to three weeks. Under such circumstances, the extent of on-going hemolysis may exceed that which might be expected on the basis of our studies, and considerably longer than two to three weeks may be required before stabilization of the rate of hemolysis occurs and before increased red cell destruction and compensatory increased red cell production reach a point of equilibration. Additional studies, particularly longer-term investigations, are needed. It is clear that in patients receiving relatively high doses of DDS, the full hemolytic impact of DDS may not be evident initially, and that even detailed observation for one or two weeks after initiation of treatment may be insufficient for adequate appraisal

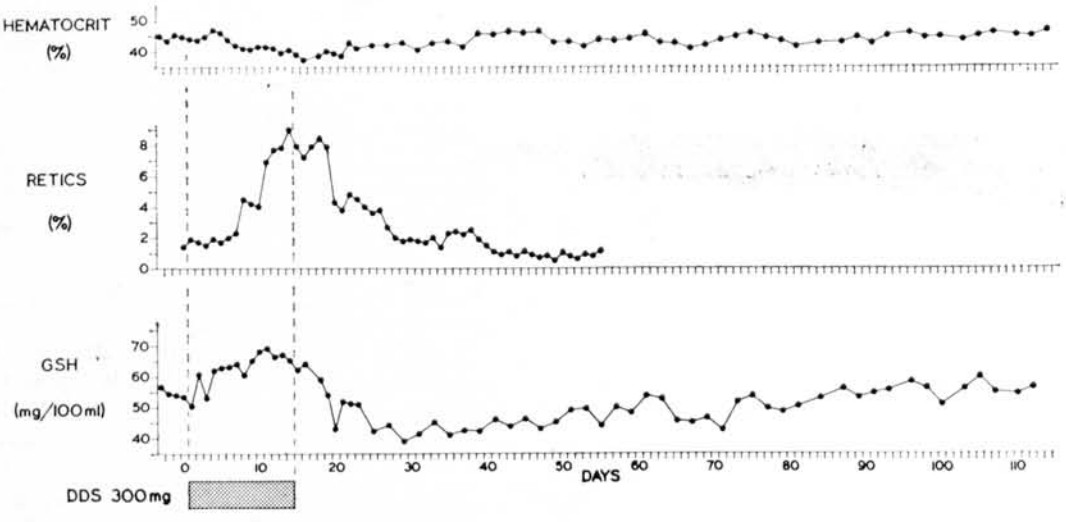

FIG. 5. Effects of DDS upon levels of GSH in red cells of a normal individual. A normal (non-G6PD-deficient), healthy, adult, male volunteer received 300 mgm. of DDS daily for 14 days as indicated. Levels of the hematocrit, reticulocyte counts (RETICS), and levels of reduced glutathione (GSH) in red cells were determined serially. Methods employed were as described previously ([20]).

of the hemolytic effects of DDS.

When employed for treatment of patients with leprosy, DDS is now usually administered initially in doses that are lower or less frequent than those used formerly. But doses of DDS considerably higher than those now used initially for treatment of patients with leprosy have at times been employed when DDS has been used for treatment of patients with certain other disorders such as dermatitis herpetiformis ([31, 33, 37]). Clearly, the administration of moderately high doses of DDS, such as 200 mgm. daily, may cause marked hemolysis in persons having fully expressed G6PD-deficiency. Alertness to the increased risk of hemolysis in G6PD-deficient persons receiving DDS is warranted.

Several different genetic variants of G6PD deficiency have been discovered. Some variants of G6PD deficiency are associated with an even more marked hypersusceptibility to drug-induced hemolysis than that noted in G6PD-deficient Negro men ([13, 46]). In addition, inherited disorders other than G6PD deficiency, but involving the pentose phosphate pathway, have been detected that are also associated with hypersusceptibility to drug-induced hemolysis ([13]). There is a need for further information concerning the hemolytic effects of DDS and of other agents in persons having inherited erythrocytic disorders other than G6PD deficiency that predispose to drug-induced hemolysis and in persons with different genetic variants of G6PD deficiency.

Both short-term and long-term studies have indicated that in healthy normal men and in healthy G6PD-deficient Negro men, marked hemolysis does not attend administration of relatively low daily doses of DDS, such as those found effective in achieving suppression of infections with some strains of chloroquine-resistant *P. falciparum* ([20, 24]). Studies involving daily administration of 25 mgm. of 50 mgm. of DDS together with weekly concurrent administration of 300 mgm. of chloroquine base and 45 mgm. of primaquine base, revealed that this combination of agents did not cause anemia in normal (non-G6PD-deficient) men, but that transient anemia did occur in G6PD-deficient Negro men when treatment with these three drugs was instituted concomitantly; however, recovery from anemia ensued, in association with reticulocytosis, in the G6PD-deficient men even though ad-

ministration of medication was continued ([24]). Further studies indicated that mitigation of hemolysis resulted when G6PD-deficient Negro men received chloroquine and primaquine weekly for several weeks *before* institution of daily administration of DDS; during these studies, prior administration of chloroquine and primaquine probably resulted in lysis of older circulating red cells so that when treatment with DDS was instituted, red cells present in the circulation had a younger-than-normal mean age and were, therefore, relatively resistant to drug-induced lysis. Serial determinations of levels of erythrocytic GSH during the latter studies yielded further evidence indicating that GSH may play an important role in the mechanism of hemolysis induced by administration of drugs such as primaquine or DDS to G6PD-deficient persons.

POSSIBLE RELATIONSHIPS BETWEEN THE ANTIMALARIAL AND HEMOLYTIC EFFECTS OF DDS

Circumstantial evidence has suggested that an intimate relationship may exist between the antimalarial and hemolytic effects of primaquine and of certain other drugs ([1]). Recent studies have disclosed that concurrent administration of para-aminobenzoic acid (PABA) vitiated the protective effect of DDS against the Malayan (Camp.) strain of *P. falciparum* ([20]). These findings are consistent with the view, based on studies with malaria parasites of lower animals, that the mechanism of the antimalarial effects of DDS and of certain other sulfones or sulfonamides may involve interference with the incorporation or utilization of PABA by the parasite ([27]). Investigations carried out to determine whether or not PABA alters the hemolytic effects of DDS revealed that PABA did not appreciably influence either the severity of DDS-induced hemolysis or the changes in levels of erythrocytic GSH that are associated with DDS-induced hemolysis in normal or G6PD-deficient persons ([35]).

The main antimalarial value of primaquine stems from the effects it exerts against exoerythrocytic forms of plasmodia, particularly those of *P. vivax*, and against gametocytes. In contrast, the main antimalarial value of DDS stems from the effects it exerts against asexual erythrocytic forms, particularly those of *P. falciparum*. Thus, although the hemolytic effects of these two agents in G6PD-deficient persons share certain characteristics, the antimalarial effects of DDS and of primaquine are quite dissimilar. Although antimalarial and hemolytic effects of primaquine and of certain other drugs may be intimately related, it appears doubtful that such a relationship exists between the antimalarial and hemolytic effects of DDS.

SUMMARY

This report summarizes information, particularly results of recent clinical and laboratory studies, concerning the antimalarial and hemolytic properties of 4,4′-diaminodiphenyl sulfone (DDS). DDS, as well as certain other sulfones or sulfonamides, may prove of increasing antimalarial value, especially for prevention or treatment of infections caused by strains of *Plasmodium falciparum* that are resistant to chloroquine and to other widely used synthetic antimalarial agents. DDS and related compounds have a relatively narrow spectrum of antimalarial activity in terms of effects exerted against different species or different stages in the life cycle of parasites that cause human malaria. For this and other reasons, during further assessment of the potential antimalarial utility of DDS or other sulfones or sulfonamides, particular emphasis should be placed upon the use of these compounds in combination with other antimalarial agents.

Results of recent studies concerning hemolysis, long recognized as a major undesirable effect of DDS, are pertinent to the clinical use of DDS for antimalarial or other purposes. Persons with glucose-6-phosphate dehydrogenase (G6PD) deficiency are considerably more susceptible to DDS-induced hemolysis than are persons who do not have this inborn error of metabolism. During clinical use of DDS, alertness to the hypersusceptibility to DDS-induced hemolysis displayed by G6PD-

deficient persons is warranted. Administration of relatively small daily doses of DDS such as those found effective in achieving suppression of infections with certain strains of chloroquine-resistant *P. falciparum* does not cause marked hemolysis in healthy normal men or in healthy G6PD-deficient American Negro men. However, additional information is needed concerning the hemolytic effects of DDS and of other drugs in persons with different genetic variants of G6PD deficiency and in persons with inherited disorders other than G6PD deficiency that involve the pentose phosphate pathway of the red cell and that also predispose to drug-induced hemolysis. Recent studies indicate that the sequence of changes in levels of erythrocytic reduced glutathione (GSH) associated with DDS-induced hemolysis in G6PD-deficient men differs strikingly from that associated with DDS-induced hemolysis in normal persons; early during the clinical course of DDS-induced hemolysis, levels of GSH decrease in red cells of G6PD-deficient persons and increase in red cells of normal persons. Changes involving erythrocytic GSH may be of importance, however, not only in DDS-induced lysis of G6PD-deficient red cells but also in DDS-induced lysis of normal red cells.

Para-aminobenzoic acid vitiates the antimalarial effects of DDS, but para-aminobenzoic acid does not appreciably alter the severity of DDS-induced hemolysis, or associated changes in levels of erythrocytic GSH, in normal persons or in G6PD-deficient persons. It appears doubtful that an intimate relationship exists between the mechanisms that underlie the hemolytic effects of DDS and those that are responsible for the antimalarial effects of DDS.

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DISCUSSION

Dr. Prabhakaran. Is it true that G6PD deficiency affords a certain degree of protection against malarial infection?

Dr. Powell. Although there are quite a few reports to that effect the evidence is not conclusive.

Dr. Prabhakaran. Has *P. falciparum* been cultivated *in vitro*?

Dr. Powell. Yes, but with relatively limited success thus far.

Dr. Thompson. In view of indications that the joint use of pyrimethamine and sulfone or sulfonamide may have an important place in the treatment of drug-resistant malaria, I wonder if you would be willing to comment on the hemolytic toxicity liabilities of such combined therapy. I am thinking particularly of the joint risks of macrocytic anemia associated with the use of pyrimethamine and hemolytic anemia associated with the use of sulfones, especially in people who are G6PD-deficient and on borderline diets relative to folic acid.

Dr. Powell. The risks or liabilities mentioned merit additional appraisal. During further assessment of the antimalarial utility of such combinations of agents, it would probably be wise to pay close attention to these aspects, particularly as they might apply to possible use of relatively high doses of certain of these drugs, to possible prolonged drug administration, or to the use of these agents in persons subject to nutritional deficiencies.