

BLOOD PICTURE IN SULFONE TREATMENT OF LEPROSY
RELATION WITH THE DOSE AND BLOOD CONCENTRATION OF THE DRUGS

LINDA NAHAS AND HANS RZEPPA
Laboratories of Hematology and Chemotherapy
Instituto Butantan

AND LAURO DE SOUZA LIMA
Padro Bento Sanitorium
São Paulo, Brazil

Anemia occurring in the treatment of leprosy by sulfones has been reported by various authors who tried different doses administered by the oral or parenteral routes. Several selected reports are cited.

Faget and Johansen (4) saw toxic reactions with daily doses of from 0.5 to 1.0 gm. of promin by mouth. When it was given by the intravenous route, however, the dose could be increased to 5 gm. per day without severe reactions, although after six weeks of treatment 46 per cent of the patients showed moderate anemia. Fernandez *et al.* (5), in a series of 140 patients treated with diasone by mouth, observed anemia with daily doses of over 1.0 gm. Because there were reticulocytosis, bilirubinemia, increase of bile pigments in the urine, and decrease of erythrocyte fragility, they considered this anemia to be of the hemolytic type. According to Lowe (6), diaminodiphenylsulfone (DDS) induced an anemia at a dose of over 300 mgm. per day, with a blood concentration of 0.8 to 1.4 mgm. per cent. When the daily dose reached 400 mgm., with a blood level of 1.0 to 1.8 mgm. per cent, the hemolysis became more marked and the drug had to be stopped. The maximal dose was attained by increasing 100 mgm. every 2 weeks. Smith (8) reported anemia in a daily dose of 2.4 gm. in patients treated with diasone. Blood examination showed an increase of red blood cells and hemoglobin and an increase of the icterus index. The same anemia appeared with DDS at doses of over 300 mgm., which Smith considers the maximal therapeutic dose. Allday and Barnes (1), who used DDS in daily doses of over 200 mgm., did not consider the hypochromic anemia an important toxic effect. Blood examinations were done only in patients with clinical complaints, such as edema, dyspnea, pallor or languor.

The several plans of sulfone treatment in leprosy actually used differ as to the daily dose, the time of continuous administration of the drug, and the resting periods. The purpose of this study was to determine the erythrocyte changes caused by the administration of different sulfones and the relation of these changes to the blood concentration of diaminodiphenylsulfone.

MATERIAL AND METHODS

Sulfone treatments were given to groups of 5 patients which had remained at rest for two weeks. The following drugs and doses were employed, all administered by mouth.

Group 1: 0.66 gm. of bis-methylene sodium sulfoxylate, daily, equivalent to 0.333 gm. of DDS.¹

Group 2: 0.90 gm. of the same compound, daily, equivalent to 0.555 gm. of DDS.

Group 3: 0.2 gm. of diaminodiphenylsulfone (DDS), 3 times a week.

Group 4: 0.4 gm. of diaminodiphenylsulfone (DDS), daily.

Hematologic examinations of heparinized venous blood were made just before treatment started. At the same time the concentration of DDS in venous oxalated blood was determined. Weekly determinations were made during the six weeks of treatment and two weeks after its interruption. The hemoglobin concentration was determined by the electrophotometer, and the erythrocyte fragility test was made qualitatively in NaCl solutions.

The blood samples for determining the DDS levels were always drawn from the patients after the same period following ingestion of the drug. Brownlee's method⁽³⁾ for the estimation of DDS was used. However, after acidification and deproteinization of the blood the filtrate was treated, for complete hydrolysis of possibly conjugated DDS, in a boiling waterbath for one-half hour. On cooling, the initial volume was restored with distilled water and the solution was diazotized and treated, after destruction of excess nitrite with N-(1-naphthyl)-ethylenediamine, according to Brownlee's method. An Evans photoelectric colorimeter with green filter ($\lambda=530$) was used for reading the color intensity.

The blood levels shown in the graphs always refer to the total sulfone (free and conjugated), expressed in terms of DDS.

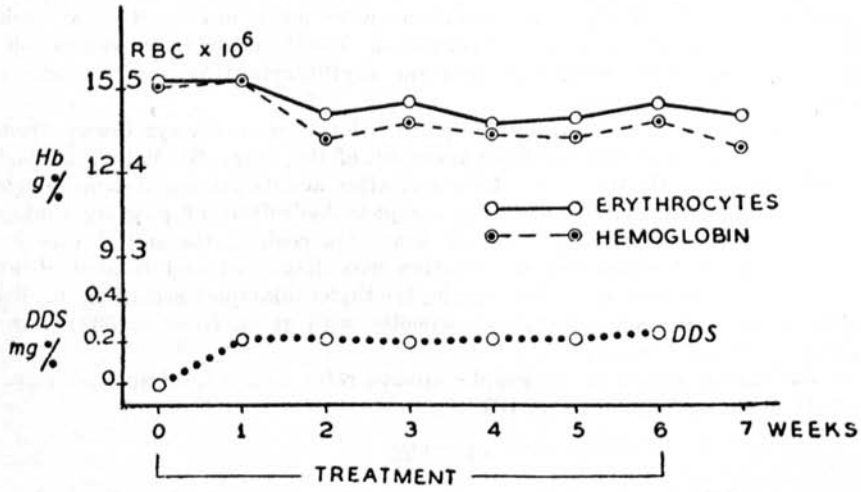
RESULTS

GROUP 1.—The mean sulfone concentrations in the blood of the five patients treated with 0.6 gm. of diaminoxil given daily for 6 weeks are shown in Text-fig. 1. The values obtained refer to minimum levels, because the blood was taken 24 hours after the last dose. Individual variations in this group ranged from 0.30 to 0.45 mgm. per cent. As shown in the same graph, the variations of hemoglobin and red cells in relation to the initial levels are within normal limits. Reticulocytes increased from 0.4 to 1.6 per cent (Text-fig. 6).

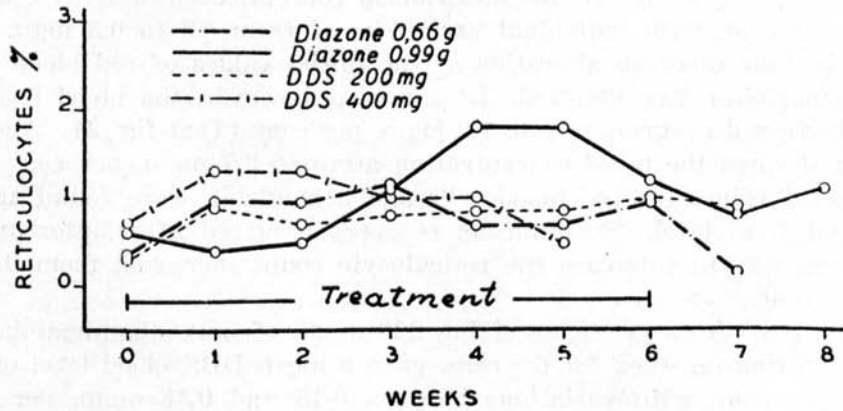
GROUP 2.—In this group, which received 0.9 gm. of diaminoxil daily for 6 weeks (Text-fig. 2), the mean blood concentration of DDS was 0.5 mgm. per cent, with individual variations of from 0.3 to 0.5 mgm. per cent. In four cases no alteration of the initial values of red blood cells and hemoglobin was observed. In one case, however, the blood level of DDS increased progressively to 1.2 mgm. per cent (Text-fig. 3). Anemia appeared when the blood concentration attained 0.7 mgm. per cent, and the lowest values for red blood cells and hemoglobin were found at the maximal DDS level. Spontaneous recovery occurred after interruption of treatment. In this case the reticulocyte count increased from 0.8 to 3.1 per cent.

GROUP 3.—This group, receiving 200 mgm. of pure diaminodiphenylsulfone 3 times a week for 6 weeks, gave a mean DDS blood level of 0.2 mgm. per cent, with variations between 0.15 and 0.35 mgm. per cent (Text-fig. 4). No variations from the initial values were seen in this group. Reticulocytes varied from 0.3 to 1.4 per cent (Text-fig. 6).

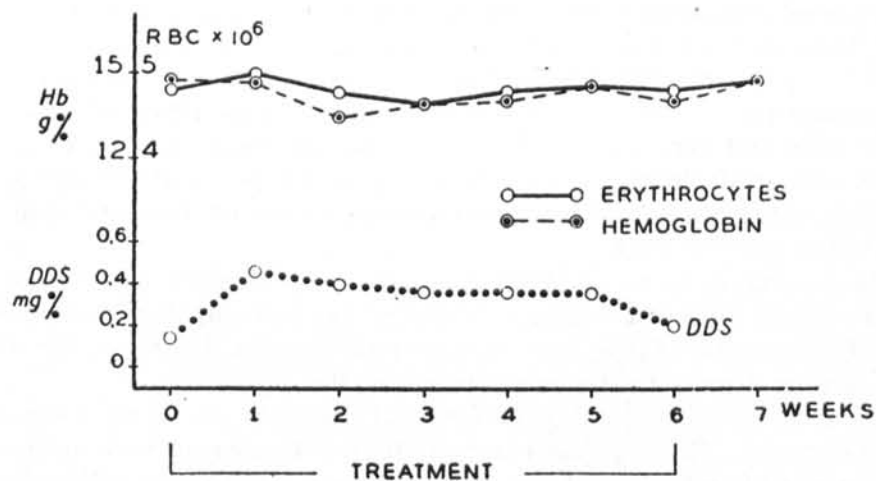
¹ "Diaminoxil," prepared by the Instituto Butantan, corresponding to diasone (Abbott) and diamidin (Parke Davis).



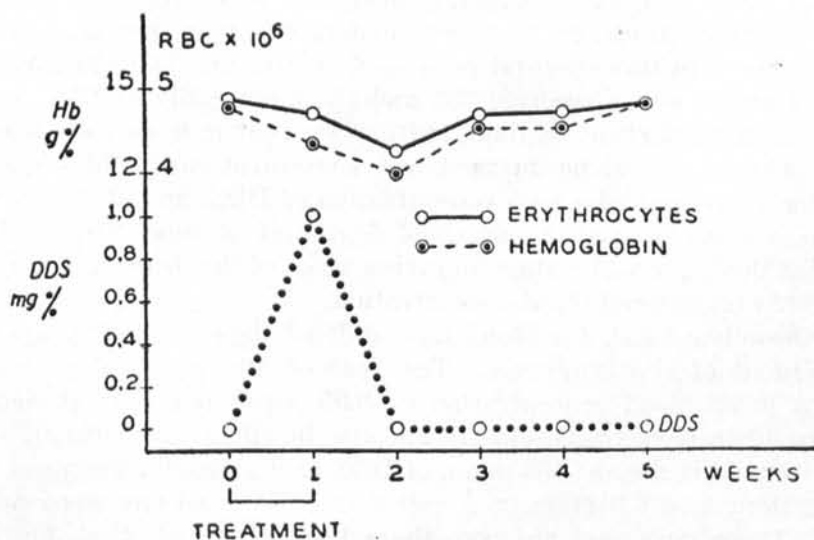
TEXT-FIG. 1. Hemoglobin, red blood cells, and DDS blood concentrations of Group 1, receiving daily doses of 0.66 gm. of diaminoxil; averages of 5 patients.



TEXT-FIG. 2. Hemoglobin, red blood cells, and DDS blood concentration of Group 2, receiving daily doses of 0.99 gm. of diaminoxil; averages of 5 patients.



TEXT-FIG. 3. One case of Group 2, in which there were marked anemia and high DDS concentrations.



TEXT-FIG. 4. Hemoglobin, red blood cells, and DDS blood concentrations of Group 3, receiving daily doses of 0.2 gm. of pure diaminodiphenylsulfone; averages of 5 patients.

GROUP 4.—In this group, with the daily dose of 400 mgm. pure diaminodiphenylsulfone, the treatment was continued for only six days. The mean blood concentration of DDS was 0.99 mgm. per cent, with individual variations of from 0.75 to 1.3 mgm. per cent. Toxic reactions were observed in all of the patients, and their severity compelled interruption of the treatment. Text-fig. 5 shows the average drop of initial values of red blood cells and hemoglobin. This drop was most marked in two of the cases. The reticulocytes varied from 0.7 to 1.5 per cent (Text-fig. 6). Further observations showed a spontaneous return of the blood values to the initial normal level.

In general, there was a slight decrease of the fragility of erythrocytes until after 38 days of treatment (Text-fig. 7), probably due to the partial hemolysis of the erythrocytes that were more fragile. However, the initial values were regained after interruption of the drug.

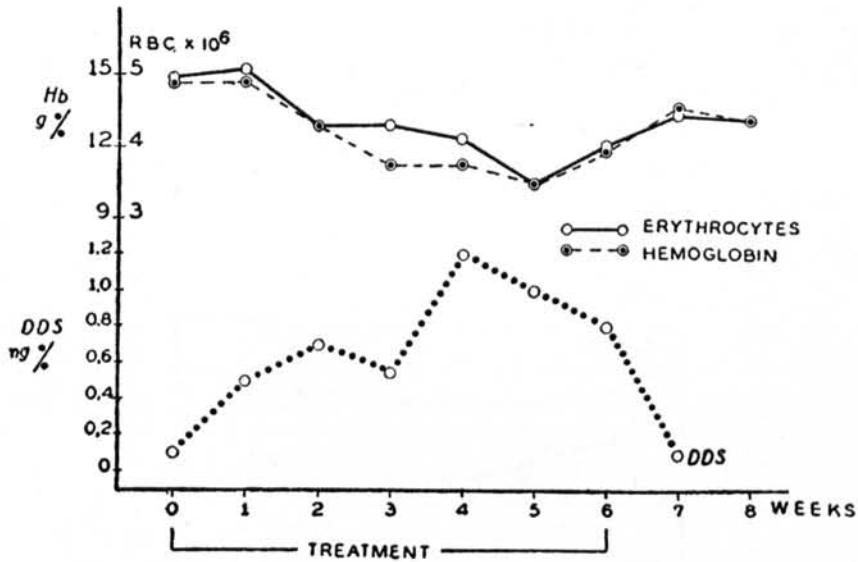
No changes in the total or differential leucocyte counts were observed in any group. Eosinophilia observed before treatment was unchanged during and after treatment. In most cases the erythrocyte sedimentation rate remained unmodified, except that in two cases of Group 4 it increased during reactions of the erythema nodosum reaction type.

DISCUSSION

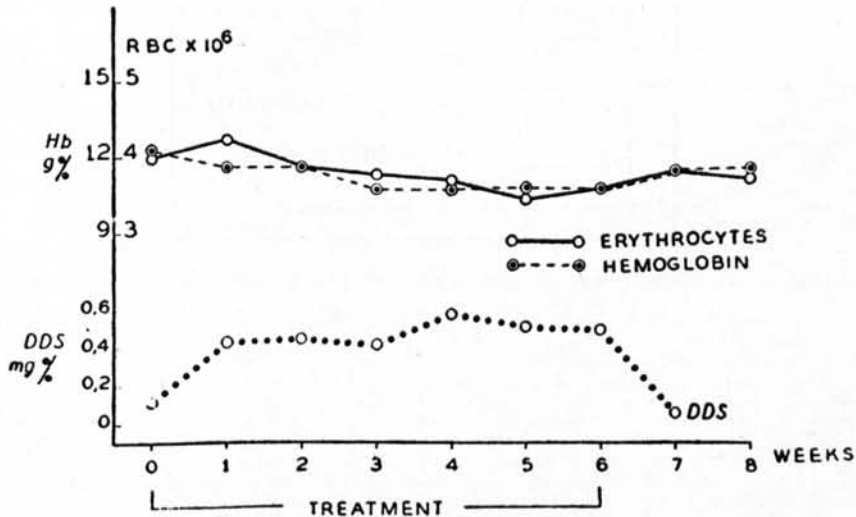
The results obtained show that blood concentrations of DDS larger than 0.6 mgm. per cent caused a moderate and progressive anemia. Smaller concentrations, up to approximately 0.5 mgm. per cent, did not alter the blood picture even on prolonged treatment. The comparison of Groups 1 and 2, which received 333 and 555 mgm. daily of DDS, respectively, as bismethylene sodium sulfoxylate (diaminoxil, or diasone) showed almost the same mean blood concentrations. Only one case (Text-fig. 3) presented a high concentration of DDS, and at the maximal level there was observed the maximal degree of anemia. This fact suggests that there is no advantage in giving more of the drug than is needed to maintain an optimal blood concentration.

On the other hand, the blood level did not depend directly upon the DDS content of the drug used. The dose of 400 mgm. of pure DDS, giving a mean blood concentration of 0.99 mgm. per cent (Group 4), provoked toxic phenomena, chiefly anemia, in all of the patients. However, with a daily dose of 555 mgm. of DDS as diaminoxil, the mean blood concentration was 0.5 mgm. per cent and toxic reactions were not observed. These data indicate, as others have observed, that the blood concentration depends more upon the molecular constitution of the drug than the amount of the active substance in it.

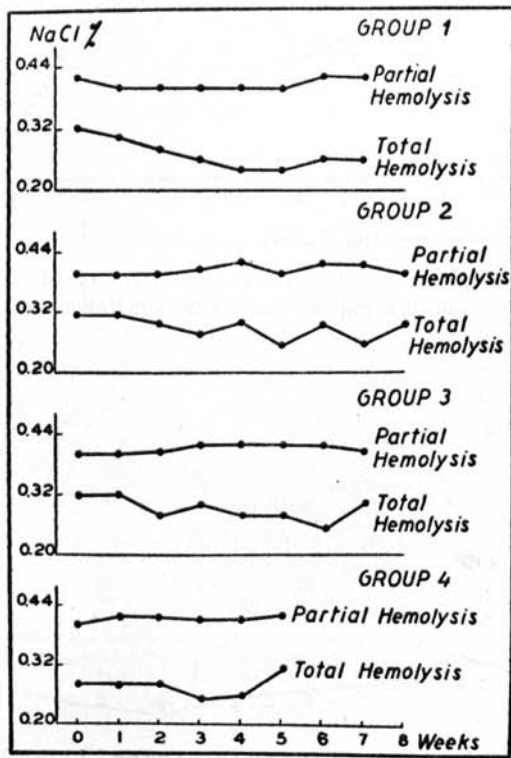
Among the toxic phenomena anemia is the most important, as has also been reported by others. It appears that it is of the hemolytic type, since reticulocytosis, bilirubinemia, increase of urinary bile pigments, and



TEXT-FIG. 5. Hemoglobin, red blood cells, and DDS blood concentration of Group 4, receiving daily doses of 0.4 gm. of pure diaminodiphenylsulfone; averages of 5 patients.



TEXT-FIG. 6. Averages of the reticulocyte counts of the 5 patients of each of the treatment groups. The larger dose of diasone (555 mgm. of DDS) provoked an initial decrease which was followed by gradual increase until the end of treatment.



TEXT-FIG. 7. Showing the results of the erythrocyte fragility tests of the several groups.

slight decrease of fragility of the erythrocytes are observed, probably due to the partial hemolysis of the erythrocytes that were more fragile.

Brownlee (2) considers that this anemia is due to lack of alimentary iron, with which sulfone forms a nonabsorbable complex. If this were so, the administration of iron to correct this anemia, as recommended by this author, would be inadequate. Experiments made in dogs by Rosenfeld *et al.* (7) indicate a hemolytic action by large doses of sulfones. This hemolysis was estimated by hemoglobin determinations in the plasma.

As there are individual variations of drug absorption, the occurrence of anemia must be watched for by hemoglobin determinations, in order to determine when a dose which is safe for most of the cases will induce hemolysis in some of them. When that happens smaller doses may be used, because the greater absorption in such individuals gives the same blood concentration as in the majority. Blood examinations made after the interruption of treatment revealed spontaneous recovery of the normal values of hemoglobin and red blood cells without the use of antianemics.

SUMMARY

Hematologic changes have been studied in patients treated with different doses of diasone—diaminoxil, the Butantan Institute equivalent of diasone, 4'-diaminodiphenylsulfone-N, N' (bis-methylene sodium sulfoxylate)—and pure diaminodiphenylsulfone—DDS—by mouth.

The results show that blood concentrations of DDS over 0.6 mgm. per cent induced a moderate, progressive anemia. Blood concentrations smaller than that amount did not cause any alterations.

Daily doses of 0.333 and 0.555 gm. of DDS in the form of diasone gave the same blood level. Thus the larger dose of this drug is excessive, presenting no advantage.

The group of patients treated with 0.4 gm. of pure DDS daily presented a mean blood concentration of practically 1.0 mgm. per cent. Toxic reactions, chiefly anemia, appeared in all of these patients.

It was also observed that the blood concentration of DDS does not depend solely upon the amount of active substance contained in the drug. Toxic reactions and anemia observed in patients who received 400 mgm. of pure DDS did not appear in the group treated with 555 mgm. of DDS as diasone. The blood level seems to be related to the chemical composition of the drug, and individual variations may be seen.

The detection of anemia by hemoglobin determinations is held to be a safe index of the optimal individual dosages. When anemia occurred, normal blood values were attained after the treatment interruption without the use of antianemics.

RESÚMEN

Se estudiaron los cambios hematológicos en pacientes tratados con distintas dosis de diasone—diaminoxil, el equivalente de diasone del Instituto Butantan, 4'-diaminodi-

phenylsulfone-N, N'(bis-methylene sulfoxylato sódico)—y diaminodiphenylsulfone—DDS—por vía oral.

Los resultados demuestran que concentraciones sanguíneas de DDS de 0.6 mgm. por 100 cc. indujeron una anemia moderada progresiva. Concentraciones menores no causaron alteraciones.

Dosis diarias de 0.333 y 0.555 gm. de DDS en forma de diasone produjeron los mismos niveles en la sangre. Por tanto la dosis mayor es excesiva, presentando ninguna ventaja.

El grupo de pacientes tratado con 0.4 gm. de DDS pura diariamente presentó una concentración promedio en la sangre de 1.0 mgm. por 100 cc. Reacciones tóxicas, especialmente anemia, ocurrieron en todos éstos pacientes.

Se observó también que la concentración sanguínea de DDS no depende solamente de la cantidad de substancia activa contenida en la droga. Reacciones tóxicas y anemia observadas en pacientes que recibieron 400 mgm. de DDS pura, no se observaron en el grupo tratado con 555 mgm. de DDS en forma de diasone. La concentración sanguínea parece relacionarse con la composición química de la droga, y variaciones individuales se pueden observar.

La detección de anemia por medio de determinaciones de la hemoglobina es un índice razonable para calcular las dosis óptimas individuales. Cuando ocurrió anemia, se regresó a valores normales luego de la interrupción del tratamiento, sin necesitar el uso de antianémicos.

Acknowledgment: The authors are indebted to Dr. G. Rosenfeld for the planning of the work and for his advice.

REFERENCES

1. ALLDAY, E. J. and BARNES, J. Toxic effects of diaminodiphenylsulphone in the treatment of leprosy. *Lancet* **2** (1951) 205-206.
2. BROWNLEE, G. Sulphetrone; therapeutics and toxicology. *Lancet* **2** (1948) 131-134.
3. BROWNLEE, G., GREEN, A. F. and WOODBINE, M. Sulphetrone, a chemotherapeutic agent for tuberculosis; pharmacology and chemotherapy. *British J. Pharmacol. & Chemother.* **3** (1948) 15-28.
4. FAGET, G. H., POGGE, R. C., JOHANSEN, F. A., DINAN, J. F., PREJEAN, B. M. and ECCLES, C. G. The promin treatment of leprosy; a progress report. *Publ. Hlth. Rep.* **58** (1943) 1729-1741.
5. FERNANDEZ, J. M. M., CARBONI, E. A., TOMASSINO, P. and GIMENEZ, M. M. Hematologic study of leprosy patients treated with diasone. *Internat. J. Leprosy* **16** (1948) 319-327; *Mem. V Congr. Internac. Lepra, Havana, 1948; Havana, 1949*, pp. 180-188.
6. LOWE, J. Treatment of leprosy with diamino-diphenyl sulphone by mouth. *Lancet* **1** (1950) 145-150.
7. ROSENFELD, G., RZEPPA, H., NAHAS, L. and SCHEMBERG, S. Hemolysis and blood concentration of sulphones *in vivo*. *Mem. Inst. Butantan* **24** (1952) 69-76.
8. SMITH, M. A pharmacological study of three sulphones. III. The specific toxic phenomena. *Leprosy Rev.* **21** (1950) 17-29.