THE TREATMENT OF LEPROMATOUS LEPROSY WITH 4:4′-DIAMINODIPHENYL SULFONE IN OIL

FINDINGS IN 100 CASES TREATED FOR ONE YEAR

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WITH A NOTE ON THE TECHNIQUE OF SULFONE DETERMINATIONS

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INTRODUCTION

The substance 4,4′-diaminodiphenyl sulfone ("DDS"), the parent nucleus of the derivatives to which the general term "sulfones" is ordinarily applied, was synthesized in 1908, first tried out in animal infections—streptococcic—in 1937, and then in experimental tuberculosis in 1940 and 1941. To overcome its high toxicity, certain derivatives were promptly made, the year 1937 seeing the production of and experimental work with a diacetyl derivative (Fourneau *et al;* "1358F"), the formaldehyde sulfoxylate derivative (Bauer and Rosenthal; "diasone"), the didextrose derivative (Parke, Davis & Co.; "promin"), and in England a product containing four phenyl rings (Burroughs, Wellcome & Co., "sulphetrone"). For a time attention was concentrated on promin, it being the first derivative to be tried out in the therapy of tuberculosis and then of leprosy.

The parent substance was not used in clinical work at all until it was tried out in leprosy by Cochrane (1), who employed it in an oily suspension given by injection. In a recent analysis of his experimental work (2) he makes the following statement:

> "We have accumulated clinical evidence that diamino-diphenyl-sulfone in a 25% suspension of ground nut oil is probably the most potent anti-leprosy remedy we have. . . . We however have found that apart from the reactions which are produced, when the remedy is continuously administered there are certain toxic signs, some of which are serious."

In our observations here reported, which confirm the efficacy of diaminodiphenyl sulfone, we have not found the complications serious with the dosage employed.

PERSONAL OBSERVATIONS

It was on the advice of Cochrane that we decided to experiment with the drug at Sungei Buloh Settlement where, particularly in the Chinese portion of the community, leprosy is of an acute and rapidly progressive kind. A majority of the cases are lepromatous, and a high frequency of minor tuberculoid and atypical cases become of that nature.

Segregation at the moment is compulsory, which results in many cases remaining hidden until so far advanced that their economic situation necessitates their coming to the Settlement. Perhaps no more than 1 case in 15 in Malaya is under some sort of treatment. The idea of a safe injectable sulfone of reasonable cost appealed greatly, and it was with a view to later outpatient treatment that this work was undertaken.

A suitable group of cases was selected, in the end totalling 100, and treatment was started in June 1948; one case only (No. 13 of this report) was started in November. Most of them were definitely lepromatous, although some had become that by conversion, but two of those illustrated here (Cases 13 and 14) were of the atypical kind. Several showed scars of the ulcerative tuberculoid condition described from this institution by Ryrie (3). Cases were selected so as to give, as nearly as possible, equal numbers in the different stages of advancement. As will be seen in Table 2, there are few cases of the lesser degree of advancement, as they do not come into the Settlement. The ages varied from 18 to 54 years, with a majority near 30; and there were included 16 children between the ages of 6 and 16 years. Most of these patients were Chinese, with some Indians and Malays. There were 59 males and 41 females.

DOSAGE AND MODE OF ADMINISTRATION

We have used throughout a 20 per cent suspension of the diaminodiphenyl sulfone in purified and deodorised neutral coconut oil with 0.5 per cent phenol, instead of ground-nut (peanut) oil which Cochrane used. The coconut oil will pass through a medium-sized (gauge 23) hypodermic needle, and has been found satisfactory. All injections were given deep in the subcutis.

Partly from caution but mostly from shortage of supply, the dosage was started as low as 1 cc., or 0.2 gm. of the substance, and for the first two months the injections were given once a week. The dosage has now been increased, in some cases to 5 cc. (1 gm.) weekly, in 2 injections per week of 2.5 cc. (0.5 gm.)

each. We intend, in cases showing good tolerance, to push the dosage still higher in the future.

All patients have received routine iron and yeast. The settlement diet is well balanced, and it provides about 3,000 calories per day, but we notice that patients who are in a position to supplement their ration with such things as eggs, butter and extra meat show less tendency to anemia than others.

All cases but one were put under treatment in June 1948, and had had treatment for a period of one year at the time of preparation of this report; Case 13 was not put under treatment until November, and so had been treated only 8 months. So far we have given no rest periods.

BACTERIOLOGICAL EXAMINATIONS

Smears taken from earlobes and other selected sites have been examined periodically. At present we are too short of personnel to enable us to determine Cochrane's "bacteriological index" on each case. Our findings in the smears are graded as follows:

3+ Heavy infection; bacilli massed, with globi, clusters, etc.
2+ Many bacilli in each field.
1+ Few bacilli in each field, less than 10.
± Very few bacilli; in many fields none (e.g., 4 in 30 fields).

These examinations were all made by the same two workers, and the results may be taken as a fair indication of conditions even if they are less accurate than a bacteriological index. The gap between 2+ and 3+ is so great that our scale might be modified by extension, changing the present 3+ to 4+ and making 3+ intermediate, for we feel that an improvement from 3+ to 2+ is greater than the figures would suggest, and vice versa.

DETERMINATIONS OF SULFONE CONCENTRATIONS

Blood levels.—Little variation has been seen in the blood levels. They have ranged between 0.1 and 0.9 mgm. per 100 cc. of blood, and have been very constant. It is to attain constancy that injections are given twice weekly, and that practice is supported by the excretion rate. Excretion is largely in the urine, and one-quarter of a 0.4 gm. dose is excreted within 36 hours after injection.

Tissue levels.—These determinations have been unsatisfactory. Even using ethyl chloride as the local anesthetic in removing the tissue, and avoiding injection sites, we have still obtained inexplicably high readings (see appendix).

Post-mortem tissues, obtained from two cases dead of pulmonary tuberculosis, gave the figures shown in Table 1. These patients, neither of whom was in the original series, had received totals of 8 and 10 gm. respectively over a period of 3 months.

TABLE 1.—*Sulfone (DDS) concentration in the tissues of two autopsied patients after 3 months treatment (mgm.%).*

Tissue	Case 1	Case 2
Skin	3.75	4.9
Lungs	3.75	3.4
Spleen	3.10	4.0
Kidney	3.70	6.6
Liver	2.10	6.1
Sciatic nerve	------	2.5
Bone marrow	------	1.8
Heart muscle	------	3.4

RESULTS

Of the 100 cases treated, 96 have shown clinical improvement, some to a remarkable extent. Selected case histories are given later on. Only 4 cases remained clinically unaltered, and not one has deteriorated—an event which, with hydnocarpus treatment, would be exceptional among our patients.

Bacteriological improvement has been seen in 27 cases, while 64 remained stationary; in 9 there was an increase in the numbers of bacilli found in the smears, yet it will be noticed that these cases had not deteriorated clinically.

TABLE 2.—*Changes of status, clinically and bacteriologically, after one year's treatment.*

Advancement	No. of cases	Improved		Stationary		Worse	
		Clin.	Bact.	Clin.	Bact.	Clin.	Bact.
Marked	34	34	4	----	30	----	----
Moderate	59	57	21	2	31	----	7
Least	7	5	2	2	3	----	2
TOTALS	100	96	27	4	64	----	9

Of the 27 cases showing bacteriological improvement, 21 were in the moderately advanced group containing 59 cases. On the other hand, of the 34 cases in the advanced group, all of whom showed clinical improvement (see Table 2), only 4 had improved bacteriologically at the end of the period.

In general, apparent clinical improvement in these cases began after 4 to 6 weeks of treatment, and was evidenced by a flattening of nodules and areas of infiltration, together with a change in color, first from red to purplish, becoming bronzed, and then beginning to fade. Superficial ulcers, even those of long duration, healed rapidly and soundly and did not tend to break down again. This change is particularly spectacular in patients with multiple superficial ulcers.

Nasopharyngeal ulcers have responded well, edema being rapidly diminished with corresponding improvement in the ease of breathing. In two cases (not in this series) with ulcerating laryngitis and gross dyspnea and aphonia, 0.4 gm. of the sulfone was given daily for a week and 0.2 gm. twice weekly thereafter. In both cases impending tracheotomy was averted, and healing was obtained with considerable improvement in voice.

Results in children.—The 16 children in the series tolerated the drug extremely well, and 15 have improved clinically. Bacteriologically, 5 have shown improvement, one is stationary, and two show increased numbers of bacilli in smears. As might be expected, however, the reaction rate was higher than in adults; 6 children needed hospital treatment and 7 more had milder attacks. The majority were given 0.2 gm. twice weekly; two of them got 0.4 gm. twice weekly.

Iritis.—Some of the patients, who had suffered from frequent attacks of iritis and iridocyclitis when under hydnocarpus therapy, have all shown improvement. None developed severe iritis after commencement of the sulfone therapy. During the last six months the incidence of iritis in this group has been almost nil.

Neuritis.—This condition we find aggravated in some cases, but except in one case we have recorded no symptoms beyond paresthesia and pain. There has been improvement of sensation in hands and feet in a few cases.

Lepra reaction.—Reactions were found to become less severe and less frequent as treatment progressed. This condition will be referred to later as a complication of treatment.

Necrosis in nodules.—In many nodular cases, nodules have softened with the formation of cold pus, heavily laden with

bacilli. These lesions have healed leaving small scars, nothing more.

Pregnancy.—Six patients who have been on this drug for 6 months or more have given birth at full term. In none of them was there any reaction following delivery, or deterioration of the leprosy during the final months of pregnancy. This had not been the case in previous pregnancies of three of these patients when on hydnocarpus oil; they had had severe postpartum reactions as well as marked progression of the disease during the final months.

Pulmonary tuberculosis.—In conjunction with lepromatous leprosy, pulmonary tuberculosis produces a vicious circle, usually quite rapidly fatal. Three patients in this group are known to have this complication.

One patient, advanced L3, had early apical tuberculosis, laryngitis and ulceration of the soft palate, and cervical adenitis with sinus. The ulceration of the soft palate and larynx healed within 6 weeks, with disappearance of the adenitis and healing of the sinus. There was no progress in the tuberculous lesion of the lung for the first six months (confirmed by x-ray), but at the end of one year there was some increased shadow with possible cavity formation.

The second patient, also L3, had advanced bilateral fibrocaseous tuberculosis with cavities. There has been marked general improvement, with gain of weight, and his frequent lepra reactions have ceased. An x-ray examination at the end of the year of treatment showed much improvement of the lung lesions, although cavities are still present.

The third tuberculous patient, a young Chinese woman, has remained stationary and has had two reactions needing hospital treatment. A far more acute case of tuberculosis than the others, she is holding her own; but we feel that the prognosis here is not so hopeful. Her leprosy has remained stationary.

COMPLICATIONS OF TREATMENT

On the whole, complications have been very light, and none except neuritis has given any difficulty.

Secondary anemia.—As stated earlier, all our cases have have received iron and yeast throughout the treatment, and we have experienced no trouble with severe anemia. It has not been necessary to interrupt treatment in any case on this account. A few injections of liver cause rapid improvement of the blood count when it drops.

Lepra reaction.—During the year 71 out of the 100 patients had reactions, and 27 of them required hospital treatment, but the treatment was not interrupted. The longest period in the hospital was 5 weeks. These reactions occurred mostly after about 8 gm. of the drug had been administered.

Neuritis.—Complaints of nerve pain have been registered by 15 patients, of whom 2 had had this trouble before; 6 had severe attacks, needing hospital treatment. In all of the cases the ulnar nerve was affected, and in 2 the peroneal as well. We find the injection of a local anesthetic in oil to be of great value in these cases.

In one case, a child of 14, operation was necessary. The sheath of the ulnar nerve was found much thickened and edematous, and it cut with a noise like tearing cloth. The sheath was split for a distance of 2½ inches, which relieved the pain for a period of 2 months, since when he has had milder recurrences.

One case developed ulnar pain with thickening of the nerve and contracture of the little finger. The drug was stopped for a week, and intensive vitamin B therapy was given, with splinting of the finger. This patient has recovered completely except for slight weakness of the finger.

Giddiness.—One-half of the patients suffered from slight transient giddiness in the beginning of treatment. None complained of nausea or vomiting.

Jaundice.—Two patients had mild attacks of jaundice. It is possible that this was of infectious origin, as there were other cases at the time. There has been no recurrence of this condition.

REPORT OF CASES

CASE 1.—Local-born Chinese, female, age 26 years. Onset of disease, and early admission into Sungei Buloh, in 1937; classified as tuberculoid neural. Treated with hydnocarpus oil and discharged in 1938. Readmitted in 1942 because of relapse. Treated for two years with large doses of hydnocarpus oil; repeated reactions. Pregnancy in 1947 with acute exacerbation in final stages and puerperium, and the same in 1948. Disease progressing.

Condition at the beginning of the sulfone treatment (June 1948): Large raised plaques on arms, legs and buttocks; skin of face thickened and nodular; loss of outer half eyebrows. Smears all 3+. After 82 cc. of the DDS suspension (i. e., 16.4 gm. of the substances)[1] had been administered she was delivered of a full term healthy baby. No reaction during the course of treatment or following delivery. Hemoglobin rose from 55 to 65% (Sahli) at the end. Red blood cell counts, 3.1 to 4.5 millions. Blood sulfone level, 0.6-0.40 mgm%. Total drug given during the year, 23.4 grams.

Result: Great clinical improvement, flattening and absorption of nodules, loss of edema. Smears now all 2+. General health good.

CASE 2.—Chinese, female, age 22. Onset in 1937; admitted to Penang colony in 1943; transferred to Sungei Buloh in 1947. On and off she had four years treatment with hydnocarpus oil, becoming worse.

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[1] Quantities of the drug stated in volume refer to the 20% suspension in oil; those stated in grams refer to the amount of the substance.

Condition: Moderately severe L2, with nodules of face, back and limbs, lepromatous infiltration in edge of scar on cheek presumably due to a previous ulcerating tuberculoid reaction. Constant reactions. Smears all 3+. During treatment a few recurrent reaction spots appeared, and there was one bad reaction after 8 gm., lasting for 4 weeks. After 16 gm. she was delivered of a full term healthy baby; no reaction. Since then treatment has been uneventful. Final hemoglobin, 65%. Red blood cells, 3.7 millions. Blood sulfone, 0.24-0.71 mgm%. Total drugs, 21.4 gm.

Result: Marked clinical improvement (see Figs. 1 and 2). Smears stationary at 3+.

CASE 3.—Tamil, female, age 40; born in India and came to Malaya age 16. Onset of disease, 1940; admitted into settlement, 1946. Treated with hydnocarpus oil; frequent reactions and nerve pain.

Condition: Gross generalized infiltrations, nodules of face, slight clawing of fingers. Smears all 3+. During treatment there were occasional slight reactions and some nerve pain, but far less than on hydnocarpus oil. Final hemoglobin, 55%. Red blood cells 2.4 millions. Blood sulfone, 0.30-0.53 mgm%. Total drug, 25 gm.

Result: Much improved. Smears now all 2+. General health good.

CASE 4.—Chinese, female, age 28. Onset of disease, 1927; admitted into settlement, 1939. Treated with hydnocarpus oil except during the war years. Leprosy progressed.

Condition: Gross, generalized, florid infiltration of entire body; nose blocked. Smears all 3+. A severe reaction occurred after 4 gm. of treatment; otherwise uneventful apart from slight anemia. Final hemoglobin, 60%. Red blood cells, 3.3 millions. Blood sulfone, 0.10-0.34 mgm%. Total sulfone, 23 gm.

Result: Infiltration subsiding, fingers becoming normal, edema decreasing (see Figs. 3 & 4). Nose unblocked, good airway, no ulcers. Smears 3+, unchanged.

CASE 5.—Tamil boy, age 18, born in Malaya. Onset of disease, 1942; admitted into settlement, 1948. Had few injections of hydnocarpus oil.

Condition: Large fleshy nodules of face and lips, arms and buttocks; macules over chest. Smears all 3+. During treatment, a severe reaction after 4 gm.; slight jaundice after 14 gm.; treatment interrupted for three weeks. Final hemoglobin, 60%. Red blood cells 4.2 millions. Blood sulfone, 0.21 mgm%. Total sulfone, 22 gm.

Result: Great clinical improvement, absorption of nodules (see Figs. 5 and 6). Smears still 3+.

CASE 6.—Male Chinese, age 42, local born. Onset of disease, 1928; admitted into settlement, 1935. Treated with hydnocarpus oil with no improvement. No treatment during the war, and the disease remained stationary; it flared up following liberation and increase of rations.

Condition: Diffuse nodular type ulcerations; the whole body affected with generalized infiltration. Nose blocked. Smears all 3+. During treatment has had no reaction or complication. Final hemoglobin, 65%. Red blood cells, 4.2 millions. Blood sulfone, 0.35 mgm%. Total sulfone, 28.2 gm.

Result: Infiltration reduced, nodules flattened, good nasal airway and ulcerations healed (see Figs. 7 and 8). Smears now 2+.

CASE 7.—Chinese, female, age 22, local born. Mother and uncle both lepromatous cases. Onset of disease, 1937; admitted into settlement, 1939. Treated with hydnocarpus oil and esters until 1942 and was improving. Disease stationary during the war, but flared up following liberation.

Condition: Diffuse infiltration of face, trunk and limbs. Perforating ulcers, left foot. Smears 2+, with one patch 3+. During treatment a mild reaction occurred, with anemia (hemoglobin 55%) after 16 gm. Final hemoglobin, 60%. Red blood cells, 3.5 millions. Blood sulfone, 0.1 rising to 0.3 mgm%. Total sulfone, 27.2 gm.

Result: Clinically improved, infiltration subsiding, edema of face cleared, ulcer healed. Smears all 3+, more than before treatment.

CASE 8.— Tamil, male, age 30, local born. Onset of disease, 1934; admitted into settlement, 1945. Received hydnocarpus oil treatment, without improvement; frequent reactions, often incapacitating, with iritis and ulcerations of hands and feet.

Condition: As stated. Smears all 3+. Treatment brought about rapid improvement and healing of ulcers. Slight reaction with anemia (hemoglobin, 55%, red blood cells, 3.8 millions) at 8 gm.; responded to liver and iron. Final hemoglobin, 60%. Red blood cells, 3.4 millions. Blood sulfone, 0.17-0.4 mgm%. Total sulfone, 17.4 gm.

Result: Ulcerations healed, nodules flattened, iritis improved, no acute attacks. Smears 1+ and 3+. General condition much improved.

CASE 9.—Tamil, male, age 31, local born. Grandmother a lepromatous case living with the family when the patient was a child. Onset of disease, 1930; admitted into settlement, 1938. Improved on hydnocarpus oil until the war; deteriorated during the war, and did not improve on hydnocarpus oil after it. Frequent reactions with iritis.

Condition: Moderately advanced L2. Diffuse infiltration of whole body, with nodules of face and arms. Smears 3+. During treatment moderate reactions at 6 gm., mild anemia at 8 gm. Final hemoglobin, 65%. Red blood cells, 4.2 millions. Blood sulfone, 0.24-0.42 mgm%. Total sulfone, 17.4 gm.

Result: Great clinical improvement, with subsidence of lesions (see Figs. 9 and 10). Smears still 3+.

CASE 10.—Chinese, female, age 23, local born. Onset of disease, 1938; admitted into settlement, 1946. Condition deteriorated with hydnocarpus oil treatment.

Condition: Moderately advanced L2. Diffuse infiltrated lesions of face, trunk and legs; nodules on arms. Smears 3+. During treatment occasional mild reactions. Final hemoglobin, 60%. Red blood cells, 3.4 millions. Blood sulfone, 0.42-0.57 mgm%. Total sulfone, 18 gm.

Result: Marked improvement, nodules of arms almost disappeared (see Figs. 11 and 12). Smears 2+.

CASE 11.—Chinese, male, age 29, local born. Mother a lepromatous case, also an inmate. Onset of disease, 1937; admitted into settlement, 1941. Deteriorated during the war, but remained stationary on hydnocarpus oil treatment from 1946 to 1948.

Condition: L2 case with infiltration of arms, face and legs, ears thickened. Smears 1+, 2+, and patches 3+. No complications during

treatment. Final hemoglobin, 65%. Red blood cells, 5.0 millions. Blood sulfone level 0.22-0.5 mgm%. Total sulfone, 18 gm.

Result: Marked improvement (see Figs. 13 and 14). Smears unaltered. Has gained in weight.

CASE 12.—Chinese, female, age 34, local born. Onset of disease, 1930; admitted into settlement, 1937.

Condition: Rapid deterioration, with severe ulcerations of legs and hands. Had been in hospital with multiple ulcers for 9 months without improvement, resisting all forms of treatment including penicillin. Smears 3+. During treatment, occasional mild reactions but nothing else. Ulcers healed rapidly and soundly. Final hemoglobin, 60%. Red blood cells, 3.8 millions. Blood sulfone, 0.7 mgm%. Total sulfone, 16.2 gm.

Result: Great improvement, ulcers healed (see Figs. 15 and 16). Airway good and voice improved. Smears still 3+.

CASE 13.—Chinese male, age 51, born in China. Onset of disease, 1947; admitted into settlement, 1948. Condition on admission, raised erythematous plaque over forehead and nose, gross swelling of lips, eye closed through edema. Multiple annular and crater-like papules over body. No nerve involvement. Smears all 3+. (A clinically "atypical" case; see Fig. 17.) Section: atypical leproma.

Sulfone treatment was begun in November 1948, without previous treatment. There were no complications. Final hemoglobin, 65%. Red blood cells, 4.1 millions. Blood sulfone, 0.4 mgm%. Total sulfone, 10.2 gm.

Result: Great improvement, clinically and bacteriologically (cf Figs. 17 and 18). Smears now 1+ and ±.

CASE 14.—Chinese, male, age 23, local born. Onset of disease, 1941; admitted into settlement, 1948. Moderately advanced L2 with thick, fleshy infiltrations of face, arms and legs. Ulnar nerves thickened. Smears 1+. (An "atypical" case; see Fig. 19.)

Under sulfone treatment, after 8 gm., mild ulnar neuritis with pain was complained of. Final hemoglobin, 60%. Red blood cells, 3.9 millions. Blood sulfone, not determined. Total sulfone, 12.4 gm.

Result: Much improved, lesions subsided and flush with surrounding skin (cf Figs. 19 and 20); nerve still thickened. Smears still 1+. Probably atypical leproma.

DISCUSSION

It does not appear that the relatively low dosage used in this series of cases—1 gram weekly, as compared with 2.5 grams weekly given by Cochrane—has lessened the effectiveness of the remedy. The reduction in numbers of bacilli in the smears of 27 out of the 100 within one year of commencing treatment is especially encouraging. Furthermore, we regard the control of reaction, particularly that associated with pregnancy, as of great importance.

The administration of the drug by injection is of value from the psychological point of view, for in Malaya all injections are considered superior to mere oral therapy. It also ensures the dose being taken.

The effect of diaminodiphenyl sulfone in healing ulcerations is a great saver of dressings. It is one of the first effects we noticed, and one which takes place even on 0.2 grams weekly, a dose which does not cause complications even in grossly anemic and debilitated patients.

We have gained the impression that, with the kinds of cases in our institution, the dosage cannot be pushed very much higher than those employed by us without causing trouble. The individual tolerance appears to vary, often with the size of the patient; as a rule the larger the patient the larger the dose he can stand. Up to 1 gram weekly, we have not been troubled with complications. What complications have occurred have been mild, and apart from the neuritis none were difficult to manage.

We have a parallel series of cases on sulphetrone which has just finished 12 months of treatment. Individual tolerance varied but the total amounts of drug taken varied between 500 and 1,000 grams. The results were very much in line with those obtained in the DDS group.

The cost of diaminodiphenyl sulfone is in the neighborhood of £6 sterling per kilogram, and even allowing for the cost of sterilizing and bottling it is still remarkably cheap, for the cost of treatment for one year is only ten shillings (U. S. $2.00) or less per patient. The cost of 1,000 gm. of sulphetrone tablets is £7-10s.

The results obtained in this group of sulfone-treated patients have raised the morale of this place greatly. The treatment is now being run almost as the routine one, with over 1,000 patients on it. We cannot personally do more than supervise that number, so the actual work has to be done mostly by the dressers; but our experience in this series has shown what difficulties may be expected and has permitted the extension of the treatment to a majority of the patients in the institution.

SUMMARY

One hundred cases of lepromatous and atypical leprosy have been treated for one year with 4,4′-diaminodiphenyl sulfone (DDS) 20% in oil. Clinical improvement occurred in 96 of them, and bacteriological improvement in 27. The matters of dosage and complication are discussed, and selected cases with photographs are recorded.[2]

[2] The author submitted photographs of all of the cases related, but it was agreed that only those of the ten cases involved in Plates (8) to (12) should be reproduced.—EDITOR.

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REFERENCES

1. COCHRANE, R. G. A comparison of sulphone and hydnocarpus therapy of leprosy. Internat. J. Leprosy **16** (1948) 139-144.
2. COCHRANE, R. G., RAMANUJAM, K., PAUL, H. and RUSSELL, D. Two-and-a-half years' experimental work on the sulphone group of drugs. Lep. Rev. **20** (1949) 4-64.
3. RYRIE, G. A. Acute ulcerative or sloughing tuberculoid leprosy. Internat. J. Leprosy **6** (1938) 153-160.

APPPENDIX. METHOD OF SULFONE ESTIMATIONS

(DR. I. A. SIMPSON)

The method which has been used is based on the development of a purplish red color by coupling the diazotized sulfone (4,4'-diaminodiphenyl sulfone) with N-(1-naphthyl)-ethylene diamine.

At first, N-sulphate-ethyl-m-toluidine was tried as the coupling agent. It was found, however, that in our hands the intensity of the color thus developed by filtrates from samples of blood to which known amounts of sulfone had been added in vitro was only about 70 per cent of that developed by aqueous solutions containing a similar amount of sulfone.

With the use of N-(1-naphthyl)-ethylene diamine, a "recovery" of 95 per cent was obtained from blood filtrates, and the intensity of color development was adequate for the concentrations handled. The method described below has given satisfactory results with samples of blood and urine; but difficulties, which are still under examination, have been experienced in the analysis of samples of tissue.

BLOOD

To 1.0 ml. of oxalated blood, diluted to 6.0 ml. with distilled water, 5.0 ml. of 2N HCl are added and the mixture is well stirred. It has been found convenient to use for this purpose a glass rod which has been flattened at one end and loosely fits the tube. After the addition of 4.0 ml. of 12% trichloracetic acid, the liquid is filtered through a 9 cm. No. 5 Whatman paper.

To 10 ml. of the clear, colorless filtrate, the following solutions are added, mixing well after each addition: (*a*) 3 drops of 0.3% sodium

nitrite,[3] after which the mixture is allowed to stand for three minutes; (*b*) 3 drops of 1.5% ammonium sulphamate, standing for a further two minutes; and (*c*) 3 drops of 0.1% N-(1-naphthyl)-ethylene diamine hydrochloride. The solution is poured into a colorimeter tube, which is kept in the dark for twenty minutes to allow full development of the color; the comparison is then made with the color developed by standards prepared from the pure sulfone.

Simultaneously with the treatment of the samples, a reagent blank is prepared by replacing the 1.0 ml. of blood with 1.0 ml. of distilled water, all other details of the procedure being followed as described. Before taking readings with the photoelectric absorptiometer (we have found a Coleman Junior spectrophotometer very convenient), the scale should be set at zero against the reagent blank.

It has been our practice, also, to prepare at the same time two standards containing known amounts of sulfone. These are submitted to the procedure as described above, and the color developed is compared with that developed by the unknown samples. The strengths of the standard solutions are as follows:

Stock solution.—0.05 gm. of sulfone dissolved in 500 ml. distilled water. This solution remains stable for several months if kept in a refrigerator.

Working solution.—20 ml. of stock solution is diluted to 100 ml. with distilled water just before use. The diluted solution then contains 20 milligrams of sulfone per milliliter.

For the stronger standard, 1.0 ml. of working solution (=20 mgm. of sulfone) is taken; and for the weaker standard, 0.2 ml. (=4 mgm. of sulfone) is used. In each case the volume is made up accurately to 6.0 ml. with distilled water, and the method is proceeded with as described.

Comparison of the developed color.—It has been found that the color developed by coupling 4,4'-diaminodiphenyl sulfone with N-(1-naphthyl)-ethylene diamine posesses maximum absorption at $\lambda = 550$. All readings with the spectrophotometer used are therefore taken at that wavelength. The galvanometer readings are taken directly from the scale:

$$\text{Optical density: "D"} = -\log T$$

where T = percentage transmission.

If 1.0 ml. of blood is taken and the weaker standard solution is used for comparison, then:

$$\left.\begin{array}{l}\text{Milligrams of sulfone} \\ \text{per 100 ml. of blood}\end{array}\right\} = \frac{0.4 \times Db}{Ds}$$

where Db = reading of sample, and Ds = reading of standard.

URINE

For samples of urine, the procedure described for blood has been found to be satisfactory, but preliminary dilution of the urine with water is

[3] The 0.3% sodium nitrite solution must be made up fresh immediately before use, by diluting 0.3 ml. of 50% sodium nitrite solution to 50 ml. with water. The 50% sodium nitrite solution remains stable if kept in a refrigerator.

necessary. With the concentrations of sulfone found in the samples of urine examined, it is convenient to use aliquots of 1.0 to 6.0 ml. of a 1:50 dilution. In the case of a few samples, however, where the concentration was low, the preliminary dilution was 1 in 10 only. Clarification with trichloracetic acid (12%) and color development was then carried out as already described for blood.

TISSUES

Some difficulty has been experienced with the examination of samples of tissue. At the beginning, fallacious results were obtained due to the fact that the local anesthetic used (procaine) also gave rise to color development when submitted to the procedure described. It appears that other workers in this field have had similar experiences. When this difficulty was recognized and overcome it was found that, in many cases, the color developed from extracts of tissue possessed a markedly pinker shade than the color developed from standards or from samples of blood. It was considered that results obtained by the comparison of two different shades of color could not be relied upon, until more information could be obtained on the causes of the change. This point is at present under examination. It is recorded, however, that extracts of tissues from guinea pigs which have received injections of sulfone in oil over a period of two or three months do not show this change in color.

DESCRIPTION OF PLATES

PLATE 8 (1)

FIGS. 1 and 2. Case 2, before treatment and after one year of treatment.

FIGS. 3 and 4. Case 4, before treatment and after one year of treatment.

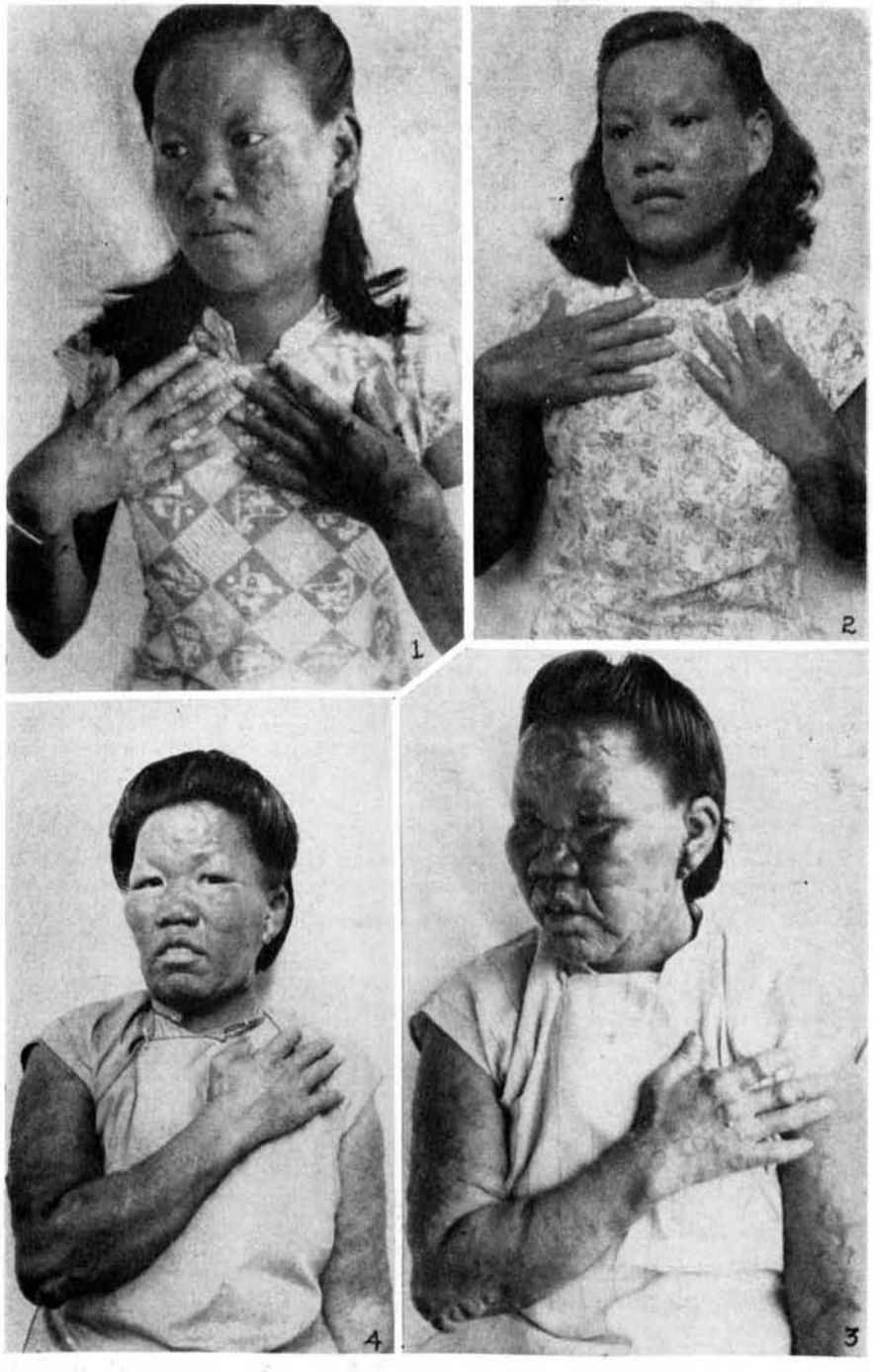

PLATE 8

PLATE 9 (2)

FIGS. 5 and 6. Case 5, before treatment and after one year of treatment.

FIGS. 7 and 8. Case 6, before treatment and after one year of treatment.

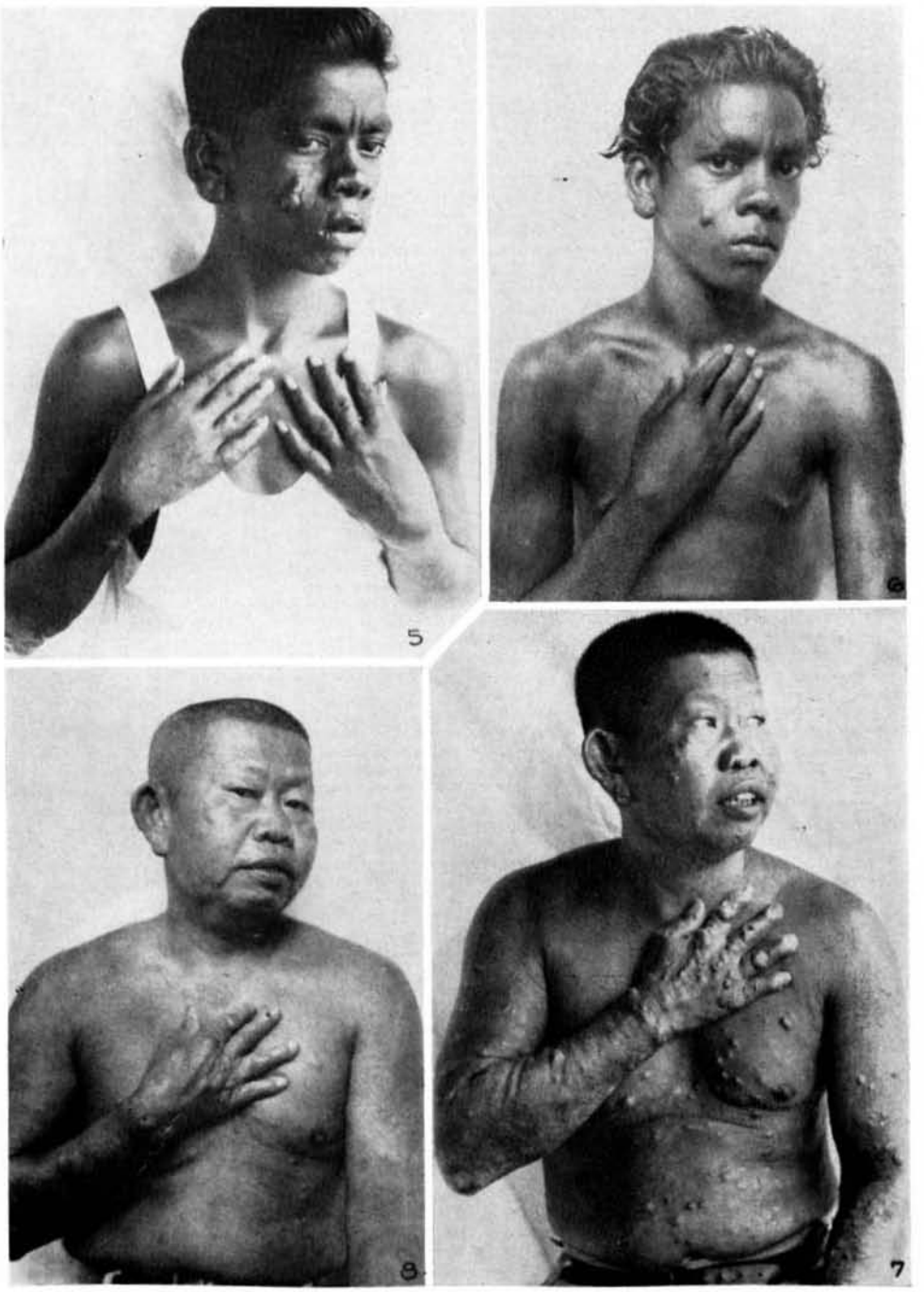

PLATE 9

PLATE 10 (3)

FIGS. 9 and 10. Case 9, before treatment and after one year of treatment.

FIGS. 11 and 12. Case 10, before treatment and after one year of treatment.

PLATE 10

PLATE 11 (4)

FIGS. 13 and 14. Case 11, before treatment and after one year of treatment.

FIGS. 15 and 16. Case 12, before treatment and after one year of treatment.

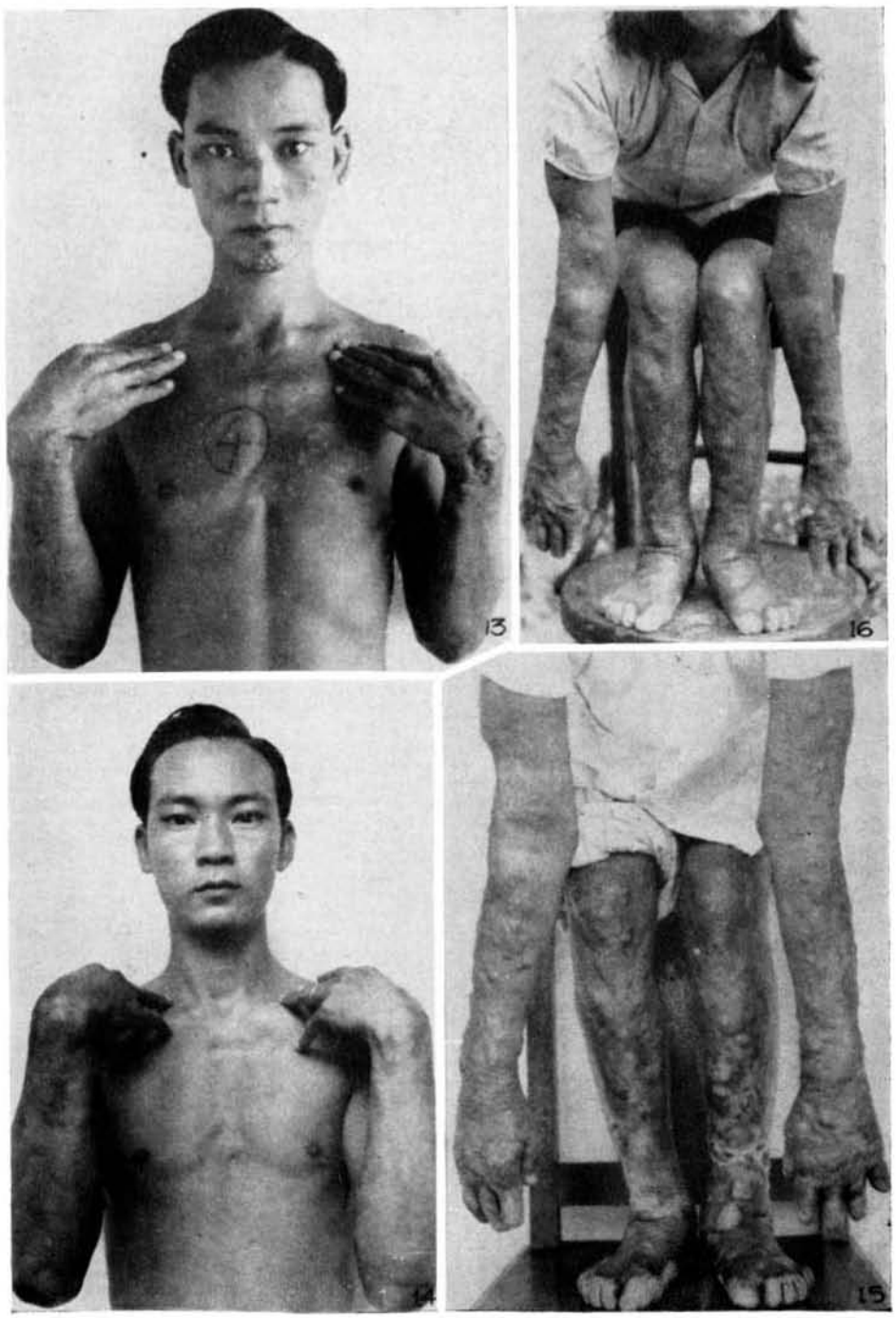

PLATE 11

PLATE 12 (5)

FIGS. 17 and 18. Case 13, before treatment and after one year of treatment.

FIGS. 19 and 20. Case 14, before treatment and after one year of treatment.

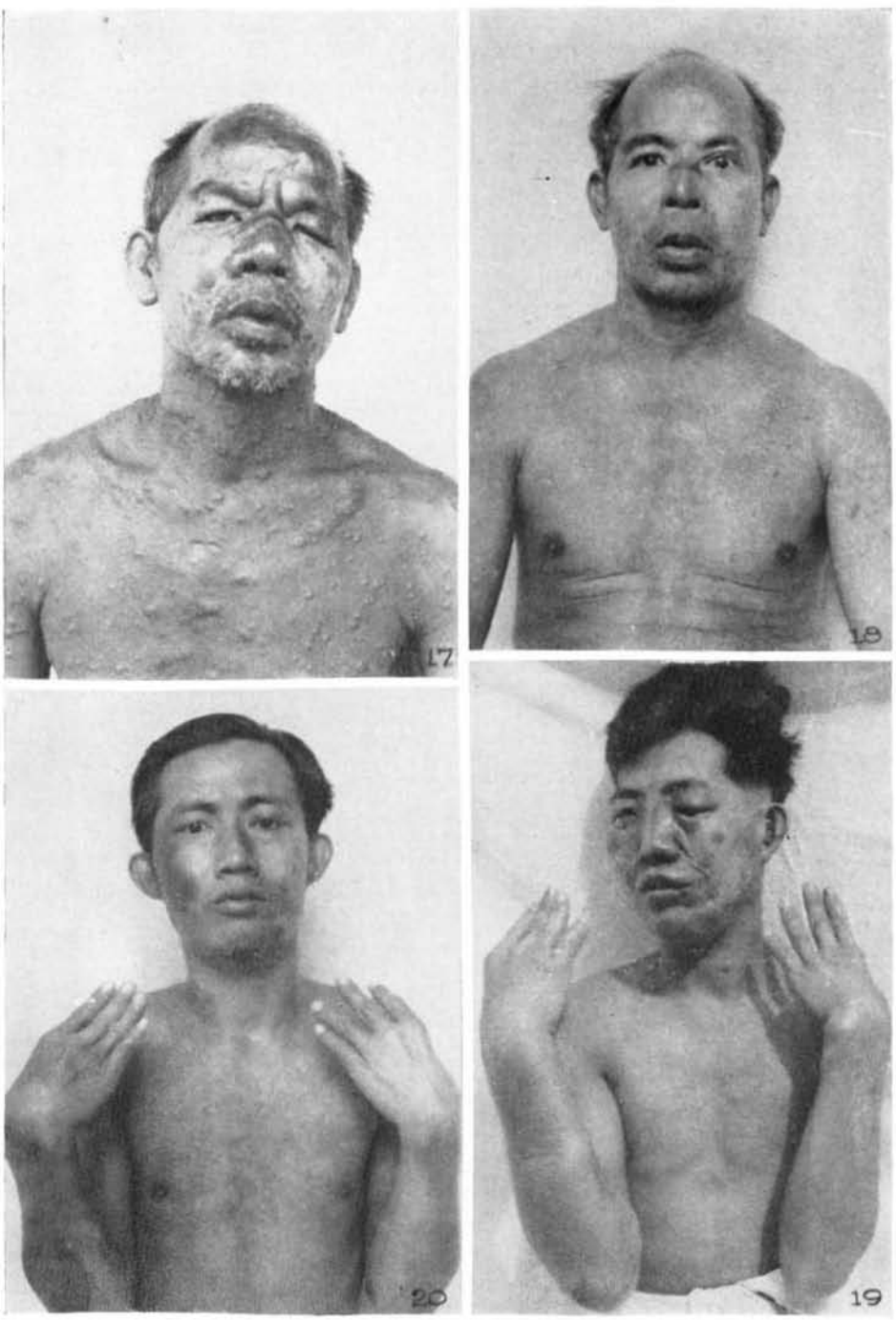

PLATE 12