ON THE USE OF FLUORESCEIN AND PHTHALLIC ACID IN LEPROSY

By Gordon A. Ryrie

Medical Superintendent, Sungei Buloh Settlements, Federated Malay States

INTRODUCTION

In a previous paper on the use of aniline dyes in leprosy the writer reported an apparently specific interference with the leprotic process that had been observed in certain proportions of cases treated by intravenous injections of trypan blue, brilliant green and fluorescein. Continued observation showed that the majority of these cases relapsed within six months following cessation of treatment. Analysis of the relapses is an involved process, and only the conclusions are given here.

(1). The relapse rates in the cases treated with trypan blue and brilliant green were very high. The only feature common to these two dyes, whether in their chemical analysis or clinical effect, appears to be the selective staining of lesions. This phenomenon is found with other dyes which in our hands have had no therapeutic effect. It was, therefore, difficult to plan out further progressive work.

(2). The relapse rate with fluorescein appeared to be less than with the other drugs mentioned.

Samples of fluorescein from different chemical firms were found to vary in color and purity and they appeared to have different therapeutic effects. From this it was decided (a) to make comparative experiments with different samples of fluorescein bought from separate firms, and (b) to carry out other experiments based on the chemical analysis of the dye.

I. COMPARISON OF FLUORESCEIN SAMPLES

For the first part of this program four samples of fluorescein were chosen, here designated A, B, C, and D. The patients chosen for experiment were all examined and classified in a uniform manner. Volunteers of varied classification were chosen, but as far as possible those cases were selected which had sharply defined skin lesions. The lesions were of over a year's duration in each case, and no patient

was chosen who had a history of lepra reaction in the preceding six months.

The cases were kept under ward supervision, and except where the nationalities varied there was no difference in food. Treatment was by intravenous injections of 20 cc. of 2 per cent solutions of fluorescein, the injections being given twice weekly for four months. No other treatment, specific or nonspecific, was given. The morning and evening temperatures were taken throughout the experiment; except for an occasional slight rise after an injection all the cases remained normal. Each patient was weighed every fortnight. His blood sedimentation rate was taken at the same intervals. The Wassermann and Kahn reactions were made in each case. Bacteriological smears from both the centers and edges of selected lesions were made at the outset and from time to time during the treatment period. The lesions were examined daily by two independent observers other than myself and notes taken. The patient's own opinion was recorded from time to time. By these means the tempting and sometimes infectious tendency towards a wish-fulfilment bias in observations was cut down as far as it is possible in dealing with human material.

Results with Sample A.—Thirty-seven cases were treated, 35 Chinese and 2 Indians, all adult males. At the end of six weeks 12 cases showed rather marked clinical improvement. Lesions had obviously receded, usually accompanied by a greater or lesser degree of exfoliation of the skin surface. In a further 8 cases improvement was definite, though not so striking. Of the remaining cases 15 showed no change and 2 were apparently worse. Smears taken at this time showed that 15 of the 37 had either become negative or contained only a few broken-up bacilli. An appreciable drop in the sedimentation rate was noted in 13 cases. The changes in weight appeared to be of no clinical interest; 8 or 9 patients gained in weight, 3 lost weight.

By the end of four months' continuous treatment it was observed that in a number of cases that showed initial results the improvement had not continued. A few had shown definite fresh lesions and others had developed erythematous edges on older lesions. In the following analysis such cases are included under "not improved." Even when a patient was definitely better than he was to begin with, but had relapsed a little from the condition that existed after six weeks treatment, he was included in the unimproved group.

Briefly, 8 cases showed a striking change for the better, the lesions having receded very definitely. Another 4 cases showed moderate improvement, less than the others but definite. On the other

hand 22 showed no improvement and 2 were worse. The analysis in in Table 1 shows the position more clearly.

TABLE 1.—*Analysis of results with Sample A after six weeks and four months treatment in 37 cases.*

Condition observed after treatment	Treatment period	
	Six weeks	Four months
Definitely improved	12 cases, 32 per cent	8 cases, 22 per cent
Slightly improved	8 cases, 22 per cent	4 cases, 11 per cent
Not improved	15 cases, 41 per cent	22 cases, 60 per cent
Worse	2 cases, 5 per cent	3 cases, 8 per cent
Sedimentation rate lowered	13 cases, 35 per cent	7 cases, 19 per cent
Weight increased	9 cases, 25 per cent	10 cases, 27 per cent
Bacilli few or negative	15 cases, 41 per cent	8 cases, 22 per cent

These figures show a total of 54 per cent clinical improvement of some degree after six weeks, which dwindles to 32 per cent when the treatment had been continued for four months. The improvement in the bacillary content of the lesions drops from 41 per cent after six weeks to 22 per cent after four months. It should be noted, too, that only four of these cases appeared to be fit for discharge at the end of the treatment. The formation of an opinion on the effects of this drug on the bacilli presents considerable difficulties.

TABLE 2.—*Analysis of results with Sample B after six weeks and four months treatment in 10 cases.*

Condition observed after treatment	Treatment period	
	Six weeks	Four months
Definitely improved	4 cases	4 cases
Slightly improved	2 cases	2 cases
Not improved	4 cases	4 cases
Worse	Nil	Nil
Sedimentation rate changed	2 lower, 3 higher	As before
Weight changed	3 higher, 1 lower	As before
Bacilli negative	1 case	1 case

On the other hand, an analysis of the cases shows that at the end of the six weeks 14 out of the "not improved" group were C3 cases; the fifteenth was N1. At the end of the four months 16 of the not improved cases were C3. It might be held in fairness that in such cases improvement in a period of weeks could hardly be expected with any drug.

Results with Sample B.—Ten cases, all Chinese adult males, were treated with the second sample of fluorescein. The experi-

ment was conducted at the same time as that with Sample A, the same conditions were observed and the same doses given. The results are shown in Table 2. The percentages are obvious. Again no improvement is discernable at the end of four months beyond that seen after six weeks.

Results with Sample C.—Fourteen cases were treated with the third sample of fluorescein, under the same conditions and with the same doses as before. The results in these cases (shown in Table 3) are of the same type as those obtained with the previous samples.

TABLE 3.—*Analysis of results with Sample C after six weeks and four months treatment in 14 cases.*

Condition observed after treatment	Treatment period	
	Six weeks	Four months
Definitely improved	3 cases, 21 per cent	3 cases, 21 per cent
Slightly improved	6 cases, 43 per cent	5 cases, 36 per cent
Not improved	4 cases, 29 per cent	5 cases, 36 per cent
Worse	1 case, 7 per cent	1 case, 7 per cent
Sedimentation rate	3 lower, 3 higher	As before
Weight changed	2 higher	As before
Bacilli negative	1 case, 7 per cent	1 case, 7 per cent

Results with Sample D.—Two cases were treated with the fourth sample, the quantity in stock being insufficient for more. One showed definite improvement, the other slight improvement.

Summary.—A study of these results leads one to the conclusion that the effects of these samples were roughly the same; the experiment failed to show that any given sample was demonstrably better than another. If anything, the "A" group seems the best, but considerable latitude must be allowed for chance in the selection of patients. In any case we failed to demonstrate a clinically inert sample which might be analyzed in comparison with an effective one.

In view of this it seemed that results of value could be obtained by considering the total results obtained with these samples. These are shown in Table 4. The laboratory results have been considered in the preparation of this table, but the effects on bacillary content of lesions have been purposely omitted as I feel that that they are misleadingly optimistic. However, one is justified in drawing the following conclusions:

(1) That six weeks seems to have been the optimum period of treatment in 64 cases, where intravenous injections of fluorescein were given twice a week in doses of 20 cc. of a 2 per cent solution.

(2) That the advanced cutaneous cases in this series did not appear to derive any special benefit within the given period of four months.

(3) That with the solutions freshly made, boiled, filtered and injected with proper technique, fluorescein seems definitely non-tonic in these doses.

(4) That while about one-half of the cases improved, only a negligible number showed complete elimination of the cutaneous lesions in four months.

TABLE 4.—*Analysis of the total results obtained with 4 samples of fluorescein in 64 cases.*

Condition observed after treatment	Treatment period	
	Six weeks	Four months
Definitely improved	19 cases, 30 per cent	15 cases, 23 per cent
Slightly improved	19 cases, 30 per cent	13 cases, 20 per cent
Not improved	23 cases, 36 per cent	32 cases, 50 per cent
Worse	3 cases, 5 per cent	4 cases, 6 per cent

II. COMPARISON OF COMPONENTS OF FLUORESCEIN

Upon completion of the comparison of the fluorescein samples it was decided to carry out the second part of the program and make experiments based on the chemical analysis of this dye. Fluorescein results from the condensation of phthallic anhydride and resorcinol. The differences in different samples are probably due to variations in the resorcinol, which is difficult to obtain in a pure or uniform state.

RESORCIN

Resorcin is already known in the history of the chemotherapy of leprosy and it seemed reasonable to start with intensive treatment by this drug. However, considerable difficulties were encountered in attempting this, and the experiment had to be given up.

RESORCIN BLUE

Resorcin blue or lacmoid contains over 95 per cent of resorcinol. This dye is easily made up by heating 100 parts of resorcinol with 5 parts of sodium nitrite and 5 parts of water. At 110°C. ammonia is given off rapidly and the mixture turns red. With a little further heating ammonia ceases to come off and the color

becomes blue. The blue preparation is water soluble, and is precipitated by acid as brown shiny powder which again turns blue on solution.

After a preliminary toxicity test on laboratory animals this dye was administered to patients who, it was found, could tolerate it easily. Nine patients were then given injections of 20 cc. of 2 per cent solution of it, two times a week, for a period of five weeks. At the end of this time no change had been detected in any of the cases, the sedimentation rate and weights showed no common tendency, and the experiment was reluctantly abandoned as a failure.

PHTHALLIC ACID

Attention was then turned to the remaining component of fluorescein, namely, phthallic acid. After preliminary animal tests, as before, sixteen patients were given intravenous injections of a solution containing phthallic acid 2 parts, potassium bicarbonate 3 parts, water to 100 parts. Each patient was given 25 cc. of this solution intravenously twice weekly for two months. The solution was freshly prepared, boiled and filtered in the usual way.

General effects.—All the patients felt a sensation of pain along the arm during the injection (which must be given very slowly) and for a minute or two afterwards; this was possibly due to the effect of the hypertonic solution. There were occasional tremors of the arm muscles during injection.

Three of the sixteen patients said that the injections caused a sensation of numbness in the hands and feet. In one instance the patient claimed that the whole left leg below the knee felt numb as a result of the injections. Seven of the sixteen said that the injections caused an itchy feeling over the leprotic lesions, though in one or two instances this statement may have been volunteered because I appeared to be particularly interested in the matter. Three patients said that the injection made them feel sleepy, two said it improved their appetites and one that his vision had become dimmer. In all cases the patients wished the injections to be continued.

Specific effects.—These are given in detail because they seem of sufficient interest to merit it. In each case are given the classification, Wassermann and Kahn reactions, sedimentation rate at the beginning and the end of the two months (e.g., "6 to 9"), weight at the beginning and the end (e.g., "120 to 118 lbs."), the bacillary content of smears from the centers (C) and edges (E) of lesions (e.g., "C++, E+ to C+++, E±," in which the ± means "few"), and the clinical finding on final examination.

(1). Cheong Kian, age 33, C3-N1. Wassermann and Kahn negative. Sedimentation 6.5 to 9. Weight 118.5 to 118 lbs. Smears: C±, E++ to C+, E−. Shows definite clinical improvement; lesions have wrinkled and their edges turned yellowish brown after surface exfoliation.

(2). Loh Seng, age 50, C2-N2. Wassermann and Kahn negative. Sedimentation 32 to 43. Weight 114 to 116.5 lbs. Smears C−, E− on both examinations. Lesions are much flatter and paler and some have disappeared.

(3). Tan Hoo, age 13, C2. Wassermann and Kahn negative. Sedimentation 38 to 29. Weight 70 to 74 lbs. Smears C±, E+++ to C+++, E±. No clinical change except that a white areola one-quarter to one-half inch in width has appeared round each macule.

(4). Low Hong, age 50, C2. Wassermann and Kahn negative. Sedimentation 47 to 48.5. Weight 114 to 113. Smears C−, E± to C−, E−. Lesions are much paler and the edges flatter.

(5). Teh Ping, age 56, 03. Wassermann and Kahn both positive. Sedimentation 8.5 to 27. Weight 110 to 112. Smears C++, E+ to C+++, E+++. Laboratory findings are bad, but the clinical improvement is very definite.

(6). Yee Sai, age 33, C3. Wassermann and Kahn negative. Sedimentation 12, stationary. Weight 140, stationary. Smears C++, E± to C+++, E−. The flatter macules on the chest have definitely improved; the chronic infiltration of the face is unchanged.

(7). Low Thong, age 28, C1. Wassermann ±, Kahn negative. Sedimentation 21 to 17. Weight 109 to 114. Smears C+++, E+ to C−, E−. Laboratory findings are good, but no change is seen in the clinical condition. That the lesion is negative is probably fortuitous.

(8). Yong Nyuk, age 32, C2-3. Wassermann and Kahn negative. Sedimentation 22 to 12. Weight 108 to 114. Smears C++, E± to C+++, E−. No improvement. The only specific change is that a pinkish areola has appeared round the macules.

(9). Lum Min, age 37, C2. Wassermann and Kahn negative. Sedimentation 11.5 to 14. Weight 161 to 164. Smears C+++, E++ to C+++, E−. Edges of lesions are much paler and flatter, and have become of a pale brown color. Centers of lesions unchanged.

(10). Alagan, age 27, C2. Wassermann negative, Kahn ±. Sedimentation 54 to 45.5. Weight 111 to 109. Smears C±, E− to C−, E−. Raised lesions are definitely flatter and now flush with the surrounding skin, after preliminary scaling on surface. Light narrow areola round the macules. Definite improvement.

(11). Yong Fong, age 46, C2. Wassermann and Kahn negative. Sedimentation 20 to 19. Weight 109 to 108. Smears C−, E−, on both occasions. Raised macules with erythematous edges which have shown no change of any kind. No improvement.

(12). Chang Kwai, age 63, C3. Wassermann and Kahn negative. Sedimentation 29, stationary. Weight 102 to 100. Smears C−, E(?) to C±, E−. Lesions are definitely paler and softer. Infiltrated parts have become mottled with paler wrinkled areas.

(13). Lo Khoon, age 43, C3. Wassermann +, Kahn ++. Sedimentation 24 to 31. Weight 111 to 112. Smears C+++, E+++ to C++, E+++. Lesions have become softer, after preliminary exfoliation. Faint, pale pink areolas have appeared round discrete macules, and paler flattened areas are beginning to show on the thickly infiltrated face.

(14). Wong Oi, age 43, C2. Wassermann and Kahn negative. Sedimentation 20 to 13. Weight stationary. Smears C++, E+ to C−, E−. Laboratory findings are good, but there is no clinical change of any kind.

(15). Ang Hak (female), age 14, C2. Wassermann and Kahn negative. Smears C+++, E+++ to C++, E+++. No observable improvement.

(16). Chah Moi, age 35, C2. Wassermann and Kahn negative. Smears C+, E+ to C−, E+. Large raised erythematous areas have become flush with the skin, much paler, with a small pale areola of normal surrounding skin. Much improved.

In considering these cases no patient has been put down as improved where satisfactory laboratory findings have not been confirmed by clinical results. Out of the sixteen cases ten, or 62 per cent, have shown changes in the lesions which are apparently a specific effect of the injections given. These changes have consisted of exfoliation of the affected skin, wrinkling and depigmentation of macules, and flattening of lesion edges. In one case has there been a change in raised indurated lesions. The sedimentation rates and body weights do not appear to show any significant tendency.

With regard to the bacillary findings, four cases (25 per cent) were negative to begin with, another four became negative during the treatment, and seven (44 per cent) have developed negative lesion edges that were previously positive. One patient appears to have a definite increase in the bacillary content of his lesions. However, examination of the slides from these cases shows that in the majority of cases the bacilli became granular and broken up in conjunction with treatment. Beaded forms and masses of acid-fast debris are frequent in the positive slides.

It is not possible to form an opinion on the therapeutic value of phthallic acid from observations of sixteen patients over a period of only two months. In none of these sixteen cases have the lesions been eliminated within the time of experiment, and there is no indication as to whether the results will be progressive or otherwise. On the other hand it seems fair to assume that the therapeutic results observed with fluorescein are due to its phthallic acid content, which has possibly a vaso-constrictor effect. Further, the results of phthallic acid administration in these cases appear to justify

a much more extended observation in the possible hope of finding a new basis of research in the treatment of leprosy.

SUMMARY

(1) In the treatment of leprosy cases with intravenous injections of aniline dyes, fluorescein has seemed to us to give the most stable effects.

(2) Sixty-four cases have been treated with intravenous injections of fluorescein over a period of four months.

(3) After six weeks treatment about 60 per cent of these cases showed greater or less specific response, based on clinical examination of the lesions and laboratory findings.

(4) The improvement rate dropped to 43 per cent on continuing the treatment for four months.

(5) Nine cases have been treated with resorcin blue (resorcin being one of the components of fluorescein). No effect was obtained.

(6) Sixteen patients have been treated for two months with intravenous injections of phthallic acid (the other component of fluorescein) with greater or less lesion response in 62 per cent of cases.

CONCLUSIONS

Fluorescein injected as described in this article appears to be worthy of trial as a six weeks' treatment between courses of the chaulmoogra preparations. We have found in a number of cases that hydnocarpus esters are better tolerated after such a course, and have gained the impression that the improvement due to fluorescein sometimes continues for some time after the drug has been stopped.

We have found little evidence of beneficial effect of fluorescein on advanced cases.

The essential ingredient in fluorescein appears to be phthallic acid, a drug which seems to justify further study.

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