

## REPRINTED ARTICLES

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### A PRELIMINARY REPORT ON THE ACTION OF CERTAIN DYES IN LEPROSY \*

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#### INTRODUCTION

This report deals with a series of cases treated experimentally by intravenous injections of synthetic dyestuffs. An attempt was made to administer these compounds in a series of their chemical classes, limiting the experiment to soluble substances. Sub-groups already used in chemotherapy include the *triaminotriphenyl methanes*; basic and acid fuchsin, methyl violet, crystal violet and gentian violet. We investigated:

1. *Azo compounds*—such as chrysoidine, bismarek brown and trypan blue.
2. *Carbonium compounds*—(a) acid: brilliant green, malachite green, crystal violet, methyl violet; (b) basic: auramine.
3. *Xanthenes*—(a) acid: eosin, fluorescein; (b) basic: rhodamine.
4. *Thiazine dyes*—methylene blue, toluidine blue.
5. *Indigoid dyes*—indigo carmine.

The essential phagocytosing cell in leprosy is the macrophage, and this shares with other cells of the reticulo-endothelial system the power of selective absorption of soluble dyes, and of particulate matter like India ink. Loading of such cells ultimately produces blockage, inhibiting their further activity and possibly interfering with antibody formation. It might seem, therefore, that reticulo-endothelial blockage of a leprotic lesion is contra-indicated, but the results

\* Condensed from the article which appeared in the *Transactions of the Royal Society of Tropical Medicine and Hygiene* 27 (1933) 85.

of work on blockage are so discordant that such a conclusion cannot be drawn. The arsenicals in spirochaetal diseases may act through concentration in the reticulo-endothelial system. The unaided macrophage does not have much effect on the *Mycobacterium leprae*, apart from phagocytosis. Lastly, by intravenous injection of these dyes we can create a selective and formidable concentration of certain drugs in the active cutaneous lesions.

Cappel (2) points out that in an animal subjected to *intra vitam* staining there may be diffuse coloration of elastic and connective tissues, and also an accumulation in certain cells in the form of granules and vacuoles. Staining occurs in forty-eight hours, there is an "equilibrium" lasting a further forty-eight hours, then gradual fading. This is in conformity with the findings of Jungeblut and Berlot (4). The period of equilibrium would seem to be the best time for repetition, and bi-weekly injections of dyes the method to obtain maximum concentration.

Many coal-tar dyes have a powerful and often selective germicidal action. Browning (1) found that brilliant green killed the *Staphylococcus aureus* in 1:10,000,000 dilution, a coefficient of 40,000. Lewis (5) found that trypan blue had a selective germicidal action on the tubercle bacillus in vitro, and that in vivo it selectively stained nodules of tuberculosis. A number of substances had a strong bactericidal action on tubercle bacilli in vitro, but none of them inhibited their growth in the living animal. Findlay (3) gives a short summary of the chemotherapy of dyes, with a detailed list of references.

#### METHODS

We have not attempted a detailed study of the action of the dyes used. In some instances we have recorded cases treated with dyes that seem therapeutically inert, partly to serve as controls.

The 85 patients were all adult male volunteers. They were wherever possible cases with clearly defined cutaneous lesions, without record of lepra-reaction within six months, of good physique, not suffering from any major complication and as far as possible capable of understanding the experiment and of expressing an opinion on it. We did not find moderate albuminuria or a positive Wassermann reaction to be contraindications.

The leprotic condition was charted before and after treatment. Serological tests for syphilis were obtained, the blood and stools were examined microscopically, and a detailed medical examination including the haemoglobin index was made. Conditions of diet, housing, etc., were similar and under institution control. Temperatures were taken twice daily. Every ten days we took (a) the sedimentation

rate, (b) the weight, (c) smears to examine for *Myco. leprae*. In some cases the serum calcium was estimated.<sup>1</sup>

#### PRECAUTIONS

Certain precautions are to be taken in the intravenous administration of unknown samples of dyes. The dyes were sealed and stored away from the light, and the solutions were freshly prepared, boiled and filtered. The patient was injected lying down and afterwards was kept in the ward. The injection was given very slowly, before food, and was stopped at once if the patient complained of pain in the chest. We were always prepared immediately to give saline intravenously and to inject strychnine; coffee and other stimulants were also kept available.

Different dyes vary greatly in their toxicity, and different samples of the same dye are markedly different in their immediate effect. Once a sample has been tested the usual precautions suffice. Solutions tend to become more toxic if left for a few days. Toxic effects may be: (a) cardiac pain and epigastric palpitation; (b) gastric and rectal irritation; (c) feeble pulse and signs of shock; (d) exceptionally, coma and even temporary failure of respiration and loss of radial pulse.

We had no deaths or permanent ill-effects. Symptoms usually pass off in about an hour. Sometimes treatment of minor degrees of shock is needed. A severe case may require immediate artificial respiration, intravenous saline, and general stimulation; this is exceptional, but it is emphasized that the first injection of an apparently reasonable dose of a new sample may give toxic symptoms.

#### IMMEDIATE EFFECTS

There is the selective concentration of the dye in the leprotic lesion, but not in pyogenic and other lesions in the same subject. Trypan blue, methylene blue, crystal violet, toluidine blue, brilliant green, and a number of other dyes, if injected in sufficient quantity, show almost at once a selective affinity for the lesions, and as a result the general appearance is sometimes remarkable. With larger doses there is a secondary infiltration of other cutaneous tissues, and the whole epidermis and mucous membranes may show varying shades of color. This usually begins to fade after 1 or 2 days, and has usually disappeared in 3 to 6 weeks.

As a rule, though not always, the bacillus-containing lesions tend to stain more intensively than those without bacilli, and such lesions tend to retrogress more rapidly. Some early lesions did not stain. Lastly, a lesion which stained selectively at first, and as a result retrogressed considerably, often failed to take the stain in the later stages of retrogression.

<sup>1</sup>By Dr. F. E. Byron, at the Institute for Medical Research, Kuala Lumpur.

## RESULTS OF TREATMENT

## TRYPAN BLUE

The analogy of Lewis' work in tubercle and the marked selective concentration in the lesions determined us to use this dye extensively. Thirty-seven cases were treated: 32 Chinese, 2 Tamils, 1 Malay, 1 Telegu and 1 Arab. In most cases 25 cc. of a 4 per cent solution was given. Some could not tolerate this, though we have given 75 cc. without apparent ill-effects. It is important to filter the solution.

In most cases, marked relative concentration in the lesions was observed. The lesions turned blue, then became scaly, softened and diminished. Sometimes scaling was apparent in 48 hours; in other cases it began on the seventh day. The average duration of treatment was 30 days. A total of 77.8 per cent showed improvement—40.9 per cent marked and 36.9 per cent less—while 15.8 per cent showed no change. In no case were the lesions aggravated. As regards the bacilli, 74 per cent showed a decrease and 31 per cent became either negative (19 per cent), at least for the time being, or showed only a few bacilli. There was an increase in the body weight with 52 per cent, and a fall in sedimentation rate in 65 per cent.

This brief treatment resulted, at the time, in the diminution of the external manifestations of leprosy in a large percentage of the cases. Observation left no doubt that the changes were a specific response to the introduction of the dye.

## BRILLIANT GREEN

This dye is much more toxic than trypan blue, and the relative toxicity of different samples varies enormously. Generally the maximum dose is 20 cc. of 1 per cent solution, and the usual tolerated dose is 15 cc. We have given a 130-lb. patient 50 cc. with only temporary ill-effects, but doses over 20 cc. tend to be dangerous. Injections cause a grayish coloration of the lesions within 30 minutes.

Nine cases, Chinese, were treated for from one to three months. Three became bacteriologically negative and five showed very definite retrogression of lesions.

## FLUORESCEIN

A fluorescein-mercury compound, mercurochrome 220, has been tried by several workers in leprosy; the results are summed up by Muir and Chatterji (6), who found it strikingly beneficial in lepra reaction. We have found that the effects of the xanthene class in

lepra reaction are of great interest, and intend to make a contribution on this subject in the near future.

A solution of fluorescein is made with an equal concentration of sodium bicarbonate. In an adult of average weight intravenous injections of 10 cc. of a fresh 2 per cent solution are well tolerated.

*Illustrative case:*—Chinese, age 49, type C-3, showing raised infiltrated lesions on forehead, face and upper part of trunk, was given 12 intravenous injections of 10 cc. fluorescein, 2 per cent, over a period of 5 months. The lesions scaled and flattened, leaving depigmented areas with a flat red erythematous margin. Improvement was very marked, the only disturbing factor being the new erythematous margin.

#### FLUORESCEIN AND BRILLIANT GREEN

After further experiments it was decided to combine fluorescein with brilliant green. Twelve cases (Chinese) were therefore injected with a mixture of 8 cc. of 2 per cent fluorescein and 16 cc. of 1 per cent brilliant green. The mixture was well tolerated, though leakage outside the vein caused painful inflammation. The average duration of treatment was 9 weeks. Four cases (33.3 per cent) became bacteriologically negative. Marked retrogression of lesions occurred in six; three showed no change.

The two points that emerge are: (1) the high proportion of cases that became bacteriologically negative, and (2) the diversity of clinical results—some cases responding rapidly, others showing no change.

#### EOSIN

This drug also belongs to the xanthene class, and its influence on lepra-reaction will be considered in a later paper. Intravenous injection of 25 cc. of a 2 per cent solution can be tolerated by adults without apparent discomfort. The cases quoted justify further study of this dye.

*Illustrative cases:*—(1) Chinese, age 32, type C-1, N-1, presented on the right leg circinate lesions with raised active margins which contained many bacilli. Given 12 injections of 2 per cent eosin, the injections rising from 5 to 25 cc. in 10 weeks, he lost 3 pounds in weight. The sedimentation rate fell from 47 to 3, the lesion became negative and has steadily diminished.

(2) Chinese, age 51, type C-2, had a raised lesion on the right cheek. Given 9 injections of 2 per cent eosin in 8 weeks, rising from 5 to 25 cc., he gained two pounds in weight and the sedimentation rate decreased from 24 to 18. The lesion was very much diminished, though still bacteriologically positive.

## OTHER DYES

The effects of a number of other dyes have been studied, mostly in single cases. They were: crystal violet, picric acid, malachite green, rhodamine, congo red, iodine green, methylene blue, acriflavine, methyl orange, toluidine blue, acid fuchsin, indigo carmine, crysordine, Giemsa's stain, auramine. Twenty-four patients were treated.

In no case were there definite signs of clinical improvement; in some cases the patient became slightly worse and the investigation was abandoned. These results were very different from those obtained with trypan blue, brilliant green, fluorescein and eosin.

## COMMENT

In the cases that improved this result often seemed to have been obtained from one single injection, when previous or subsequent injections produced comparatively little effect. Often improvement was preceded by a marked rise in the bacillary content of the lesions. The action of the effective dyes often seemed haphazard and irregular. The results obtained so far, and within the stated times, compare very favourably with those obtained in this hospital with chaulmoogra derivatives.

## SUMMARY

(1) A number of indications and analogies suggest the trial of coal-tar dyes and their derivatives in leprosy.

(2) Intravenous injections of a large number of dyes have been given to 85 patients.

(3) A number of these dyes show marked selective concentration in the leprotic lesions, a phenomenon not seen in certain non-leprotic lesions.

(4) With trypan blue, brilliant green, fluorescein and perhaps eosin a definite diminution of the external manifestations of leprosy has been observed, accompanied by other signs of clinical improvement.

(5) With the other dyes administered no signs of clinical improvement were observed.

(6) This report is a study of the immediate reaction of the lesions; we have as yet no knowledge of the later effects of the injection of these dyes.

We consider from this preliminary report that further trial and study of these dyes is definitely indicated in the hope that it may lead to further possibilities in the chemotherapy of this disease.

(Acknowledgement is made of indebtedness to Dr. A. Neave Kingsbury, of the Institute for Medical Research, for help and advice, both with the experiments and with the preparation of this paper. Appreciation is expressed of the work of Mr. Soon Tin and Mr. Teck Heng, inmate dressers in charge of the experimental wards.)

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