A Note on the Action of Chaulmoogra (Hydnocarpus) Preparations on *M. Leprae Muris*

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WE seek in this note to examine the grounds for the common belief that the preparations of chaulmoogra and hydnocarpus oils have an inhibitory and bactericidal action on *M. leprae,* to point out how unsatisfactory is the evidence on which the belief is founded, and to describe certain experiments which do not support this belief. This note is therefore mainly destructive criticism, but we hope it may help in clearing the ground for a more rational view of leprosy treatment.

Chaulmoogra oil inunction was used empirically for many centuries in the treatment of leprosy. According to Tomb (1933) injections of the oil were first given by Tourtoulis Bey in 1894; the ethyl esters of the oil were first used by Engel Bey about 1908; sodium salts of the fatty acids were made by Moss in 1879 and were used orally from 1891 and by injection from about 1912. All these developments were purely empirical, and the first work done in investigating the action of these preparations on *M. leprae* was done by Walker and Sweeney (1920). They investigated the action of soluble preparations of chaulmoogra oil on acid-fast bacilli in a liquid culture medium (glycerol-veal broth). They found that the presence in the medium of the sodium salts of the total fatty acids of the oil in high dilutions (up to 1 in 75,000) inhibited growth and made subculture impossible. The acid-fast bacilli used were "*B. leprae muris* (*Hollmann*)" and "*B. leprae hominis* (*Levy*)" in addition to *B. smegmatis,* and the bacilli of human, bovine, and avian tuberculosis. They considered that these findings indicated the antiseptic and bactericidal

activity of chaulmoogra oil on the acid-fast group of organisms. This activity was found to be one hundred times greater than that of phenol.

There are various criticisms which may be made of this work. Firstly, the cultures of leprosy organisms which Walker and Sweeney used, are not generally accepted as genuine. In fact, "*B. leprae muris (Hollmann)*" was considered by Hollmann himself (Currie and Hollmann, 1913) as being not a true culture but a contamination. Secondly, the impossibility of subculture is not a sound criterion of death of the bacilli. A far sounder criterion would be the failure of the bacilli to produce the disease when injected into susceptible animals. Walker and Sweeney attempted to demonstrate this with *M. tuberculosis*, but their results were, in our opinion, inconclusive.

This work of Walker and Sweeney stimulated other work of a similar kind. Such work is reported by Schobl. (1923 and 1924) who confirmed Walker and Sweeney's findings; his work is open to the same criticism that we have made of Walker and Sweeney's work. Recently Cummins and Weatherall (1933) investigated the action on *M. tuberculosis* of *Alepol*, a preparation of a selected fraction of the lower-melting-point fatty acids of hydnocarpus oil. They found that the presence in Besredka's fluid egg medium of alepol in a dilution of 1 in 1,000,000 inhibited growth; after thirty-five days material from the culture tube was injected into a guinea-pig which was sacrificed nine months later and showed no disease. A control tube of the same medium showed growth which, when injected into a guinea-pig, caused general tuberculosis. A similar result was obtained in a second experiment. Attempts to treat rabbits suffering from experimentally induced tuberculosis by intravenous injections of alepol gave inconclusive results.

The work of Walker and Sweeney, while it is suggestive of a bactericidal action of chaulmoogra preparations on *M. tuberculosis*, fails to prove any bactericidal action on *M. leprae*, for they were almost undoubtedly not dealing with *M. leprae* or *M. leprae muris*. We therefore fail to see what grounds they have for the statement :—" These facts supply a scientific basis for the use of chaulmoogra oil and its products in leprosy." The acceptance of this statement makes necessary three assumptions: (1) that the hydnocarpus preparations have an inhibiting and bactericidal action on *M. tuberculosis* in vitro; (2) that because *M. leprae* and *M. tuberculosis* are similar in structure and staining properties, the action of hydnocarpates on both

these organisms is the same, and (3) that the action of hydnocarpus preparations in vivo is the same as in vitro. While the first assumption may, on the evidence available, be considered a possible one, the second and third assumptions are mere guesses and based on no sound evidence.

Before expressing any definite opinion regarding the action of hydnocarpates on *M. leprae* in vitro, we must obtain evidence regarding their action on genuine *M. leprae*, using the method of injection of treated bacilli into susceptible experimental animals, in order to investigate whether the treated bacilli are alive or dead. We have no satisfactory method of culturing *M. leprae*, but since we can obtain large numbers of bacilli from the nodules of leprosy patients, this difficulty can be overcome. There still remains, however, the insuperable difficulty presented by the fact that we have no experimental animal susceptible to human leprosy. Therefore the action of hydnocarpus preparations on *M. leprae hominis* cannot be properly investigated at present.

With rat leprosy, however, the major difficulties disappear. We can get enormous numbers of *M. leprae muris* from the tissues of infected rats, and we have in the rat an animal highly susceptible to this organism. We have therefore been able to carry out a careful investigation of the action of hydnocarpus preparations on *M. leprae muris*, and the results of this work we report here. We should be quite clear, however, that since the relation between *M. leprae* and *M. leprae muris* is undetermined, we do not know whether the results of these experiments can be interpreted as indicating what is the action of the hydnocarpus preparations on *M. leprae hominis*.

Experiment 1.

A rat in the advanced stages of rat leprosy was sacrificed and portions of the spleen and liver were emulsified in normal saline. To different lots of the emulsion was added sodium hydnocarpate (the sodium salts of the total fatty acids of hydnocarpus wightiana oil) in dilutions 1 in 200, 1 in 2,000, 1 in 20,000, 1 in 200,000, and 1 in 2,000,000. The emulsions showed large numbers of acid-fast bacilli. The various emulsions were kept at room temperature for three hours. At the end of this time some of the treated bacilli were injected into rats. (With the lower dilutions the sodium hydnocarpate was washed out before the bacilli were injected into rats.)

In all, 32 rats were used, five lots of four rats being

inoculated with bacilli treated with five different dilutions of sodium hydnocarpate and twelve control rats being inoculated with untreated bacilli.

Of the experimental rats, three died in the first month, showing acid-fast bacilli in the tissues. These we will ignore. The remaining 17 all lived for three months or more and at death all showed a generalised infection with rat leprosy.

In connection with the interpretation of post-mortem findings we would point out that the findings of a few acid-fast bacilli is no proof that the animal has rat leprosy. Bacilli may be found for more than a year after the injection of killed bacilli. The presence of a progressive systemic infection, with very large numbers of multiplying bacilli, is the criterion we use in all our rat leprosy work.

Experiment 2.

In a similar way bacilli were treated with a 5% solution of sodium hydnocarpate, for periods of 3 hours and 20 hours, and were then injected into rats. Control rats were injected with untreated bacilli.

Of four rats injected with bacilli treated for three hours, all developed a generalised rat leprosy infection.

Of seven rats injected with bacilli treated for 20 hours, all developed a generalised rat leprosy infection, but in several of them the development of the disease was much retarded.

Experiment 3.

Eleven rats were injected with bacilli treated with 5% sodium hydnocarpate for three hours. Four died within two months. The remainder lived for three months or more, and all showed a massive general infection with rat leprosy.

In our experience the only fact suggesting a bactericidal action is that in a few rats injected with bacilli treated with 5% sodium hydnocarpate acting for 20 hours, a generalised rat leprosy did not develop till very late, while in all rats injected with bacilli treated with the same dilution of sodium hydnocarpate for three hours, generalised rat leprosy developed fairly quickly. This suggests that the time factor may be of importance, and that even weak dilutions might kill the bacilli if longer time were allowed. Even if this is so, we have to be sure that such bactericidal activity is peculiar to hydnocarpus preparations, and is not due to a simple soap action. We are therefore making further experiments, treating bacilli with sodium hydno-

carpate in various dilutions for long periods before injection into rats, and using as controls rats injected with bacilli similarly treated with sodium oleate. One such experiment has already shown little or no difference in the bactericidal powers of these two preparations.

If we compare our results with those reported by Walker and Sweeney we see a great difference. According to our experiments, even 5% sodium hydnocarpate acting for 20 hours did not give a complete bactericidal action while, according to Walker and Sweeney, a supposed culture of *M. leprae muris* was killed by a dilution of 1 in 75,000 acting for 24 hours. The reasons for the difference we judge to be (1) we were dealing with true *M. leprae muris* direct from the tissues of an infected rat, while Walker and Sweeney were using a culture of a bacillus of doubtful nature isolated from rat leprosy material many years previously, (2) the criterion of death of bacilli used by Walker and Sweeney was failure to grow in subculture. We used the test of pathogenicity in susceptible animals.

We cannot help feeling that our results are more reliable than those obtained by Walker and Sweeney.

Conclusions.

We are led to the conclusion that up to the present no conclusive evidence has been obtained of any special inhibiting or bactericidal activity of sodium hydnocarpate on *M. leprae muris* in vitro. We believe that " the scientific basis for the use of chaulmoogra oil and its products in leprosy " provided by the work of Walker and Sweeney is unsound.

The apparent lack of bactericidal activity of sodium hydnocarpate on *M. leprae* in vitro does not prove that it has no effect in vivo. Quinine does not kill malaria parasites in vitro, emetine does not kill *Entamoeba histolytica* in vitro, antimony does not kill *Leishmania donovani* in vitro, yet the efficacy of these preparations in treatment is established. We should note, however, that these are delicate protozoa, the killing of which is a very different matter from the killing of an extremely resistant acid-fast bacillus, such as *M. leprae*. *M. leprae muris* can be treated with 5% sulphuric acid for several hours and still remain viable (Marchoux and Chorine, 1932).

We think it is unlikely that hydnocarpus preparations have any special inhibitory or bactericidal action on *M. leprae* either in vitro or in vivo. Nevertheless we believe that hydnocarpus preparations are valuable in leprosy

treatment. The action is probably indirect. If this is so, the proof of the efficacy of any preparation is to be obtained only by accurate observation of the results of treatment of patients. In such a chronic disease as leprosy this is not easy, but under the circumstances it is probably the nearest we can get to a scientific basis for the use of hydnocarpus or any other preparations in treatment.

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