Observations

ON

THE USE OF HYDNOCARPATES AND
MORRHUATES INTRADERMALLY
IN LUPUS VULGARIS

BY

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THE GROWTH OF TUBERCLE BACILLI IN
BESREDKA'S FLUID EGG MEDIUM

BY

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When reporting the beneficial action of soluble preparations of the fatty acids of chaulmoogra and hydnocarpus oils in leprosy in 1916,¹ I expressed the opinion that "the reactions produced by gynocardates in leprosy tissues, and the apparent destruction of Hansen's bacillus, raise the very important question as to whether some similar reaction may not be obtained in the case of another human acid-fast bacillus—namely, that of tuberculous disease."

In 1918² I reported the use in tuberculous disease of sodium hydnocarpate, and also of sodium morrhuate, which I had made on similar lines from the fatty acids of cod-liver oil, and in the following year³ I recorded promising results obtained by several observers with injections of sodium morrhuate in various forms of tuberculous disease. In the winter of 1920-1, with the kind help of the Medical Research Council, I tested the action of hydnocarpates and morrhuates in the rapidly fatal acute miliary tuberculosis in the inoculated disease in rabbits and guinea-pigs, with negative results; but I concluded that the possibility of benefit in the much more chronic tuberculous disease of man was not thereby excluded. In 1924, in my fourth Croonian Lecture,⁴ I summarized with references the early literature and reported trials of sodium morrhuate in surgical tuberculosis, kindly carried out at my suggestion at the large Carshalton and Fazakerley children's hospitals with somewhat variable results—results which indicated the advisability of further trials. In 1920 E. L. Walker and Marion A. Sweeney⁵ reported that the sodium salts or soaps of the lower melting point fatty acids of chaulmoogra oil—consisting mainly of hydnocarpates—which I had found to be most effective in leprosy, had the power of inhibiting the growth of various acid-fast bacilli which will grow beneath the surface of fluid media, including that of avian tuberculosis, in dilutions of 1 in 100,000 to 1 in 1,000,000, but sodium morrhuate had very little such action. It will be seen from the note below that Professor Lyle Cummins has extended this important observation to the human tubercle bacillus, using "alepol," which consists mainly of sodium hydnocarpate. As this preparation can be injected intravenously in considerable doses of a 3 per cent. solution without toxic symptoms, the further trial of hydnocarpates in tuberculous disease appears to be indicated, but it should be used with caution in pulmonary cases as it can produce both general febrile and focal reactions, with softening of the tuberculous lesions.

There is now an extensive literature on the use of these preparations in tuberculosis, but I can only refer here

to a very few which have a special bearing on my present subject. Colloidal copper morrhuate, prepared by Dr. Paulo Sebra, has been used extensively in both pulmonary and surgical tuberculosis in South America, and a number of favourable reports have been summarized by Dr. J. Mouzon.⁶ These include successful injections into the cold abscesses of gland and bone disease, and also in lupus cases. Cases of lupus treated by injections of "iodo-morrhuate" have been reported by O. Blatt and by Jaczewski in Poland,⁷ but a reference to Blatt's original paper shows that he used iodine dissolved in potassium iodide mixed with cod-liver oil. In my early work on leprosy in Calcutta I found that extensive nodular lesions were best treated by injecting hydnocarpates directly into them, and this intradermal method has recently been used more extensively in leprosy by C. B. Lara, C. Nicolas, and others⁸ with great benefit—confirmed by E. Muir in Calcutta, and by myself in unrecorded cases in this country.

CASE RECORD

I was thus led to try the method of intradermal injections of ester morrhuates and hydnocarpates in the following very chronic case of lupus vulgaris.

A female, aged 42, gave a history of having suffered when a child from "abscesses" on her head and over the base of the spine, and later from ulceration of her face. After various treatments with ointment, etc., by private practitioners and at three hospitals in London, she stated that she underwent the Finsen light therapy at the London Hospital for thirteen years, with nearly 2,000 hours' application in all. This resulted in some temporary improvement, but she abandoned this treatment after seeing "cancer" develop in other cases of lupus. The disease was again spreading when she was sent to consult me in May, 1931. At this time it affected the whole of both cheeks, extended across the forehead and chin, and over both sides of the neck, and had a raised, thickened margin surrounding the whole of the diseased area. There was much scar tissue over her cheeks, while the lower right eyelid had become everted by its contraction. In addition, there was a less active patch of the disease, about two inches square, over her right shoulder. This enabled me to test the effect of the injections first in an unexposed part of the body.

Her progress is a record of steady improvement. I commenced treating the thickened edge of the shoulder lesion by injecting a drop or two of ethyl ester morrhuate intradermally at several neighbouring points at each sitting, so as to produce slightly raised whitish swellings at each point. Different areas were dealt with at each weekly visit. Except for the prick of the needle no material pain or irritation resulted, the temporary swelling at the points of injection rapidly subsided, and within two weeks or so the original thickening of the treated areas showed definite reduction, without any breaking down ever occurring at the sites of the injection. I very soon extended the treatment to the thickened margins on the face and neck, with similar beneficial results. As progress with the morrhuates was somewhat slow I proceeded to use instead the more active ethyl ester hydnocarpate in the form of "moogrol," combined with 4 per cent. creosote as an antiseptic. This also caused very little irritation and no breaking down of the injected areas, while it proved decidedly more effective in quickly reducing the thickening of the treated areas. Moreover, the administration of the hydnocarpates was followed by the appearance of some fresh ulceration in the older, uninjected, central parts of the cheeks. This I regarded as a reaction in the diseased tissues very similar to the swelling and softening of leprosy dermal lesions following intravenous or intramuscular injections of the same drug, with, ultimately, very beneficial results in the skin lesions. I therefore returned to the morrhuates for a time, and watched the progress of the reaction in the uninjected lupus lesions with great interest. Only a simple dressing was applied to the ulcers on small strips of linen, such as the patient had long relied on for this purpose. The exposed lupus-diseased tissues in the floors of the ulcers soon came away and the small superficial ulcers healed, leaving much more healthy-looking skin than before the breaking

down had taken place, thus bearing out the analogy with the leprous reactions produced by the hydnocarpates.

It became apparent that, in addition to their local effect, the hydnocarpate injections into the spreading margin of the disease had a generally beneficial effect in the uninjected, long-affected, central portions of the lupus patches. This made me much more hopeful as to the ultimate result. Consequently I continued the treatment with the hydnocarpates alone, and found that the reactions in the central portions became milder, and an increasing number of points could be injected at a sitting, with more rapid improvement. At the end of a year the thickened diseased areas had nearly all disappeared, and the intervals between the injections were increased from one week to two, and later to three weeks. Breaking down of the central areas ceased to take place, with great improvement in the appearance of all the affected skin, while the marginal thickening had nearly all disappeared, so that the edge could no longer be felt, although some redness still remained, except in the central parts, which presented smooth, whitish scar tissue. The shoulder patch has now lost all thickening, and shows only thin scar tissue. Occasional injections are still given, and the patient will be kept under observation to determine if the improvement proves to be permanent or not.

So far the result of this simple method of treatment in a very resistant and extensive case of lupus vulgaris encourages me to recommend its further trial by others with more clinical material than I can command. I may also mention that Dr. T. A. Henry has made a copper hydnocarpate and an iodohydnocarpate. These are now being tried by experts in skin and surgical forms of tuberculosis, but the simple creosoted ester hydnocarpate proved so satisfactory in the above case that I did not have occasion to use them.

REFERENCES

- ¹ *British Medical Journal*, 1916, ii, 550.
- ² *Indian Med. Gaz.*, February, 1918, p. 73.
- ³ *Indian Journ. Med. Research*, November, 1919, p. 236.
- ⁴ *Lancet*, 1924, ii, 1297.
- ⁵ *Journ. Infect. Dis.*, 1920, xxvi, No. 3, 238.
- ⁶ *La Presse Méd.*, 1929, xxxviii, 358.
- ⁷ *Lancet*, 1930, ii, 1410.
- ⁸ *Journ. Philippine Islands Med. Assoc.*, 1929, ix, No. 9, 321.

INHIBITORY ACTION OF "ALEPOL"

As far as we can ascertain, previous experiments designed to test the inhibitive or bactericidal effects of chemotherapeutical preparations on the tubercle bacillus have been carried out by making surface cultures on fluid media impregnated with the substance under test, or by adding the test substance to nutrient media, which were then solidified and employed as a culture substrate on the surface of which the growth, if any, of the tubercle bacillus could be observed. Examples of these methods are to be found in the works of Möllgaard, Madsen and Mörch, Sweany and Wasick (on sanocrysin), and Walker and Sweeney (on the effects of the hydnocarpates and morrhuates on the growth of acid-fast organisms). The fact that all these methods involve surface growth introduces a possible source of error, since there is a tendency for the tubercle bacillus to heap up its growth vertically as well as to push out laterally, and certain layers of established cultures may be exempt from the action of the chemical substances included in the media.

To avoid this difficulty we decided to work with Besredka's fluid egg medium. A few preliminary experiments with this substance showed that, with the necessary care in its preparation and the use of new-laid eggs, good growth, in the shape of numerous fine balls of culture, could be obtained in the depths of the medium in the course of two or three weeks. A supply of this medium, freshly prepared, was distributed into small Roux bottles—40 c.cm. to each—and the appropriate amount of test substance added, after which the bottles and their contents were sterilized in the autoclave. These Roux bottles,

containing sterile Besredka medium, together with varying quantities of the chemical substance under investigation, were now inoculated with equal amounts of an emulsion of *T.B. constantinides*, a human strain of average virulence, one inoculated flask being left without chemical addition as a positive control and one left uninoculated as a negative control. The latter was regarded as important, as a certain amount of precipitation takes place in this medium after some weeks at 37° C., and this might be confused with a fine growth unless a negative control flask were included.

IN VITRO EXPERIMENTS

The substances tested in our first experiment were alepol (B. W. and Co.), a preparation of the sodium salts of a selected fraction of the lower melting point fatty acids of hydnocarpus oil, and an emulsion of cod-liver oil reduced to very fine dispersion in saline. The two latter preparations were supplied to us through the kindness of Sir Leonard Rogers, F.R.S., who informs us that the cod-liver oil emulsion has been prepared by Dr. V. G. Walsh for experiments in the treatment of tuberculosis. Sir Leonard Rogers had not suggested that this emulsion possessed bactericidal properties, nor did this appear probable, but we made use of it so as to exclude any such possibility.

Our results, after incubation of the Roux bottles for thirty-five days, are shown below.

TABLE I

Test Substance	Serial Dilutions			
	1 in 10,000	1 in 100,000	1 in 1 million	1 in 10 million
Alepol	Nil	Nil	Nil	Contamination
Cod-liver oil ...	Growth	Growth	Growth	—

The cod-liver oil was further tested in dilutions of 1 in 100 and 1 in 1,000, a copious growth occurring in both. The positive control flask also contained copious growth, but the negative flask was sterile. To make sure that there were no living tubercle bacilli in the highest dilution of alepol noted as free from macroscopic growth, a volume of 0.5 c.cm. was taken from the 1 in 1,000,000 flask and inoculated into guinea-pigs on January 20th, 1931. At the same time 0.5 c.cm. of the 1 in 10,000 flask of the cod-liver oil emulsion was similarly tested. The cod-liver oil guinea-pig died of generalized tuberculosis on March 18th, 1931, while the animal inoculated from the alepol flask remained in good health until October 9th, 1931, when it was killed, examined, and found free from infection. So far as tubercle bacilli were concerned this experiment appeared conclusive. We found, however, on microscopical examination of our flask cultures, that a non-acid-fast rod, similar in size and certain cultural characters to the tubercle bacillus, which has been a source of trouble in some of our stock cultures during recent years, had appeared in the *constantinides* strain used, and was visible in the positive control contents. We thus felt that the work should be repeated.

In our next experiment, which was carried out on similar lines, the cod-liver oil preparation was left out and only the alepol tested. The results of our second investigation are given in Table II.

TABLE II

Test Substance	Serial Dilutions			
	1 in 10,000	1 in 100,000	1 in 1 million	1 in 10 million
Alepol	Nil	Nil	Nil	Very slight growth