Hydnocarpus Oil and its Ethyl Esters. How to prevent trouble with Injections.

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INTRODUCTION.

THE two preparations for injection in most common use in leprosy treatment are hydnocarpus oil and its ethyl esters. The advantages and disadvantages of these may be summarised as follows :—

1.—*Cost.* Oil is cheaper, esters dearer.

2.—*Ease of Injection.* This is of importance where large numbers of patients have to be treated in a limited time. Esters being more fluid are more easily injected especially by the intradermal method. Oil being much less fluid at ordinary temperatures is more difficult to inject, but if heated it can be given subcutaneously, intramuscularly or intradermally.

3.—*Rapidity of Absorption.* The esters being more fluid are more rapidly absorbed than the oil. With intradermal injections this is a doubtful advantage.

4.—*Local Reaction.* The oil if pure and fresh causes little local reaction. The esters if properly prepared and used cause little reaction, but otherwise local reactions may occur.

5.—*Therapeutic Effect.* It is a matter of opinion which of these two preparations is more beneficial. The writer's own experience of subcutaneous and intramuscular injection favoured the esters. Only the esters have been extensively used for intradermal injection, but Dr. Muir has pointed out that oil heated to 50° C. can be given by intradermal injection and that the comparatively slow absorption of the oil may be an advantage rather than a disadvantage, for the local effect may be of longer duration. The oil injected intradermally is beneficial but whether it is as good as the esters remains to be seen.

Painful Injection and their Prevention.

Leprosy treatment is a long slow job. Many patients fail to continue treatment long enough. One factor which causes this is pain and trouble with injections. It is therefore most important that we should reduce this pain to a minimum.

From time to time we have ourselves experienced the difficulty of producing oil and esters which are comparatively painless. We have from time to time received reports about the painfulness of commercial supplies of oil and esters. We have therefore investigated the matter carefully, and the results of our enquiries are incorporated in this brief paper.

In general the factors tending to give pain are :—

1.—Free fatty acids. These appeared to be of two kinds, first the free natural fatty acids found in perfectly good fresh oil and second fatty acids produced by oxidation in oil which is old and not carefully stored. The first appears to cause little irritation, fresh oils containing 6 per cent. of free acid sometimes giving little pain, but the oxidation products are much more irritating and if present in only small amounts may cause much pain on injection.

2.—Volatile impurities of doubtful nature produced by oxidation.

3.—Suspended impurities.

These irritating products can be prevented or removed by using only carefully stored fresh oil, by neutralising the acid present, by steaming to remove volatile impurities and by filtration. There are five requirements for comparatively painless injections of oil or esters. The oil must be pure and fresh, esters must be properly made, oil or esters must be properly sterilised ; they must be properly stored, and the injections must be properly given.

Fresh Pure Oil.—Oil must first of all be hydnocarpus oil unadulterated. Other oils or adulterated oils are often sold as pure hydnocarpus oil. Supplies should be purchased only from reliable firms. The freshest oil is obtained direct from the firms preparing it in the months of June and July. It may be advisable to purchase a year's supply in these months.

Oil can be tested by the polarimeter which will show if it is genuine hydnocarpus oil and will also detect adulteration, and it can also be tested for free acid and oxidation products. These tests are unnecessary if the oil is obtained from a reliable source.

Proper Storage.—Oil must be stored so as to prevent oxidation. Clean glass bottles with stoppers should be filled to exclude air and keep in a cool dark place. The same applies to storage of esters. Only remove from the store just sufficient for immediate requirements. Esters or oil

left over at the end of a day's work should not be poured back into stock bottles. For out-patient clinics it is usually better to have the oil or esters sterilised in four-ounce bottles, one bottle being emptied and used before the next bottle is opened. This prevents waste and facilitates the maintenance of sterility. Small amounts left over can be utilised for purposes other than injection.

The Proper Preparation of Esters.—The details of one satisfactory and simple method of making esters, a method which can be used even in small institutions is given in Appendix I. Methods of testing the purity of esters are given in Appendix II.

Sterilisation.—Oil and esters should be sterilised by heating to 120° C. for half an hour. This may be done in an oil bath or in an autoclave. Old hydnocarpus oil or any other cheap oil can be used for the oil bath. If it is done in an autoclave the bottles used must be good and be very securely stoppered. Otherwise the stopper may blow out and steam will get into esters, or the bottle may burst. Sterilisation should be done once only. Repeated sterilisation causes increase in irritating properties.

Before sterilisation creosote 4 per cent. may be added. Double distilled creosote of a reliable make should be used. Inferior creosote may contain impurities in large amount which may cause pain on injection.

The Technique of Injection.—This need not be described here. It is described fully elsewhere. We would emphasise one or two points which are sometimes overlooked. The needle and the syringe should be free from spirit or other disinfectant which may cause pain. By the intradermal technique only minute quantities (about 1 minim) should be injected at each puncture. Larger quantities may cause severe local reaction. Intramuscular injections are best not given just at the place on which the patient sits down.

If the precautions outlined in this note are carefully followed there should be little or no trouble with painful injections.

Any readers wishing for further information regarding sources of supplies, etc., may communicate with the Leprosy Department, School of Tropical Medicine and Hygiene, Calcutta.

I am indebted to Mr. N. K. De, the chemist of the Leprosy Research Department for information concerning the chemistry of the hydnocarpus preparations.

APPENDIX I.

The Manufacture of Ethel Esters of Hydnocarpus Oil.

1.—*Requirements.*

(*a*) Apparatus

3 Litre flasks.
Reflux condensers.
Measuring cylinders 500 c.c.
Hot water bath, *see* Fig.
Heating apparatus (electricity, gas or primus stove).
Retort stand with clamps.
Rubber tubing.
Separating funnels, 2,000 c.c.
Large funnel for filtration.
Filter papers large size.
Large double saucepan.
Burette (10 c.c.) and stand.
Erlenmeyer flasks 100 c.c.
Pipettes 5 and 10 c.c.

(*b*) Other supplies

Hydnocarpus oil.
Absolute alcohol.
Rectified spirit.
Sulphuric acid pure.
Caustic soda.
Creosote (double distilled).
Phenolphthalein indicator.
Common Salt crystals.
Normal solution of Caustic Soda.
Soda Ash (exsiccated Sodium Carbonate).

2.—*Methods.*

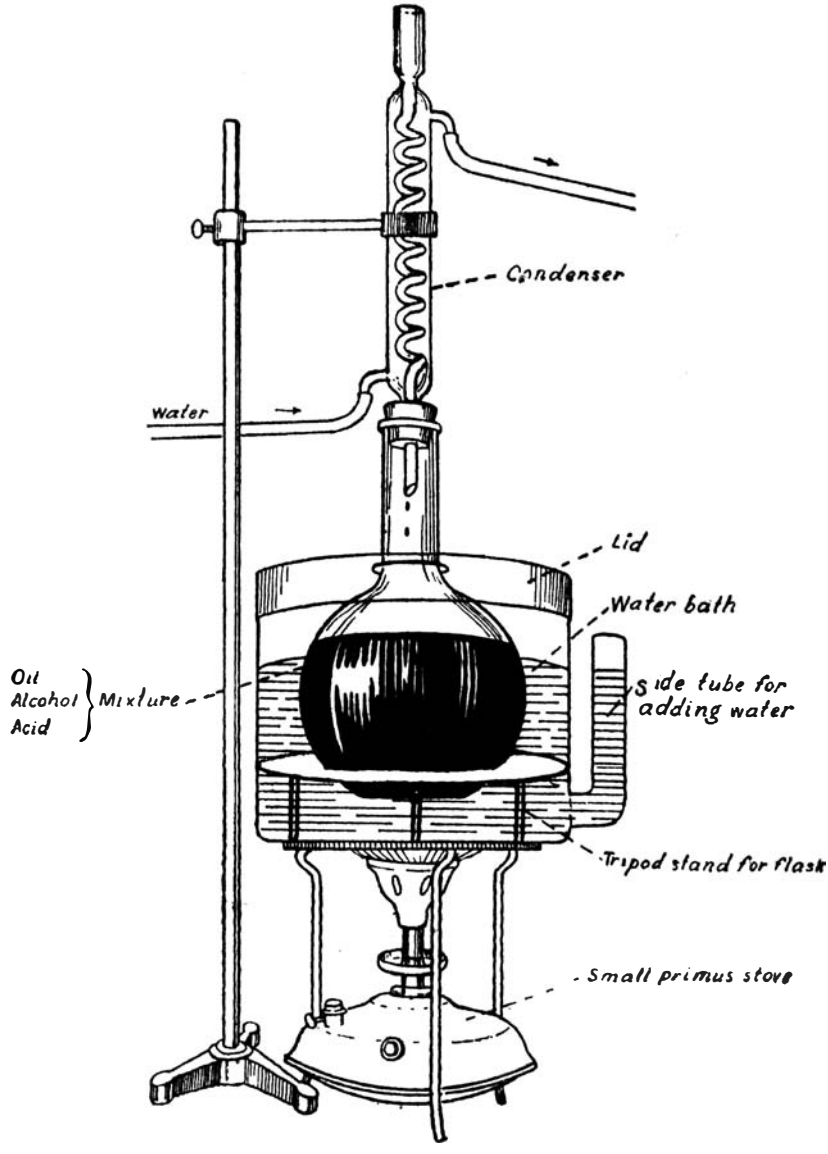
In a 3,000 c.c. flask pour alcohol 95 per cent. by volume (*see* note) 1,300 c.c., sulphuric acid pure (specific gravity 1·84), 75 c.c., and when these are thoroughly mixed add hydnocarpus oil 1,090 c.c. After the mixing the oil falls to the bottom of the flask. Heat on hot water bath with a reflux condenser attached to flask so that there is just a steady drip of condensed alcohol from the bottom of the condenser. The esters when formed are a light brown oily fluid which rises to the top of the fluid in the flask. On this apparatus the esters separate in about three to four hours. Having seen the time needed for separation to start, say four hours, allow an equal time, four hours, for completion. Allow to cool.

Testing for Complete Esterification.

If necessary this can be done by mixing 2 c.c. of the esters with 2 c.c. of 95 per cent. alcohol. If esterification is complete, complete solution occurs with a little shaking and without heat.

Washing Esters and Neutralising Free Acid.

Pour the esters into two separating funnels of 2,000 c.c. capacity. Run off lower layer leaving the esters. (About 70 per cent. of the fluid removed is absolute alcohol which can be recovered by distillation). Wash the esters three times with equal quantities of cold water. Then add an equal quantity of hot caustic soda 1 per cent. solution (dissolved in water and filtered). This forms a very thick emulsion. At once fill up the funnels with boiling water, add to each funnel about 4 drams of ordinary table salt, place the stopper in the funnel and without shaking rotate



APPARATUS FOR MANUFACTURING ETHYL ESTERS.

the funnel in the horizontal position to bring the salt into contact with the emulsion. Rotate for about one minute, then gently replace funnel in stand and leave till separation is fairly complete. This may take one or two hours. If separation is slow it can be accelerated by running out what water has separated at the bottom and refilling the funnel with boiling water. After complete separation the esters should be washed three times with boiling water. At the end of this process the esters are light brown and opaque because of a considerable amount of water remaining in emulsion.

Steaming.

The irritating volatile impurities can be largely removed by steaming, that is by passing steam through the esters for about two hours. This is best done between the washing and the drying as steaming introduces water into the esters. Steaming makes the esters lighter in colour and reduces the irritating smell of the esters. Steaming should be continued until the irritating smell has practicallydisappeared. The water introduced by steaming can be removed by a separating funnel.

Drying.

The esters are heated over boiling water. A double saucepan is very convenient. After about a quarter of an hour's heating the top pan containing the esters is removed and allowed to stand for a few minutes. Nearly all the water settles to the bottom and the esters at the top can be gently poured off and the water with a little esters at the bottom can be removed. The esters are again heated on the double saucepan, and the process of water removal can be repeated, if necessary. Within half-an-hour of the heating being started, the esters can be rendered perfectly free from water, the small amount of water left towards the end being driven off as steam. The esters finally should be clear and free from emulsion, though there may be fine particles in suspension.

Filtration.

The dried esters are now allowed to cool and they are then filtered. A large funnel and filter paper 1-ft. in diameter greatly accelerates filtration. With such apparatus from 8 to 10 lbs. of esters can be filtered through one funnel in an hour or two. The resulting esters are light brown in colour and perfectly clear.

3.—*Quantities.*

By the method here outlined from 1,090 c.c. of oil 1,150 c.c. of esters are produced. 1,300 c.c. of alcohol

are used and 60 per cent. of this can be recovered redistillation (*see* next section).

4.—*Note on Alcohol.*

Strength. The alcohol for making esters should have a concentration of about 95 per cent. by volume of alcohol. Absolute alcohol purchased from a reliable source contains 99 per cent. by volume of alcohol. Rectified spirit (B.P. standard) contains 90 per cent. by volume of alcohol. The alcohol recovered by distillation after making esters contains about 93 per cent. of alcohol. It is therefore quite satisfactory to mix equal parts of (1) absolute alcohol, and (2) either rectified spirit or recovered alcohol. This gives a mixture containing about 95 per cent. of alcohol.

Method of Recovery of Alcohol.

When esterification is complete the esters rise to the top and the lower layer consists mainly of (1) alcohol (2) sulphuric acid (3) glycerol. This lower layer is removed with a separating funnel. The acid is then neutralised and the alcohol is distilled off. The method is as follows. To 1,000 c.c. of this alcohol mixture add 140 grammes of dry commercial washing soda (exsiccated sodium carbonate). Leave over night. Transfer the mass to a flask containing a few pieces of pumice stone. To this flask attach by glass and rubber tubing a condenser. Heat the flask on a water bath and distil the alcohol over. The same apparatus which is used for ester making can be modified by tubing to do distillation also. Sometimes a thick emulsion will form in the flask and distillation will be arrested. If this occurs add to the emulsion about 50 c.c. of water and distillation will then recommence. About 60 per cent. of the alcohol used, can be recovered by distillation. This recovered alcohol can be mixed with absolute alcohol for making more esters.

APPENDIX II.

Method of Testing Esters.

Esters should be yellow or light brown. They should be perfectly clear. They should have a characteristic odour which should not be too pungent. If a specimen passes these tests, it can be tested also by the following methods, dissolving in absolute alcohol, by testing for free acid, and by giving small experimental injections.

1.—*Test for Complete Esterification.*

If esterification is complete the resultant esters should be completely soluble in an equal amount of 95 per cent. alcohol without heat.

Partial solution indicates incomplete esterification.

2.—*Test for free acid.*

The suitability of esters for injection depends very largely on the amount of free acid they contain. The amount present can be estimated by titration, the esters being dissolved in absolute alcohol. Since absolute alcohol is itself usually acid in reaction, it has first to be neutralised.

Pour into a burette N/20 sodium hydroxide solution. Into a small conical flask pour about 10 c.c. of absolute alcohol and about 4 drops of phenol phthalein indicator. The alcohol being acid, no colour results. Run in the N/20 sodium hydroxide drop by drop until a faint pink colour is produced. This indicates that the alcohol is neutralised. Then add to the alcohol 5 c.c. of esters shaking to produce complete solution. The pink colour disappears. Read the level of the sodium hydroxide in the burette, then titrate drop by drop until the pink colour re-appears in the flask, shaking the flask all the time. Read the amount of sodium hydrate used. The amount of N/20 sodium hydrate necessary to neutralise 5 c.c. of esters should be less than ·5 c.c. and we find that such esters are satisfactory for injection. If on testing, the acidity is found to be too high, further washing and neutralisation of the esters by caustic soda is necessary. By the method here described we produce esters needing only ·2 c.c. of N/20 sodium hydroxide to neutralise 5 c.c.

3—*Test for Pain on Injection*, by giving small test injections by the intradermal method. Local reaction should be confined to slight induration lasting only a few days and there should be no ulceration.