THE IRRITANT ACTION OF SOME DRUGS DERIVED FROM HYDNOCARPUS OIL[1]

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Although a large number of drugs has been tried or suggested for use in leprosy, the oil from the seeds of *Hydnocarpus wightiana*, and in some parts of the world *H. anthelmintica* and *Taraktogenos kurzii* (chaulmoogra), still provide the only antileprosy drugs in general use. This is perhaps in some degree due to the difficulty of measuring clinical progress in leprosy and of estimating the curative value of new drugs. The nature of the disease is such that treatment has to continue over long periods. If the patient is to be encouraged to persevere with it, the drugs used must be of low toxicity and the discomfort attending their application must be reduced as far as possible.

The present paper describes some of the work which has been carried out in these laboratories in the past ten years with a view to securing a drug which is effective and easy to administer, and at the same time to eliminating as far as possible the pain attending its use. The method of estimating the degree of irritation consisted in determining the minimal concentration causing necrosis on intradermal injection into shaved guinea pigs, dilutions being made in liquid paraffin, as described by Paget, Trevan and Attwood (6).

CAUSES OF IRRITATION IN ANTILEPROSY DRUGS

Excessive pain on intravenous, intramuscular or intradermal injection of drugs derived from hydnocarpus oil has been attributed to:

1. The low solubility of the soaps.

[1]J. W. Trevan and A. M. P. Attwood were responsible for all experiments on animals.

2. The degree of acidity of the oil or esters, or of alkalinity of the salts.
3. Hemolytic action of soap solutions.
4. Products of autoxidation of fatty acids.
5. By-products of manufacturing processes.

Rogers (8), as a result of a study of the action of the so-called "gynocardates"—sodium salts of fractions of the acids of chaulmoogra oil—found that in a short time the veins became blocked near the site of intravenous injection, an occurrence attributed to deposition of the sodium chaulmoograte, which is of low solubility. The salts of the lower melting acids, containing a higher proportion of hydnocarpic and liquid acids, are more soluble, and these are now used for intravenous or intradermal injection instead of the salts of the total acids.

It has been shown (6) that the acids of hydnocarpus oil cannot themselves be injected, diluted with liquid paraffin, without causing necrosis. The minimal necrosing concentration is 1.5 percent, equivalent to an acid value of 3, as compared with a minimal necrosing concentration of 100 percent for the ethyl esters and 2.25 percent in water for the sodium salts. On the other hand Jackson (3) tested for the authors in patients a series of 3 percent aqueous solutions of sodium salts of the lower melting acids having pH values ranging from 8.3 to 9.5, and found no correlation between alkalinity and the degree of irritation.

HEMOLYSIS BY SODIUM HYDNOCARPATE

Solutions of the sodium salts of hydnocarpus acids, in common with all soap solutions, exert a hemolytic action on red blood cells in vitro, a 0.5 percent aqueous solution hemolysing a 5 percent suspension of red cells in normal saline in 2 minutes at 37°C. Rogers (7), however, considered that the degree of hemolysis produced by sodium "gynocardate" was negligible from the practical point of view. In Table 1 are shown the rates of hemolysis in vitro by dilutions of sodium salts of (*a*) the total hydnocarpus acids, (*b*) the liquid hydnocarpus acids, and (*c*) oleic acid. These were determined by adding 1 cc. of the soap in various solutions or in normal horse serum to 1 cc. of a 5 percent suspension of washed red cells in normal saline or in serum, and in a thermostat at 37°C. The tubes were rapidly shaken and the time required for hemolysis was measured.

From these results it appears that the concentration of sodium

hydnocarpate which just fails to hemolyse red blood cells in normal horse serum is about 100 times that required when normal saline is used as the solvent. It seems unlikely that, under the conditions of intravenous or intradermal injection, hemolysis is an important factor in the production of irritation, since if a 3 percent solution is injected slowly and with a small needle, the circulating blood will rapidly reduce the concentration of soap at the site of injection below that required for hemolysis in vitro in presence of serum.

TABLE 1. *Hemolysis by sodium hydnocarpate solutions.*

Solvent	Concentration of soap, percent	Time taken to hemolyse, minutes		
		Total acids	Liquid acids	Oleic acid
Normal saline	0.05	15	10	10
	0.005	—	33	30
Citrated saline	0.05	40	5	5
	0.005	—	45	30
1% Disodium phosphate	0.05	15	5	1
	0.005	—	60	60
2.5% Glycerol in saline	0.05	50	5	—
0.5% Phenol in saline	0.05	3	2	—
50% Serum[a]	0.10	—	—	—
	0.50	3	2	—
95% Serum[b]	0.50	—	10	—
	0.75	40	10	—
	1.00	8	8	—

[a] = 1 cc. of 5% suspension of red cells in saline +0.1 cc. of 2% or 10% soap solution +0.9 cc. serum.

[b] = 1 cc. of 5% suspension of red cells in serum +10% soap solution and serum to make 2 cc.

Minus signs indicate no hemolysis in 60 minutes at 37°C.

REMOVAL OF IRRITANT DECOMPOSITION PRODUCTS

It has been shown beyond question (2, 4, 6) that the principal cause of excessive irritation by hydnocarpus preparations is the presence of oxidation products of the unsaturated and unstable acids, chaulmoogric, hydnocarpic and dehydrochaulmoogric. Their presence is due either to the use of deteriorated seed as a source of the oil, or to exposure to light and air of the oil or of the esters or salts of the acids. Once formed, these products cannot be completely removed by washing or by distillation, and they give rise to volatile and irritant products in the course of manufacture of the esters.

An attempt has been made to identify among the latter formic acid, which has been suggested (9) as one such irritant product. A

sample of ethyl esters was freely exposed for several months, until it became yellow and viscous. It was then steam distilled. The distillate was passed into a suspension of barium carbonate, and formic acid was estimated by mercuric chloride. Formic acid was found to amount to 0.56 percent, equivalent to about one third of the acid value of the exposed esters. The data given in Table 2 show that two or three times this amount of free formic acid, added to ethyl esters of hydnocarpus or oleic acids, which are themselves bland, can be injected without causing more extensive necrosis than the exposed esters.

TABLE 2. *Irritant action of formic acid.*

Preparation tested	Acid value	Necrosis caused by concentration [a]		
		100%	50%	25%
Ethyl esters of hydnocarpus acids				
Freshly distilled	0.5	±	±	0
After exposure	40.0	++	+	+
With 1.36% formic acid	17.5	++	+	0
Ethyl oleate				
Distilled	0.5	0	0	0
With 1.9% formic acid	24.0	+	±	±

[a] Dilutions in liquid paraffin.

ESTERS OF HYDNOCARPUS CRYSTALLINE ACIDS

While there is little evidence to show that any one of the three characteristic acids of hydnocarpus oils is more effective than the others in the treatment of leprosy, hydnocarpic acid is more rapidly oxidized than chaulmoogric, and dehydrochaulmoogric acid containing two ethylenic linkages is least stable of all. This last acid constitutes, with oleic acid and some hydnocarpic acid, the low-melting or liquid fraction, which also contains the irritant tarry acid products of oxidation. It is possible to remove these tarry products by means of the copper salts which are insoluble in acetone or ether (5). The ethyl esters of the liquid acids refined in this way when washed and dried are quite bland, but they become irritant on distillation at 200-240°/2 mm. (see Table 3).

In view of these facts, a series of esters was prepared from the higher-melting hydnocarpus acids from which the liquid acids had been eliminated by crystallizing the total acids from 80 percent alcohol. Of the esters shown in Table 4, the ethyl esters were prepared in the usual way with sulphuric acid as catalyst; the

cyclohexanyl, benzyl and phenylethyl esters were made by refluxing a mixture of the acids and the alcohol for 8 hours; and the cinnamyl, thymyl and menthyl esters were made by means of the acid chloride. The esters were freed from acid by washing with

TABLE 3. *Effect of distillation on ethyl esters of hydnocarpus liquid acids.*

Preparation of ethyl esters	Necrosis produced by concentration		
	100%	50%	25%
Unrefined	++	+	±
Refined, undistilled	0	0	0
Refined, distilled	++	0	0

an equal volume of hot 50 percent alcohol containing a small excess of sodium hydroxide, then with 30 percent alcohol, and finally with hot water until the oil separated sharply and water-bright. In this way troublesome emulsions were avoided. The mixed alcohols of the crystalline acids were made by reduction of the ethyl esters with sodium and amyl alcohol, and the alcohols were esterified with benzoyl or cinnamoyl chloride.

TABLE 4. *Irritant effects of various esters of hydnocarpus crystalline acids.*

Esters	b.p.	Necrosis produced by concentration		
		100%	50%	25%
Ethyl	205-220°/12mm.	+	0	0
Cyclohexanyl	240-255°/15mm.	±	0	0
Benzyl	250-265°/12mm.	±	0	0
Phenylethyl	255-270°/12mm.	0	0	0
Cinnamyl	230-255°/1mm.	0	0	0
Thymyl	205-225°/1mm.	trace	0	0
Menthyl	215-245°/1mm.	0	0	0
Benzoate [a]	225-235°/3mm.	++	++	±
Cinnamate [a]	240-270°/3mm.	++	0	0
Ethyl of total acids	205-220°/12mm.	±	±	0

[a] Esters of hydnocarpus alcohols.

As will be seen from Table 4, the aralkyl esters of the crystalline acids are less irritant than the ethyl esters of the crystalline or total acids. The menthyl esters are bland, producing practically no reaction, when injected without dilution. The phenylethyl and cinnamyl esters caused lumps which subsided without necrosis; with the thymyl esters there was a trace of necrosis. The degree of irritation caused by the esters of the mixed alcohols is in marked contrast to that from the esters of the mixed acids, and is possibly

due to the insoluble nature of the alcohols liberated as a result of hydrolysis in the tissues.

STABILIZATION OF IODIZED ETHYL ESTERS

The methods generally adopted to render the ethyl esters as bland as possible consist of steam distillation and iodization of the dried esters by the addition of 0.5 percent of iodine at a temperature of 145-150°C. in an open vessel (1). Such a preparation of ethyl esters is almost black in color and on injection causes a blue, bruise-like stain which may last for some years. These

TABLE 5. *Stabilization of iodized hydnocarpus ethyl esters.*

Preparation	After 3 months exposure:			
	Color	Necrosis caused by concentration		
		100%	50%	25%
Iodized ethyl esters of total acids, without additive	light brown	++	+	+
Iodized ethyl esters of crystalline acids, without additive	golden yellow	++	++	++
Same, with 0.1% catechol	deep brown	0	0	0
Same, with 0.1% quinol	deep brown	++	+	0
Same, with 0.1% pyrogallol	deep brown	0	0	0
Same, with 0.1% phloroglucinol	deep red brown	+	0	0
Same, with 0.2% guaiacol	light brown	++	++	0
Same, with 1.0% creosote	deep red brown	+	0	0
Same, with 1.0% phenol	light brown	.	.	+
Same, with 1.0% thymol	light red	.	±	0
Same, with 5.0% ethyl oleate	yellow	+	±	±
Same, with 10% ethyl esters of liquid acids	light brown	.	+	±
Same, with 5.5% ethyl esters of tarry acids	red brown	+	+	±
Same, with 5.5% ethyl esters of keto-acids	red brown	+	±	±
Control: iodized ethyl esters of total acids, not exposed	very dark	0	0	0

iodized esters will remain bland for several months if stored in a full, closed bottle in diffused daylight. If, however, the bottle is frequently opened, especially under tropical conditions, the color becomes lighter and irritant properties develop. The iodized esters of the crystalline fraction of acids cause a less pronounced stain, but are less stable to oxidation than those of the total acids, and in the course of one or two months storage become of a golden yellow color. Addition of the constituents of the liquid

acids does not greatly increase the stability of these iodized esters, but the presence of 0.1 percent of certain phenols, which have been shown to inhibit autoxidation of drying oils, notably catechol and pyrogallol, prevents oxidation for several months. The ethyl esters listed in Table 5, to which the substances indicated were added after iodization, were exposed for three months in open bottles under tropical conditions and tested at the end of that time by intradermal injection.

SUMMARY

1. An acid value of 3 is sufficient to render hydnocarpus preparations irritant.

2. Hemolysis is caused rapidly by salts of hydnocarpus acids in normal saline but it is so far inhibited by the presence of serum that it cannot be considered an important factor in causing pain on injection.

3. The amount of formic acid present in ethyl esters which have deteriorated is insufficient to account for more than a small part of the irritation.

4. Aralkyl esters (thymyl–, phenylethyl–, cinnamyl–, and menthyl–) of the crystalline acids are less irritant than the ethyl esters, causing no necrosis on intradermal injection.

5. Iodized ethyl esters of the crystalline acids are unstable on exposure to air, but oxidation is inhibited by addition of 0.1 percent of catechol or pyrogallol.

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