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RELATION OF IRRITATION TO METHOD OF
MANUFACTURE OF ETHYL ESTERS
OF CHAULMOOGRA OILS

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The causes of irritation of the tissues upon injection of chaulmoogra ethyl esters have generally been ascribed to the presence of (a) a small amount of a strongly irritant compound of unknown nature, (b) free fatty acids, (c) irritant compounds produced by heating of the drug, and (d) an inherent irritant quality of the esters. It has been assumed that the greater the degree of purification the less the irritant quality, but experiments to prove this have not heretofore been made. To determine whether distilled esters are less irritant than undistilled, and whether removal of free fatty acids and volatile impurities actually reduces irritation, the following experiments were carried out.

EXPERIMENTAL WORK

Ethyl esters of *Hydnocarpus wightiana* oil were prepared by our standard method (1): they were boiled for 8 hours, washed four times with twice their volume of hot water, and dried. One-half of the resulting "crude" dry esters was distilled at 15 mm. pressure. Both of these lots (crude and distilled) were divided into four parts which were treated separately in the following manner:

- (a) Used without further treatment.
- (b) Blown out with steam for two hours to remove volatile impurities, filtered and dried.
- (c) Neutralized with an excess of lye to remove free fatty acids, washed free of soap and dried.
- (d) Combined (b) and (c) treatment; i.e., neutralized, washed, blown and dried.

To each of these eight lots of esters was added 4 percent of creosote U.S.P. at room temperature, the mixtures were filtered and put into 5 cc. ampules, the latter were sealed and all were sterilized at one time in an autoclave for 20 minutes at one atmosphere pressure.

TABLE 1.—Comparison of irritant quality of creosoted H. Wightiana esters made by various methods.

Lot No.	Number of patients showing irritation after															Ulceration after two days					
	Two days					Four days					Eight days					No.	Percent				
	Marked		Slight			Marked		Slight			Marked		Slight								
	+++	++	+	Total	---	+	Total	+++	++	+	Total	+	—	Total	+++	++	+	Total			
9	8	8	15	6	21	4	3	7	16	14	30	—	—	0	—	—	11	26	37	8	21.6
10	7	9	18	3	21	4	3	7	20	10	30	—	—	0	—	—	10	27	37	7	18.9
11	4	8	12	4	25	4	—	4	20	13	33	—	—	0	—	—	11	26	37	4	10.8
12	5	4	9	20	8	28	2	4	6	17	31	—	—	0	—	—	4	33	37	3	8.1
13	4	10	14	13	10	23	4	4	8	12	17	—	—	1	1	7	29	36	4	10.8	
14	4	10	14	15	8	23	5	2	7	13	17	—	—	0	—	8	29	37	4	10.8	
15	3	8	11	21	5	26	3	1	4	16	17	—	—	0	—	8	29	37	3	8.1	
16	2	4	6	20	11	31	2	1	3	14	20	—	—	0	—	7	30	37	1	2.7	

Key: +++ papule with intense erythema or ulceration; ++ papule more than 1 cm. in diameter;
+ papule less than 1 cm. in diameter; — complete absence of reaction.

These lots were numbered (Nos. 9 to 16) and tested clinically at the Curupaity leprosarium.¹

CLINICAL TESTS

Skin irritation tests of these eight lots of creosoted ethyl esters were made on each patient, four injections being given in the upper part of each arm. Injections were made intradermally with a short, Muir-type needle and tuberculin syringe graduated in 0.01 cc., exactly 0.1 cc. of each drug being injected. The effects were observed immediately upon injection, one-half hour after injection, and two, four and eight days later. The degree of pain upon injection and the appearance after one-half hour showed so little variation that the results are not included here. None of the drugs was very painful on injection. Table 1 shows the results of the tests on 37 cases of leprosy. Table 2 and Text-fig. 1 show the characteristics of the drugs tested, as well as the order of increasing irritation.

TABLE 2.—*Properties of creosoted H. Wightiana esters. Lots listed in order of increasing irritation.*

Lot No.	Method of purification	Free fatty acid percent ^a	Optical rotation, degrees ^b	Refractive index at 25°C.
16 ^c	Distilled, neutralized and blown.....	0.08	47.98	1.4578
12	Crude, neutralized and blown.....	0.16	47.76	1.4586
15	Distilled, neutralized.....	0.08	47.98	1.4579
11	Crude, neutralized.....	0.16	47.76	1.4586
14	Distilled, blown.....	2.00	47.96	1.4581
13	Distilled, untreated.....	2.10	48.12	1.4581
10	Crude, blown.....	2.60	47.98	1.4591
9	Crude, untreated.....	2.90	47.90	1.4591

^a Free fatty acid as percent oleic.

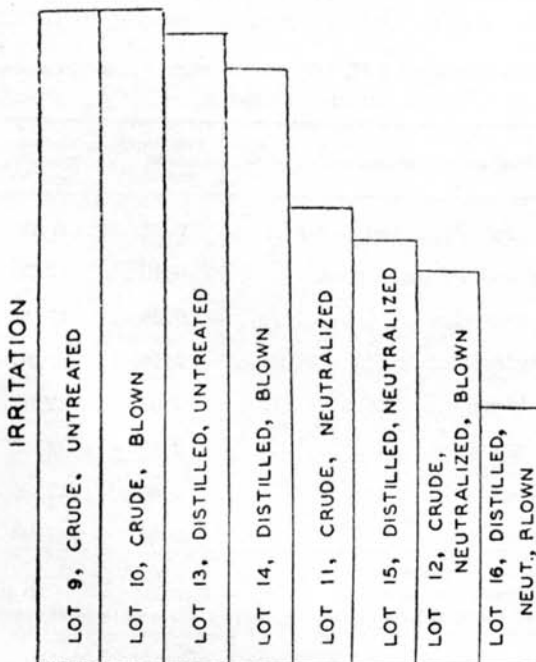
^b Actual rotation in 10 cm. tube.

^c Lot 16 is our standard method of preparation and is shown to be the least irritant of the methods tried.

Attempts were made to use iodized instead of creosoted esters, but those preparations gave very inconstant results, probably because the conditions of heating the esters with iodine in small lots could

¹The clinical tests mentioned in this paper were made by the medical staff of the Curupaity leprosarium. Several thousand tests were made, but for conciseness they are not included here. The authors wish to acknowledge their indebtedness for the cooperation of the director, Dr. d'Almeida, and Drs. Campos Mello, Ferreira da Rosa, Moura Costa, Portugal Rodriguez and Souza Araujo.

not be regulated closely enough. Several methods of preparation were tried, but the iodized products were always far more irritating than our standard drug. One method of combining the iodine by heating in sealed ampules is described as it gave interesting though unsatisfactory results. One-half percent of iodine was dissolved in the esters by stirring without heating. This yielded a dark green liquid. One-tenth cc. injected intradermally without sterilizing (to avoid heating) immediately gave rise to large wheals and an intense burning sensation. The same product placed in 5 cc. ampules, sealed and heated in the autoclave for one hour at two atmospheres pressure (134°C.) was still very irritating. Another sample, heated for two hours at the same pressure produced no pain upon injection but was decidedly irritating for at least six days after injection. The last two products were of a clear brown color.



TEXT-FIG. 1. Reduction of irritation of *H. wightiana* esters of distillation, neutralization, and removal of volatile impurities. (From Table 1, columns 4 and 10.)

INFLUENCE OF METHOD OF ESTERIFICATION

To determine whether variation in methods of esterification influences the irritant effect, esters were prepared by the 48-hour

boiling method used at Culion (2) and the 8-hour boiling method used here (1). No differences in irritation were observed. Esters prepared by the cold sunlight method (3) were only slightly more irritant. Any one of these three methods yields quite satisfactory esters.

PRINCIPAL CAUSE OF IRRITATION

Experiments made at Culion (4) several years ago seemed to lead to the conclusion that a large part of the irritation was due to the ethyl hydnicarpate and chaulmoograte themselves. Recently we have been able to prepare these compounds in an even purer state than those used at Culion. These have been tested intradermally.

Ethyl hydnicarpate made from absolutely pure hydnicarpic acid and then washed, neutralized and immediately injected, produced no pain and only a very slight reddening of the skin. After this product had been distilled at 10 mm. pressure it was still not painful, but after standing in a glass-stoppered bottle for three months 0.1 cc. of it was extremely painful when injected intradermally; it caused intense erythema that lasted more than two weeks. This product was then redistilled at 10 mm. pressure in a Podbielniak high-temperature fractionation apparatus (5), three equal fractions being taken. The boiling point was lower than that of pure ethyl hydnicarpate. The three fractions were tested intradermally. The first fraction (lowest boiling point) was at least as painful as the ester before it was distilled. The middle fraction was less painful, and the last fraction was only slightly irritating, less so than our standard iodized esters.

Since pure ethyl hydnicarpate when freshly distilled was non-irritating but became intensely irritating after standing for three months, it seems likely that we do not have to seek further for the cause of most of the irritant effect of ethyl esters of chaulmoogra oils. Ethyl chaulmoograte acts like ethyl hydnicarpate except that it is more stable, but it also becomes irritating on standing. There is decomposition, probably for the most part an oxidation process, as these esters soon absorb all of the air in a tightly sealed bottle. The irritant compounds cannot easily be separated by fractionation. Even in a high precision fractionating apparatus each fraction taken produces some irritant effect, though it tends to concentrate in the lower boiling fractions.

DISCUSSION

From Tables 1 and 2 it would appear that our present standard

method of preparation (lot No. 16) yields a product which is superior in every way to those resulting from the other methods tried. Each step in purification helps. Distillation removes some of the inactive substances as shown by increase in optical rotation, and it lowers somewhat the free fatty acids; the product is almost colorless. Blowing out with steam removes decomposition products such as aldehydes, and also the small amount of volatile free fatty acids (see Table 2) which might cause irritation. Thorough neutralization (to less than 0.2 percent free fatty acids) not only removes most of the free fatty acid but undoubtedly has a general purifying effect. The presence of free fatty acid up to 2 or 3 percent is only a minor cause of irritation, but the neutralized esters are distinctly better than those that are not neutralized. In fact neutralization appears to be the most important of the purification processes.

Text-figure 1 illustrates these points graphically. It shows that blowing out with steam (for two hours) has no effect on reducing the irritant properties of the crude esters, but does lower them to some extent in the distilled or neutralized esters. This measure appears to have some effect on lowering the number of cases of ulceration, even in the crude esters (Table 1). Distillation to produce its greatest effect must be accompanied by neutralization and blowing out with steam, as shown by the small drop between lots 9 and 13 and the large drop between 13 and 16. Of the three treatments, neutralization is shown to have the greatest effect on both the crude and the distilled esters, as indicated by the large drops between lots 9 and 11 and between lots 13 and 15.

Unless creosote, iodine or some other irritation-reducing substance is added, however, these purified esters are still far too irritating for injection purposes. This leads to the conclusion that a large part of the irritant effect must be due to one of two factors, either (a) the ethyl esters themselves or (b) the presence of a small amount of a highly irritant compound not removed by any of our present methods and only partly rendered nonirritant by addition of iodine or creosote. But we have seen that very pure ethyl hydno-*carpate* and *chaulmoograte* are nonirritant, becoming extremely irritating upon standing, because of partial decomposition. We believe that the main irritant effect is due to these decomposition products of ethyl hydno-*carpate* and *chaulmoograte*.²

²Ethyl oleate after distillation and standing for three months is not very irritant and, when present in the *chaulmoogra* preparations, cannot be responsible for much of the irritant effect.

SUMMARY

1. Various methods of preparing *H. wightiana* ethyl esters have been investigated with regard to their irritant effect.

2. There is not much variation in irritant effect of esters prepared by the Culion 48-hour method of esterification, our 8-hour method, and the cold sunlight method.

3. In the purification of these esters, neutralization is shown to have the greatest effect in reducing irritation; distillation comes next, and blowing out with steam has the least effect.

4. Even the combination of these three processes yields a product that is too irritating to use without the addition of iodine, creosote, or some other similarly acting substance. This irritation is shown to be due, not to the esters of hydnocarpic and chaulmoogric acids themselves, but, at least in great part, to their decomposition products.

5. Methods of rendering these decomposition products non-irritating without the use of iodine are being investigated.

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