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Comparative Biological Activity of Seven New Water-soluble Chaulmoogric Acid Derivatives.*

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Rogers[1] introduced the first water-soluble chaulmoogra derivative intended for intravenous injection in leprosy, which was recognized to be a systemic disease most rationally treated by administering drugs for systemic effect. Due to the hemolytic, local irritative, sclerosing and other undesirable properties of sodium gynocardate, its intravenous use in humans was generally abandoned even though promising results had been obtained in a considerable number of lepers. No other soluble chaulmoograte has been developed sufficiently free from the disadvantages of "Alepol" (sodium hydnocarpate) or sodium gynocardate to warrant its continued intravenous use. This report deals with several compounds which appear from various theoretical considerations to be preferable to the chaulmoogra soaps.

Seven new water-soluble derivatives of chaulmoogric acid† have been examined pharmacologically and 3 of these have been tested further by a standardized method for antileprotic efficacy as measured by their influence on the course of an experimental leprous infection in rats. The strain of rats, infection, the time and route of administering the drugs, the diet, size of treated and untreated groups, and all other controllable experimental conditions were identical with those used in work previously described.[2] Further experimental treatment is being continued for a more extended period with the more promising agents.

* Part of a cooperative study of the chemotherapy of leprosy conducted by the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund for Medical Research.

[1] Rogers, L., *Lancet*, 1916, **1**, 288; *Brit. Med. J.*, 1916, **2**, 550.

† Those compounds designated by numbers are preparations synthesized and generously supplied by Dr. Richard Wrenshall, of the Chemistry Department of the University of Hawaii; those designated by a letter were synthesized by one of us (G. A. E.) from chaulmoogric acid obtained from redistilled pure ethyl chaulmoograte supplied by Dr. Wrenshall.

[2] Anderson, H. H., Emerson, G. A., and Leake, C. D., *Internat. J. Leprosy*, in press.

TABLE I.
Biological Activity of Water Soluble Chaulmoogric Acid Derivatives.

Drug % of drug in water	Toxicity, acute subcutaneous in rats	Toxicity, acute intravenous in rats	Total tolerated dose* in treated leprous rats in 6 mo.	No. deaths during treatment	Aver. wt. variation in treated leprous rats	Aver. size of leproma at end of treatment	Total no. of sloughs occurring during treatment	Bactericidal concentration in vitro†	Minimum hemolytic concentration on human blood in vitro	Local effects noted of acute toxicity in surviving rats
	Gm./Kg.	Gm./Kg.	Gm./Kg.			Sq.cm.				
No. 104 3% "Alepol" (Na hydnocarpate)	2.0 7/7 died in 24 hr.	0.1-0.125 3/5 died in 2 min.	0.445	3/12	+8	7.4	22	1:20,000	1:20,000 in 3 hrs.	Necrosis in 9/18 rats
Drug "A" 3% (Na dichaulmoogryl-β-glycerophosphate)	2.0 3/5 died in 6 to 24 hr.	1.25 3/6 died in 2 min. to 30 hr.	2.475	0/8	+65	4.2	4	1:20,000	1:500 in 20 hr.	No necrosis at injection site when given subcutaneously
Drug "B" 5% (Na chaulmoogryl glutamate)	2.0 3/5 died in 24 to 48 hr.	0.15-0.25 3/6 died in 10 min. to 24 hrs.	not tested	not tested	—	—	—	1:1,000	1:40,000 in 3 1/2 hr.	No necrosis at injection site when given subcutaneously
No. 1141 3% (Na chaulmoogryl glycinate)	1.0 4/5 died in 24 to 48 hr.	0.15-0.20 3/4 died in 6 min. to 10 min.	0.420	3/8	+56	5.4	5	1:1,000	1:10,000 in 30 min.	Necrosis in 4/12 rats
No. 661-K 3% (K-iodo-dihydrochaulmoograte)	0.25 4/5 died in 10 to 24 hr.	—	0.410	2/8	+52	9.8	6	1:20,000	1:10,000 in 60 min.	No necrotic effect
No. 2001 5% (Na diethylethanolammonium chaulmoograte)	1.5 3/5 died in 3 to 24 hr.	—	not tested	not tested	—		—	1:10,000	1:20,000 in 45 min.	Necrosis in 7/9 rats
No. 1601 3% (Na chaulmoogryl o-aminobenzoate)	0.5 4/8 died in 24 hr.	—	" "	not tested	—	—	—	1:1,000	1:100,000 in 15 min.	Necrosis in 8/8 rats
No. 2211 5% (choline chaulmoograte)	0.5 5/5 died in 5 min.	—	" "	not tested	—	—	—	1:1,000	1:100,000 in 2 hr.	Necrosis in 1/8 rats

* Infiltrated lesion site with drug; dose expressed in chaulmoogric acid equivalent. Rats were infected with a standard strain of *Mycobacterium lepra muris* obtained from Drs. Walker and Sweeney.

† *Mycobacterium lepra hominis*, Mary Puhulahula strain (isolated by Dr. E. L. Walker) was grown on Long's liquid media (*Amer. Rev. Tuberc.*, 1926, **13**, 393).

In Table I may be found the tolerated and minimum lethal doses on subcutaneous and intravenous injections in rats, the *in vitro* bactericidal concentration and the effects in experimental rat leprosy of K-iodo-dihydrochaulmoograte (No. 661-K), Na-chaulmoogryl-glycinate (No. 1141) and Na-dichaulmoogryl-β-glycerophosphate (Drug "A")[3] in comparison with those of "Alepol" (No. 104). The average variations in body weight, size of leprous lesion and incidence of sloughing lesions in each group of infected rats during a 6-months treatment period are included. The toxicity and *in vitro* bactericidal activity of Na-chaulmoogryl-o-aminobenzoate (No. 1601),[4] diethyl-ethanolammonium chaulmoograte (No. 2001), choline chaulmoograte (No. 2211) and Na-chaulmoogryl glutamate (Drug "B") are also shown.

Among infected but untreated control groups observed in earlier studies the mortality from the disease, pneumonia and other causes was 33% in a year, while here 3 of 8 infected control animals have died in 7 months. The mortality ratios of similar leprosy infected rats under treatment with the various drugs are shown in Table I. Average calculated doses of chaulmoogric acid per drug for each of the 3 treatment periods per group of treated rats were: "Alepol," 200, 125 and 120 mg./kg.; K-iodo-dihydrochaulmoograte, 75, 135 and 200 mg./kg.; Na-chaulmoogryl-glycinate, 70, 125 and 225 mg./kg.; and Na-dichaulmoogryl-β-glycerophosphate, 625, 1150 and 700 mg./kg. The apparent superiority of the latter compound in comparison with the others may be due to the fact that we were able to give it in larger amounts, especially with reference to chaulmoogric acid content, without toxic or local effects. This drug is probably hydrolyzed more slowly than the others in the body, liberating a relatively non-toxic solubilizing group and prolonging the presence of the presumably effective chaulmoogra remainder. The modifying factor of availability of the chaulmoogric acid must be considered, however, since both drugs 1141 and "A" rank with the ethyl esters in therapeutic effectiveness although given in much smaller amounts. Our findings in this study with new water-soluble chaulmoograte support our previous conclusions.[2] The importance of unsaturation and optical activity of chaulmoogra derivatives in probable therapeutic value is emphasized by the ineffectiveness of the saturated HI substituted soap, drug 661-K.

[3] Emerson, G. A., in press.

[4] Santiago, S., and West, A. P., *Philipp. J. Sci.*, 1927, **33**, 265; independently prepared by Dr. Wrenshall, through a synthesis giving greatly improved yields (personal communication, 1932).

Since these agents were studied from the standpoint of their possible intravenous use it was felt advisable to test their hemolytic activity on human blood *in vitro*. Dikshit[5] has shown that the degree of hemolysis produced by "Alepol" and other chaulmoogra soaps is the same as that brought about by the Na oleate of ordinary washing soaps. This action is modified by the formation of insoluble Ca salts, if the drugs are dissolved in Locke's solution or if serum is added to Locke's solution as Dikshit found.[5] We compared the hemolytic properties of the 8 derivatives using concentrations from 1:500 to 1:100,000 in 0.9% NaCl solution. One-tenth of a cc. of washed human red cells was added to 10 cc. of the drug solution and observations were made over a 48-hour period. Na stearate, used as control, hemolyzed blood cells at the same concentrations as "Alepol". No hemolysis occurred in any of the Drug "A" solutions within the first 12 hours. Other results are indicated in Table I.

Studies on the metabolism of chaulmoogryl derivatives which contain a physiologically inert or harmless solubilizing radicle rather than a toxic one are in progress.

Summary. On the basis of *in vitro* bactericidal and hemolytic action, intravenous toxicity, tolerance, and effectiveness in experimental rat leprosy, Na-dichaulmoogryl-β-glycerophosphate ("chaulphosphate" for convenience), appears superior for possible use in the intravenous treatment of leprosy than other representative water-soluble chaulmoogra derivatives, such as Na-hydnocarpate ("Alepol"), K-iodo-dihydrochaulmoograte, Na-chaulmoogryl-glycinate, Na-chaulmoogryl-o-aminobenzoate, diethyl-ethanolammonium chaulmoograte, and choline chaulmoograte.

[5] Dikshit, B. B., *Ind. J. Med. Res.*, 1932, **19**, 775.