

## SOME PHARMACOLOGIC EFFECTS OF THE CHOLINE ESTER OF CHAULMOOGRIC ACID

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During a study of chemotherapy of experimental rat leprosy under the direction of Professor C. D. Leake, from 1932 to 1934 (2, 3), one of many chaulmoogra derivatives considered was chaulmoogrylcholine, the chaulmoogric acid ester of choline. This was prepared by Professor Richard Wrenshall and later by Professor J. H. Payne of the University of Hawaii, who supplied it for our use. It was hoped that this soluble ester might show specific affinities for nervous tissue and mycobacteria, but preliminary toxicity tests indicated that only minute amounts of chaulmoogra could be given safely in the form of this compound. Thus no chemotherapeutic studies were made with it. Its pharmacologic effects were not analyzed at the time, but over the past six years a few pertinent data have accumulated.

This work emphasizes, first, the hazard of serious physiologic effects of compounds of this type and the necessity for thorough pharmacologic study of new antileprosy agents before they are considered for clinical use. Secondly, this ester is of pharmacologic interest since it represents a type of choline compound not yet adequately studied. Dale (4) and others (5) have noted the rapid decrease in muscarine-like activity of choline esters as the molecular weight is increased. The palmitic acid ester of choline is reported to be practically devoid of effect on the autonomic nervous system. Alles and Knoefel (1) have reported similar findings with alkyltrimethylammonium compounds. Certain parasympathomimetic actions of chaulmoogrylcholine were therefore studied.

*Physical properties.*—Professor Payne supplied the ester as the iodide (33D1) and chloride (33D2) and listed the following properties. For 33D1: decomposes at about 150°C; pH of saturated aqueous solution, 4.6; soluble in CH<sub>3</sub>OH, insoluble in ether, acetone and water; iodine number 51.65 (calculated,

51.38). For 33D2: softens at 65°C, decomposes slowly; pH of 5 percent aqueous solution, 6.4; very soluble in water, CH<sub>3</sub>OH and hot acetone; slightly soluble in cold acetone; insoluble in ether; iodine number 62.17 (calculated, 62.17).

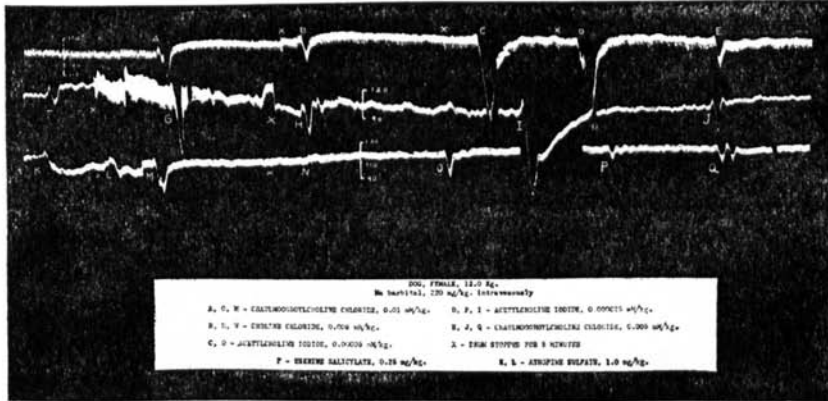
Despite exceptional agreement of values for iodine absorption with the theoretical amounts, Payne's statement (7) that "nitrogen and halogen determinations are likewise in good agreement" is better evidence of purity and identity. Contaminating amounts of acetylcholine greater than 0.1 percent would invalidate the pharmacologic results, but presumably even minute traces are absent because of the method of preparation. The relative solubilities of the two salts of chaulmoogrylcholine present an analogy to the relative deliquescentences of acetylcholine salts. Although it was found that 1 percent opalescent emulsions of 33D1 are fairly stable, biologic activity is always much less than with equivalent amounts of 33D2. Therefore only pharmacologic effects of the chloride are described.

*Effects on circulation.*—Chaulmoogrylcholine chloride produces equivalent depressor responses in rabbits and dogs when given intravenously in amounts about one-third the molecular equivalent of choline chloride. It causes depressor responses in dogs that show only pressor responses to choline, and is strongly potentiated by eserine. Its action is decreased by hydrolysis on standing.

Its depressor effect, however, is not completely antagonized by atropine in doses abolishing the depressor response to acetylcholine. Also, with larger doses of chaulmoogrylcholine there sometimes occurs a moderate fall of carotid pressure of long duration, instead of a greater depressor response. These observations suggest that part of the depressor effect is not mediated by the autonomic nervous system but is due to direct harmful effects on the heart or vessels. Pressor effects are frequently noted if chaulmoogrylcholine is given immediately after response to a large dose of epinephrine, but probably not through a nicotine-like action.

Text-fig. 1 presents consecutive records of carotid pressure in a dog given the ester, choline and acetylcholine. The quantitative comparison of its effects with those of choline and acetylcholine is not typical of the majority of experiments, but the record is given to show the influence of eserine and atropine on response to these three agents. In all, ten dogs and four rabbits were given chaulmoogrylcholine.

*Smooth muscle.*—Twelve strips of rabbit jejunum were tested by the Magnus technique. In comparing molecular amounts, chaulmoogrylcholine has slightly more than 0.1 percent of the stimulant activity of acetylcholine, or slightly more activity than choline. Similar to other cholines, amplitude of contraction is increased by small doses without much effect on tonus; larger doses increase tonus and decrease amplitude, while still



TEXT-FIG. 1. Influence of eserine and atropine on carotid pressure responses to chaulmoogrylcholine, choline and acetylcholine. Tracings from above: 1, control; 2, after eserine; 3, after atropine. Lines 4, 5 and 6 (faint, straight) are base-lines for 1, 2, 3 resp., b.p. 0 mm. Time intervals, 5 seconds, noted at left on base-lines. Animal: dog, female, 12.0 kg., given Na barbital, 220 mg./kg. intravenously. Letters on curves signify: A, G, M, chaulmoogrylcholine chloride; B, H, N, choline chloride; C, O, acetylcholine iodide; D, P, I, acetylcholine iodide; E, J, Q, chaulmoogrylcholine chloride; X, drum stopped for 5 minutes; F, eserine salicylate; K, L, atropine sulfate.

larger doses cause spasm. Relative to effects of acetylcholine, the stimulant action is somewhat greater on amplitude of contraction than on tonus. In one strip showing rhythmic cycles of contraction after acetylcholine, chaulmoogrylcholine  $2.5 \cdot 10^{-5}$  converted activity to an even level of large contractions with slightly greater tonus. As with the depressor effect, aged solutions are less active, presumably through hydrolysis. Solutions applied to three strips were carefully buffered, without changing the response; i.e., effects noted were not due to pH changes.

*Chromodacryorrhea.*—Shedding of red tears in rats may be used as a test for acetylcholine. Pilocarpine and dacryorrhetin also cause this phenomenon but choline does not, even in eserized rats (8). Ten rats were injected with acetylcholine. Three of five showed a definite response after 20 mg./kg. were given

intraperitoneally, and three of five after 0.15 mg./kg. was given intravenously. A week later, these six reacting rats were given intravenous injections of chaulmoogrylcholine chloride. None of five reacted after 100 mg./kg. and the other did not react after 200 mg./kg. Thus if any acetylcholine is present as a contaminant in the preparation of chaulmoogrylcholine studied, it occurs in amounts certainly less than 0.15 percent and probably less than 0.08 percent. Further tests were limited by the toxicity of chaulmoogrylcholine.

*Hemolysis.*—Chaulmoogrylcholine chloride causes immediate hemolysis of washed erythrocytes of the cat at a concentration of 1:20,000, but no hemolysis in twelve hours at 1:50,000. In comparison, diethylethanolammonium chaulmoograte causes hemolysis in less than fifteen minutes at 1:10,000, but not 1:20,000; chaulmoogryldiethylethanolamine (antileprol, Hoffmann-La Roche) causes a delayed hemolysis at 1:1,000; and ethanolammonium chaulmoograte produces hemolysis within fifteen minutes at 1:10,000. As previously reported (6), sodium hydncarpate (alepol) hemolyzes human cells *in vitro* within three hours at 1:20,000, choline chaulmoograte within two hours at 1:100,000, and diethylethanolammonium chaulmoograte within 45 minutes at 1:20,000. The high hemolytic activity of choline chaulmoograte and the low activity of antileprol suggest that in solution chaulmoogrylcholine may be converted in part to the soap. Solutions of chaulmoogrylcholine chloride are slightly soapy when first prepared but become strongly so on standing.

#### SUMMARY

X The choline ester of chaulmoogric acid has a small but definite muscarine-like activity, slightly greater than that of molecularly equivalent amounts of choline. In addition it has direct harmful effects upon tissues. A fraction of the ester may be rapidly hydrolyzed to form choline chaulmoograte. Chaulmoogrylcholine is unsuited for antileprosy use since its toxicity precludes administration of significant amounts of chaulmoogric acid in this form.

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