A PHARMACOLOGICAL EVALUATION OF CERTAIN ANTILEPROTIC DRUGS[1]

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INTRODUCTION

The introduction of new therapeutic agents into medicine requires the cooperation of three types of workers: chemists, pharmacologists, and clinicians. Chemists have been active in preparing chaulmoogra derivatives, salts of heavy metals, and various synthetic agents for the treatment of leprosy. Clinicians have been willing and anxious to try these drugs in man; and they have done this without any special regard for their pharmacologic action. Such a procedure is to be condemned for several reasons: First, human life is endangered with drugs of possible toxic effect; second, if ineffective agents are used much time is lost for the patient, who should have had the best of the tried therapeutic agents; and third, clinical studies are always inadequate because a complete investigation of pharmacologic action can be done readily only in animals. As has been pointed out (6):

> Clinical trial of new chemical substances should be made only after critical disinterested pharmacologic study has estimated (a) the probable toxicity, (b) the type and mode of action, (c) the worthiness of application to human beings, and (d) the reasonableness of replacing existing drugs.

Text-figure 1 illustrates this matter graphically.

Perhaps the most important step in a pharmacologic study is the determination of toxicity, acute and chronic, in normal and diseased animals. Before drugs are used in patients there should also be obtained from normal human volunteers as much data as possible on the rate of absorption, local irritation, and excretion. Next in im-

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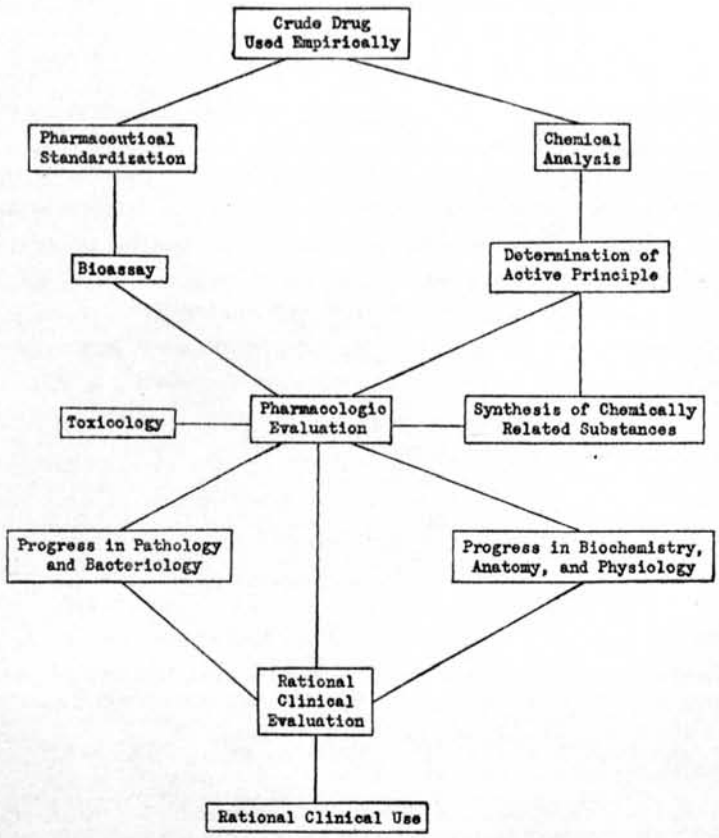
portance is the ratio of lethal effect for the pathogenic organism, both in vitro and in vivo, to toxicity for the host—that is, the postulated therapeutic index. A pharmacologic investigation including these and other studies is tedious but absolutely necessary, in our opinion, before clinical trials are made.



TEXT-FIG. 1. *Logical and historical position of pharmacology in the rational use of chemical agents in medicine.*

We have attempted such a laboratory study of four chaulmoogra derivatives and one other compound recently proposed for use in leprosy. The chief purpose was to elaborate a laboratory method for evaluating chaulmoogra derivatives and other agents proposed for use in the treatment of human leprosy, in an effort to predict their probable therapeutic value in man. All but one of the five agents

tested have been tried in man, and two are in general use today. Two of those studied were water-soluble synthetics prepared by Wrenshall,[2] No. 921 (sodium chaulmoogryl p-phenetidine sulphonate) and No. 923 (sodium di-hydro-chaulmoogryl p-phenetidine sulphonate). A third was one of those of Adams,[3] di-N-heptyl acetate, which he found on chemical and bacteriological examination to be one of the best of the many proposed antileprotic drugs that he has prepared over a number of years (17). The other two drugs were "alepol"[4] and the ethyl esters of chaulmoogra oil.[2]

Rats were used as test animals for both the toxicity determinations and the therapeutic trials, since *Myco. leprae muris* causes in them a chronic infection that is not unlike human leprosy in its effects (22). Examinations of tissues were made by Dr. James Rinehart, and in vitro experiments using a supposed culture of *Myco. leprae hominis* were carried out with the aid of Dr. E. L. Walker and Miss M. A. Sweeney.[5] Certain physico-chemical and biological tests were made also in an attempt to determine the mechanism of action of these agents.

ANIMAL TOXICITY STUDIES

Read (12) maintains that the toxic effects of chaulmoogra oils are due chiefly to hydnocarpic acid. Its compounds cause hemolysis, renal irritation with hemoglobinuria, and fatty infiltration of the liver. Changes in the kidneys and liver occur when any chaulmoogra derivative is administered, and are important in chronic toxicity. According to Read, death in experimental animals results from respiratory failure due to central effects of chaulmoograte, and not to multiple emboli in the lungs. Before considering chronic toxicity, which is of major importance, it is desirable to present our acute toxicity findings.

ACUTE TOXICITY IN NORMAL RATS

Normal, healthy, adult rats were used for the acute toxicity studies. They were kept in small cages in the same room at approx-

[2] Dr. Richard Wrenshall, of the University of Hawaii, upon whose suggestion this work was initiated, and who supplied his synthetics and also the samples of alepol and chaulmoogra ethyl esters that were used.

[3] Dr. Roger Adams, of the University of Illinois, who kindly supplied the substance tested.

[4] Manufactured by Burroughs and Wellcome, London.

[5] The three investigators named are at the University of California Medical School, San Francisco.

mately uniform temperature, and maintained on a diet of carrots, barley, bread, milk, water and yeast.

Intravenous experiment.—In the single intravenous toxicity experiment four water-soluble chaulmoogra derivatives were tested. They were alepol or "sodium hydnocarpate" (Rogers)[6], "sodium gynocardate" (Rogers)[6], and Wrenshall's compounds Nos. 921 and 923. Solutions were made in physiological saline and injected into the external jugular vein, without anesthesia, from 30 to 60 seconds being allowed for giving each cubic centimeter of solution. No food was given on the morning the tests were made. The results are summarized in Table 1.

TABLE 1.—*Toxicity in rats of single doses of four water-soluble chaulmoogra derivatives in physiological saline, injected intravenously.*

Drug	Solution, Per cent	Dose in mg./kg.[a]	Mortality ratio [b]	Time of death	Lethal range
No. 102. Sodium gynocardate (Rogers)	3	120	0/4	—	300 mg./kg.
	3	200	1/5	2 minutes	
	3	300	2/5	6 hours, 16 days	
	3	400	2/2	2 minutes	
No. 104. Alepol or sodium hydnocarpate	3	60	0/3	—	100-125 mg./kg.
	3	100	1/3	2 minutes	
	3	125	2/2	2 minutes	
No. 921. Sodium chaulmoogryl p-phenetidine sulphonate (Wrenshall)	5	200	1/3	6 days	200-300 mg./kg.
	5	300	3/4	6 hours, 24 hours, 3 days	
	5	400	3/3	2 minutes, 1 hour, 5 days	
	5	500	3/3	4 minutes, 5 minutes, 3 hours	
No. 923. Sodium dihydrochaulmoogryl p-phenetidine sulphonate (Wrenshall)	3	50	0/3	—	75-100 mg./kg.
	3	75	1/3	5 minutes	
	3	100	2/3	2 minutes	

[a] Milligrams per kilogram of weight.
[b] Showing the number of animals that died out of the number injected.

Half of the animals, that died did so within 2 to 5 minutes after the drug was administered, and of the remainder all but three died within 24 hours. Preceding death there occurred respiratory difficulties such as labored breathing, dyspnœa, hiccough, and irregular gasping respiration, followed by hyperesthesia to touch and clonic convulsions. Death was due to respiratory failure. Necropsies performed immediately after death showed, in the animals dying within

[6] Manufactured by Smith, Stanistreet Co., Ltd., Calcutta.

5 minutes, no gross lesions except marked pulmonary congestion. The lethal range for sodium gynocardate given intravenously in this group of rats is about one-third that of rabbits (Rogers [13], Walker, McArthur and Sweeney [21]). Alepol is but half as toxic for rats as for rabbits, in the same percentage dilution, but about three times as toxic for dogs and cats (Dikshit [3]).

TABLE 2.—*Toxicity in rats of single doses of antileprotic drugs in aqueous solution, injected subcutaneously.*

Drug	Solution, Per cent	Dose in gm. or cc./kg.[a]	Mortality ratio [b]	Time of death	Lethal range
No. 103. Ethyl esters of chaulmoogra oil	undiluted	4 cc.	0/6	—	
		6 cc.	0/5	—	
		12 cc.	2/8	1 and 2 days	
		20 cc.	0/10	—	
		30 cc.	2/10	2 days	
		40 cc.	5/5	1 day	40 cc./kg.
No. 105. Ethyl di-N-heptyl acetate	undiluted	2 cc.	0/6	—	
		4 cc.	2/5	18 and 23 days	
		10 cc.	1/5	4 days	
		15 cc.	0/5	—	
		20 cc.	3/5	4, 16 and 17 days	20 cc./kg.
No. 104. "Alepol" or sodium hydnocarpate	2	0.8 gm.	1/6	23 days	
		1.6 gm.	1/8	12 days	
		1.8 gm.	0/8	—	
		2.0 gm.	7/7	1 day	2.0 gm./kg.
No. 921. Sodium chaulmoogryl p-phenetidine sulphonate (Wrenshall)	6	0.3 gm.	0/3	—	
		0.5 gm.	2/5	3 days	
		0.6 gm.	0/3	—	
		0.7 gm.	3/3	1, 2 and 10 days	
		1.0 gm.	3/3	1 day	0.5—0.7 gm./kg.
No. 923. Sodium dihydro-chaulmoogryl p-phenetidine sulphonate (Wrenshall)	2	0.2 gm.	1/3	2 days	
		0.3 gm.	0/3	—	
		0.4 gm.	2/7	2 and 7 days	
		0.6 gm.	3/3	1 day	
		0.8 gm.	3/3	1 day	0.4—0.6 gm./kg.

[a] Grams or cubic centimeters per kilogram of body weight.
[b] Showing the number of animals that died out of the number injected.

Subcutaneous experiments.—The toxicity in rats of single doses injected subcutaneously was determined for three of the above chaulmoogra derivatives, and also the chaulmoogra ethyl esters and ethyl di-N-heptyl acetate. The solutions of the water-soluble chaulmoograte used in this work were of the same concentration as those used later in treating leprous rats and in the chronic toxicity determinations. Though single doses were used the actual injections were multiple, not more than 0.05 cc. of the drug solution being put at each injection point; all were given into the subcutaneous tissues of the abdominal wall. Animals were observed over a thirty-day period and necropsies were performed on rats dying during this time. Table 2 shows our results.

The lethal dose for the ethyl esters of chaulmoogra oils given subcutaneously was found to be 40 cc. per kilo. The rats gained weight during the observation period. Valenti (19) reports a lethal dose of 5 to 10 cc. per kilogram in rabbits, 10 to 20 cc. per kilogram in guinea pigs, and 5 cc. per kilogram in dogs. Ethyl di-N-heptyl acetate is twice as toxic as the chaulmoogra esters, i. e., the average lethal dose was found to be 20 cc. per kilogram. Apparently both agents are relatively non-toxic in single injections. Alepol is acutely toxic at 2.0 grams per kilogram, killing all animals within 24 hours. Dikshit (3) found it toxic in 0.4 gram per kilogram doses in cats, and in 0.45 gram per kilogram doses in guinea pigs, using 3 per cent solutions hypodermically. Drug No. 921 is about three times as toxic as alepol, killing at 0.5 to 0.7 gram per kilogram, animals dying from one to ten days after injection. Drug No. 923 is lethal in the same range, that is, from 0.4 to 0.6 gram per kilogram, but rats die within 48 hours with these doses. Intravenously, however, single doses of drug No. 923 are three times as toxic as of drug No. 921. Necropsies did not reveal any marked gross tissue changes, except at the site of injection where drugs No. 921 and No. 923 caused tissue breakdown and necrosis. None of the other antileprotic drugs studied caused gross changes at the injection site, an important point in human treatment.

In examining our results in comparison with those of others it is apparent that a marked species variation exists as well as considerable individual variation. We used rats throughout our work to obviate the difficulty of species variation, enabling us to apply our acute toxicity figures in determining treatment doses used in leprous rats. It is also very likely that there are distinct differences in lots of supposedly the same product made in different places with different materials and methods.

Chronic toxicity in leprous rats

Chronic toxicity of chaulmoogra derivatives has been considered chiefly by Frazier (5), who carefully described the pathological changes caused by repeated administrations of small doses of sodium hydnocarpate. The chief findings were non-inflammatory degenerative changes in the renal tubular epithelium, with fatty infiltration. Liver damage also occurred, with beginning necrosis of the parenchymal cells and varying degrees of fatty infiltration. The total

amount of drug used was 1.8 grams per kilogram, given intravenously in divided amounts bi-weekly over the period of a year. The total dose used by Frazier is smaller than amounts used clinically over a similar time.

Carthew (1) gave to a group of men in the Bangkok prison approximately 0.5 gram per kilogram intravenously in seven months, in addition to about 9 grams per kilogram orally during the same period. Since Walker and Sweeney (20) have shown that 95 to 98 per cent of the drug is absorbed when given by mouth, it is not unlikely that these prisoners suffered kidney damage, although there were apparently no studies made to check this point. In view of the apparent species variation of toxicity in animals it may be that no damage occurred, although one would expect that five times the chronic lethal rabbit dose based on body weight would be injurious to man when administered in about half the time. In view of the possibility of tissue damage with continued antileprotic treatment with chaulmoogra derivatives, we believe that dosage should be more carefully adjusted to body weight.

TABLE 3.—*Chronic toxicity in leprous rats of antileprotic drugs given subcutaneously in repeated doses over a six months period.*[a]

	Mortality of treated groups, per cent	Smallest amount after which death occurred	Dose range at which most deaths occurred [b]	Average amount received by survivors
No. 103. Chaulmoogra esters	8	5.4 (7.0 cc.)	7.2	9.4
No. 104. Alepol	33	0.24	0.32—0.48	0.54
No. 105. Ethyl di-N-heptyl acetate	54	2.0 c	4.12—10.26 c	10.5 c
No. 921. Sodium chaulmoogryl p-phenetidine sulphonate (Wrenshall)	33	0.27	0.28—0.38	0.47
No. 923. Sodium di-hydrochaulmoogryl p-phenetidine sulphonate (Wrenshall)	25	0.09	0.09	0.17

[a] Of the untreated, uninfected controls 50 per cent died during the period of observation, and 65 per cent of the untreated leprous rats.

[b] Total dose received by each animal before death.

c Expressed in cubic centimeters of ethyl di-N-heptyl acetate per kilogram.

Experiment.—We are not concerned here with the pathologic effects of chronic chaulmoogra poisoning, but rather with the chronic toxicity of antileprotic drugs in leprous rats. Infections were produced in 80 albino rats using material from spontaneous leprosy in wild rats originally obtained from the U. S. P. H. S. Plague Laboratory in San Francisco. Transfers had been made by Dr.

TABLE 4.—*Inhibition of growth in culture of* Myco. leprae hominis (*Mary Puhulahula strain*) *with various antileprotic drugs.*

Drug	Time after inoculation	Dilution, in thousands					
		1	10	20	40	75	100
No. 102. Sodium gynocardate	72 hours	x	x	—	—	++	+
	96 hours	x	x	+	+	++	++
	160 hours	—	—	+	+	++	++
	1 month	—	—	—	—	+++	+++
	Transfer [a]			—	+		
No. 104. Alepol	72 hours	x	x	x	—	++	+
	96 hours	—	—	—	+	++	++
	160 hours	—	—	—	+	++	++
	1 month	—	—	—	—	+++	+++
	Transfer [a]				—	+	
No. 921. Sodium chaulmoogryl p-phenetidine sulphonate	72 hours	++	—	++	++	++	+
	96 hours	++	+	++	++	++	+
	160 hours	++	+	++	++	++	++
	1 month	+++	+	+++	+++	+++	+++
	Transfer [a]	+	—				
No. 923. Sodium dihydrochaulmoogryl p-phenetidine sulphonate	72 hours	++	++	+	+	++	++
	96 hours	++	++	++	++	++	++
	160 hours	++	++	++	++	++	++
	1 month	+++	+++	+++	+++	+++	+++
	Transfer [a]	+					
Sodium di-N-hyptyl acetate.	72 hours	x	—	—	++		

[a] Transferred after 72 hours to fresh medium.

Explanation of symbols:

x Chemical precipitation; no growth.
+ Slight growth.
++ Heavy growth.
+++ Very heavy growth; controls gave such growths in 30 hours.

Walker and Miss Sweeney about every 6 months in the Burlingame strain of albino rats, without apparent alteration in virulence in ten generations. Lesions were definitely palpable before treatment was started. A group of 12 animals was used for each drug. A control group of 20 untreated infected rats was kept under identical conditions, which were the same as in the case of normal rats already described. As an additional control group 12 normal untreated, uninfected rats were maintained during the same period. Table 3 shows the lowest dose at which death occurred, the average chronic lethal range, the average tolerated dose, and the percentage mortality for each treated group.

Five of the twelve uninfected and untreated controls died (two of pneumonia) during the 12 months observation period, and one was killed because of a middle ear infection. Thirteen of the twenty untreated leprous rats died during the same time, five of pneumonia and one of pulmonary edema. Only one of the twelve rats given the ethyl esters of chaulmoogra oil died, while of those treated with the three water-soluble chaulmoogrates one-fourth to one-third died. With ethyl di-N-heptyl acetate, six of the eleven treated rats died. The chaulmoogra esters were tolerated much better than the water soluble chaulmoogrates, but of the latter alepol was best tolerated. Drugs Nos. 921 and 923 caused necrosis at the site of injection, so that larger doses could not be given. Other changes which occurred will be noted later when treatment is discussed.

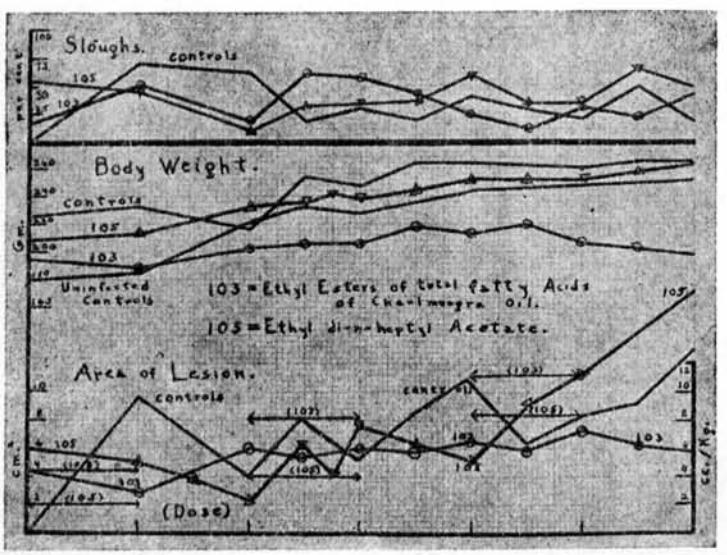
LEPROCIDAL ACTIVITY OF CHAULMOOGRA DERIVATIVES

Growth inhibition in vitro.—Estimation of the effect of drugs upon the leprosy organism is extremely difficult to evaluate with any degree of certainty. Adams, et al. (17), Walker and Sweeney (20), and Schöbl (15) have all tried to determine the activity of various drugs in vitro on acid-fast organisms. While these studies offer inconclusive evidence of therapeutic activity they point the way and afford a means for comparing various agents in the test tube. In our work the Mary Puhulahula strain of an organism believed to be the human leprosy bacillus (*Myco. leprae hominis*) was grown on Long's liquid medium (7) with varying amounts of the water-soluble drugs added. In Table 4 the inhibition of culture growths by various drug dilutions is shown.

Control cultures, without added drug, showed heavy growths within thirty hours. It is apparent that alepol is the most active of the chaulmoogrates tested, inhibiting growth at 1:20,000 dilution in 72 hours. Sodium di-N-heptyl acetate also is lethal at this dilution. On the other hand Walker (20) using comparable methods, found that the sodium salts of the mixed acids of chaulmoogra oil inhibit a supposed leprosy-bacillus culture in vitro at 1:60,000 dilution.

THERAPEUTIC EFFECT ON LEPROUS RATS

It is our opinion that in vitro studies alone will not furnish enough information to be of value in determining the clinical usefulness of antileprotic drugs. Therefore we established for experimental therapy an experimentally infected colony of rats harboring *Myco. leprae muris*. Infections were produced and allowed to develop

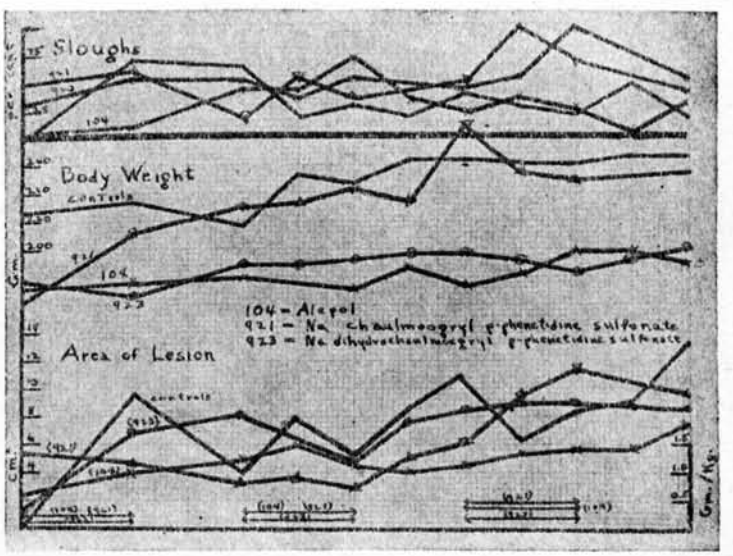


TEXT-FIG. 2. *Results in leprous rats treated with alepol and drugs No. 921 and No. 923, and in infected untreated controls. Percentage of sloughs, average body weight, area of lesion (in square centimeters), and amount of drug given in grams per kilogram of weight). Twelve animals in each treated group.*

for about four months, until lesions were definitely palpable, before treatment was started. The rats were caged in groups of six or less and kept under the best possible sanitary conditions. Bacteriologic examinations were made of affected tissues from time to time before treatment, during the treatment period, and at the death of the

animal. Injections of drugs were made as aseptically as practical, first swabbing the area with tincture of iodine and using a freshly sterilized needle for each animal.

In evaluating our therapeutic results we used the following criteria: (1) the weight of each animal before, during, and after treatment; (2) the area of lesion measured (with calipers) in two dimensions and expressed in square centimeters; (3) the tolerated amount of drug determined as the equivalent in chaulmoogric acid as calculated from the formula and corrected by the iodine number actually found; (4) the recorded incidence of sloughing lesions; and (5) the incidence of fatalities with cause based on necropsy findings. Treatment was confined to periods of 3 months, giving animals five injections each month at weekly intervals and allowing one month's rest between treatment periods.



TEXT-FIG. 3. *Results in leprous rats treated with mixed ethyl esters of chaulmoogra oil, ethyl di-N-heptyl acetate, and in untreated, infected and also in untreated, uninfected controls. Percentage of sloughs, average body weight, area of lesion (in square centimeters), and amount of drug given (in grams per kilogram of weight). Twelve animals in each treated group.*

The progress of the animals in the treated and untreated groups and in the uninfected controls is illustrated in Text-figs. 2 and 3. In the former the water-soluble chaulmoogrates are compared. With respect to body weight alone, the greatest gain was with drug No.

TABLE 5.—*Summary of therapeutic studies. Relation of effects of drugs studied on* Myco. leprae hominis *in vitro and* Myco. leprae muris *in vivo, showing the relation of the dose given and of the unsaturation of the drug to the therapeutic effect.*

	Chaulmoogra ethyl esters, undiluted	Alepol, 2 per cent solution	Sodium chaulmoogryl p-phenetidine sulphonate, 6 per cent solution	Na dihydro-chaulmoogryl p-phenetidine sulphonate, 2 per cent solution	Ethyl di-N-heptyl acetate, undiluted	Infected untreated controls	Uninfected untreated controls
Number of rats in each group	12	12	11	12	11	20	12
Age of lesions at start of treatment, in days	161	161	161	153	153	161	—
Total dose of chaulmoogric acid, grams per kilogram of weight	8.8 [a]	0.35	0.43	0.18	8.4 cc.[b]	—	—
Average variation in weight	+20	+8	−19	−3	−3	+37	+45
Percentage mortality during treatment	8	33	33	25	54	35	25
Average maximum size of lesion [c]	5.5	7	11.5	9	17.5	14.1	—
Total number of sloughs during treatment	20	22	30	32	18	17	—
Bactericidal concentration in vitro	1:60,000 [d]	1:20,000	1:1,000	1:1,000	1:20,000	—	—
Theoretical therapeutic indices	+10.00	+4.05	+0.65	+1.50	+3.50	—	—
Iodine number (calculated)	82.5 [e]	83.8 [e]	50.6	0	0	—	—
Iodine number (found)	94.6	101.2	12.2 [f]	0.5	0	—	—

[a] 11.4 cubic centimeters.
[b] of ethyl di-N-heptyl acetate.
[c] In square centimeters.
[d] Walker's results (11) determined for sodium salts of mixed chaulmoogric acids.
[e] Calculated for ethyl chaulmoograte and sodium chaulmoograte, respectively.
[f] Insoluble.

921. However, when the area of lesion is considered it is to be noted that the animals given drug No. 921 did as badly as the controls, while animals treated with alepol apparently had smaller lesions. In figures for sloughs alepol again favorably ranked with the controls in having a smaller number of tissue necroses. Drug No. 921 was given in largest amounts, alepol next, and drug No. 923 was given in smallest quantity.

When we consider Text-fig. 3 it is seen that the uninfected controls gained more weight than the infected group, and the ethyl di-N-heptyl acetate animals showed more gain in weight than the group treated with chaulmoogra esters. However, the lesions at the end of the treatment were largest in the ethyl di-N-heptyl acetate rats and next largest in the controls, while those receiving the chaulmoogra esters showed an average increase in size of less than 2.0 square centimeters. There were more sloughs in the ethyl di-N-heptyl acetate animals than in either of the two other groups. We were able to give about 1.5 cc. per kilogram more of the chaulmoogra esters than of the esters of di-N-heptyl acetate, with only 8 per cent mortality for the group in contrast to 54 per cent mortality with the latter drug. Pneumonia or pulmonary edema was the cause of death in the majority of animals that died. These therapeutic studies in leprous rats are summarized in Table 5.

We were able to give more chaulmoogric acid in the form of ethyl esters than as water soluble salts. The ethyl esters group showed the greatest average gain in weight of the treated animals, though less than that of the two control groups. Also, the percentage mortality was smaller in this group, and the lesions were smaller at the end of therapy. More sloughs occurred in animals receiving drugs No. 921 and No. 923 than in any others. In vitro activity was greatest with chaulmoogra esters. Alepol and ethyl-di-N-heptyl acetate were about one-third as active, while drugs No. 921 and No. 923 were relatively inactive in vitro.

We estimated the relative effectiveness of these various compounds by calculating "therapeutic indices" for them. Since the chaulmoogra esters are of known value in the treatment of human leprosy, and since in this study we found them to be the best agent in controlling rat leprosy, we used them as a standard for comparison, giving them an arbitrary value of 10. Of this figure, 5 was given to the bactericidal activity in vitro (see Table 5), and a value of 1 to each of the following: lowest (chronic) dose causing death (see

Table 3), average variation in weight, percentage mortality during treatment, average maximum size of lesion, and total number of sloughs during treatment. When we consider these factors together and postulate a theoretical therapeutic index of +10.00 for the chaulmoogra esters, we obtain +4.05 for alepol, +3.50 for ethyl di-N-heptyl acetate, +1.50 for drug No. 923, and +0.65 for drug No. 921. In this study, then, the best therapeutic agents are the ethyl esters of chaulmoogra oil and alepol. It is to be noted especially that these two agents have by far the highest iodine numbers of the drugs considered, and also that the actual iodine absorption of these compounds is higher than theoretical for sodium chaulmoograte and ethyl chaulmoograte. This might indicate the presence of a therapeutically active, highly unsaturated compound of the gorlic acid type (MacDonald and Dean [8]).

MECHANISM OF ANTILEPROTIC ACTION

While it is not necessary to include physico-chemical data in pharmacologic studies of this character, certain investigators have contended that such data are helpful in determining therapeutic effect. Unfortunately, as experienced workers admit, estimates of therapeutic activity cannot be based entirely upon bacteriological tests on the growth of leprosy organisms in test tubes containing various drug dilutions. Adams et al. ([17]) maintain that an active antileprotic agent should contain from 16 to 18 carbon atoms and should have a molecular weight of about 256, that unsaturation is not necessary, that a five-membered carbon ring structure is not essential (as held by Walker [20]), and that in addition to other physical characteristics it must have a marked effect of lowering surface tension.

We have attempted to correlate physico-chemical data with our estimated therapeutic indices, as is indicated in Table 6. The therapeutic indices are based on our treatment results in rats, on the relative toxicity of drugs used, and on in vitro action, as has been discussed. For the compounds studied it is apparent that the most therapeutically efficient are those that have optical activity and the greatest degree of unsaturation, namely, the mixed ethyl esters of chaulmoogra oil and alepol.

Our findings support the conclusions of Walker and Sweeney ([20]) who maintained that the cyclopentenyl group must be present, and also Schöbl ([15]) who emphasized the importance of unsaturation. Perkins ([11]) believed that the activity of oils other than those of

the chaulmoogra group depends upon their degree of unsaturation. Chopra (2) and Read (12) both state that chaulmoogra oils of high optical activity are most effective. Muir (9) also contends that unsaturation and the presence of a five-membered ring are necessary. MacDonald and Dean (8) suggested that salts or esters of mixed acids are more effective because of the presence of a highly unsaturated acid. All of these impressions, together with the experimental results cited, tend to show an inter-relation between unsaturation and therapeutic efficacy.

TABLE 6.—*Some physico-chemical data on the compounds studied.*

	Chaulmoogra ethyl esters	Alepol	Sodium chaulmoogryl p-phenetidine sulphonate	Na dihydrochaulmoogryl p-phenetidine sulphonate	Ethyl di-N-heptyl acetate
No. of carbon atoms	16-18	16-18	18	18	16
Molecular weight	300	305	502	504	270
Specific rotation [a]	+49.5	+52.0	—	0	0
Iodine numbers					
calculated	82.5	83.8	50.6	0	0
found	94.6	101.2	12.2	0	0
Surface tension 25°C. dynes/cm.	38.4	36.5	47.4	46.3	33.6
Percentage solubility					
Water at 20°C.	insol.[b]	20.0	3.0	10.0	insol.[b]
Oil at 20°C.		insol.	insol.	insol.	
Calculated therapeutic indices	+10.00	+4.05	+.65	+1.50	+3.50

[a] At 20°C., sodium flame; ethyl chaulmoograte in 2 per cent alcohol solution, alepol in 2 per cent water solution.
[b] All proportions.

One other controversial point that we have considered in this study is the possibility of antileprotic action being dependent upon stimulation of non-specific lipolytic activity of infected tissues, as suggested by Walker and Sweeney (20) and others. We have previously reported on the lipolytic action of various tissues in leprous and normal rats as estimated by the percentage hydrolysis of ethyl butyrate (4). Briefly, our results showed that: (a) leprous tissue is significantly lower in lipolytic activity than other tissues in infected and normal rats; (b) leprous tissue from different animals is remarkably constant in lipolytic action in comparison with other tissues; and (c) the presence of "late stage" leprosy significantly lowers the lipolytic activity of most tissues of the host, with the probable exception of the spleen, in comparison with tissues from non-infected normal rats.

Treated animals of the groups reported herewith exhibited but slight variations in lipolytic activity during and after treatment.

The lepromata of the treated animals caused about the same degrees of hydrolysis as did early lesions, but because the statistical variation was so large the significance of the mean was lost. Other tissues of treated rats produced, in general, a percentage of hydrolysis which lay within the range of the values found for the tissues of early- and late-stage untreated leprous rats. There was no tendency to approach a normal hydrolytic effect. Rogers (14) reported that the blood of treated human lepers had a greater lipolytic action in vitro than that of untreated leprous humans, but Neill and Dewar (10) in a much larger series of cases failed to confirm this finding and showed, as we did in the tissues of leprous rats, that chaulmoogric acid derivatives cause no increase in blood lipase.

SUMMARY

A pharmacologic method for evaluating antileprotic drugs is presented which includes the following considerations: (a) acute toxicity in normal animals; (b) chronic toxicity in leprous rats; (c) rate of absorption, local irritation and excretion in human beings; (d) lethal action on *Myco. leprae hominis* in vitro; (e) therapeutic effectiveness in a supposed culture of *Myco. leprae muris* in rats; (f) an arbitrary therapeutic index based on treatment results in infected rats and ratio of toxicity to action in vitro.

When we estimate relative effectiveness in this way, the five drugs considered may be ranked as follows, the best agent being placed first: ethyl esters of chaulmoogra oil, alepol, ethyl di-N-heptyl acetate, di-hydrochaulmoogryl p-phenetidine sulphonate, and chaulmoogryl p-phenetidine sulphonate.

The mechanism of antileprotic action is discussed. For the compounds studied the most therapeutically efficient are those having optical activity, the greatest degree of unsaturation and the cyclopentenyl group. We were unable to demonstrate an increase in lipolytic activity of the tissues of leprous rats with treatment. An important factor in the effectiveness of treatment with chaulmoogric acid derivatives is the amount of chaulmoogric acid that can be given safely. To give a sufficient amount is difficult with water-soluble chaulmoogra derivatives.

It is recommended that drugs be subjected to pharmacologic study before clinical use, inasmuch as the relative value in human leprosy of four of the drugs (those which have had clinical trial) could have been predicted if a critical laboratory investigation had been

made beforehand. Such a procedure would obviate possible toxic effects in humans and loss of time with ineffective or unsatisfactory agents; further, more complete data on the action of drugs in this disease would be available if animal experimentation were resorted to more frequently.

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