filamentous polymorphonuclear neutrophiles equally. During recovery there is a marked increase in the non-filamentous neutrophiles. There is a latent lymphopenia which reaches its maximum about 7 hours after inoculation. The monocytes are depressed and do not return to normal during the period of the experiment. A peculiar cell with pyknotic nucleus and intensely basophilic staining cytoplasm appears in about one-half hour, and reaches its maximum concentration approximately 2 hours after inoculation. This cell has been designated as the "D-Cell". It probably represents a degeneration form. There is noted a progressive increase in normoblasts which reaches a maximum after 7 hours. These are still present in appreciable numbers after 28 hours.

Summary. Potassium antimonyl tartrate produces a characteristic leucopenia without subsequent leucocytosis when administered intravenously to normal rabbits. It also produces a moderate normoblastic response. It does not affect the platelet count. It is suggested that antimony, like arsenic, might be used to depress abnormal leucocytoses of myeloid cells.

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In vitro Leprocidal Activity of Some Non-chaulmoogryl Compounds.*

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Little emphasis has been placed upon the *in vitro* bactericidal activity against *Mycobacterium leprae* of compounds other than the fixed and essential oils and various fatty acids examined by Walker and Sweeney, Schöbl, and Adams and his co-workers.[1] In view of the recent interest in the clinical use of non-chaulmoogryl compounds, particularly certain dyes, as antileprosy drugs, a report of

* 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 Walker, E. L., and Sweeney, M. A., *J. Inf. Dis.*, 1920, **26**, 238; Schöbl, O., *Phil. J. Sci.*, 1923, **23**, 533; *Ibid.*, 1924, **24**, 23; Stanley, W. M., Coleman, G. H., Greer, C. M., Sacks, J., and Adams, R., *J Pharmacol. Exp. Therap.*, 1932, **45**, 121.

our accumulated findings on the action of these and other agents against *M. leprae* is made herewith. These observations have been made during the past year as a tentative guide to therapeutic efforts on experimentally infected leprous rats using a standardized technique previously described.[2]

The leprocidal activity of over 200 chemical agents falling into 4 general types has been studied by the 2 appropriate techniques described by Adams *et al.*,[1] with careful adherence to the precautions noted by these authors. A single strain of test organism was used, this being *M. leprae hominis,* Mary Puhulahula strain, isolated by Dr. E. L. Walker.† The media used as substrate in the tests was that of Long and Seibert,[3] recommended for our use by Dr. Walker, rather than a 5% glycerol broth as used by Adams[1] in his tests. No other variations from Adams' method were made. Most of the tests were carried out in Dr. Walker's laboratory and in conjunction with the Bacteriological Laboratory of the University of California. By closely following the bacteriological technique worked out by Adams, his estimate of a probable error of ±10% appears justified.

The first group of agents studied was a series of dyes. The magnitude of the effects with the more potent of these is noted in Table I. Among those found ineffective, a few are of special interest and deserve comment.

Trypan blue was found to be ineffective in solutions of less than 0.1%, a concentration which will be referred to as "ineffective" in this paper since from 50-75 gm. of the agent in question would be required to furnish this concentration in the body even if no segregation occurred. Since Ryrie[4] has reported trypan blue to be of value when given intravenously in human lepers in much smaller doses (1.0 gm.), it is not probable that trypan blue acts therapeutically by virtue of its bactericidal action even though some concentration of the dye may take place in the lepromata, as reported.[4] In regard to this selective absorption of trypan blue by the leproma, a similar phenomenon was noted in leprous rats by Oliver,[5] who further observed in sections of the tissues that only the healthy macro-

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

† Whether or not this organism is the pathogen of leprosy does not concern us here. Adams has shown the susceptibility of different strains of *M. leprae* to various drugs to be the same.

[3] Long, E. R., and Seibert, F. B., *Am. Rev. Tuberc.,* 1926, **13**, 393.

[4] Ryrie, G. A., *Trans. Royal Soc. Trop. Med. and Hyg.,* 1933, **27**, 85.

[5] Oliver, J., *J. Exp. Med.,* 1926, **43**, 233.

TABLE I.

Minimal Certain Leprocidal Concentration of Various Agents in Long's Media.*

Substance	pK_{lep}†	Substance	pK_{lep}†
Crystal violet	5.7	Merthiolate	6.3
Methyl violet	5.7	Metaphen	6.0
Brilliant green	5.7	Mercurochrome	5.7
Malachite green	4.7	Solganol	5.3
Methylene blue	5.3	Cu morrhuate	5.0
Safranin	5.1	Ag proteinate	5.0
Toluidin blue	5.1	Fuadin	4.7
Thionin	5.1	Arsphenamine	4.7
Pyronin	5.1	Emetine Bismuth iodide	5.0
Rose bengal	4.0	Emetine Sb iodide	5.0
Octyl resorcinol	5.3	Isoamyl cephaelin phos.	4.7
Heptyl resorcinol	5.0	Yatren	4.3
Hexyl resorcinol	5.0	Vanillin	5.0
Na glycocholate	4.7	Na thioglycolate	5.0
Na taurocholate	4.7	Thymol	4.0
Na F	4.3	Carvacrol	4.0
Salicyl aldehyde	4.3	Eugenol	4.0

* Single strain of *M. leprae* used throughout, against which Na hydnocarpate is active in concentration of 1:40,000, *i. e.*, $pK_{lep} = 4.6$.

† pK_{lep} = neg. log concentration in gm./cc.

phages about the lesion stored the dye in quantity, while the infected so-called "lepra cells" took up smaller amounts in inverse proportion to their content of lepra bacilli.

Eosin and fluorescein, found by Ryrie to be unsuited to leprosy treatment, were found to be without leprocidal activity, while mercurochrome (dibromoxymercurifluorescein) used intravenously with success by Denney,[6] has a high activity (Table I). Acriflavine, used clinically with poor results,[7] is active only in concentrations above 0.01%. Other ineffective dyes were: alizarin, tropaoelin 00, indigo, methyl orange, carmine, Bismarck brown, methyl red, purpurine, vital red, bromcresol green, congo red, azolitmin and rosaniline. Neutral red and rose bengal inhibited growth in concentration of 0.01%. Selective absorption of the dye from solution by the growing colonies was noted chiefly in the case of congo red, rose bengal, and brilliant vital red.

The simpler phthalein dyes were also found ineffective, which is of interest since soaps of fatty acids assayed for leprocidal activity by Adams' technique are necessarily contaminated with a small amount of phenolphthalein. Halogenation does not increase their activity, since tetraiodo-, tetrabrom-, and tetrachlor-phenolphthaleins are ineffective also. Phenolsulphonephthalein and thymolsulpho-

[6] Denney, O. E., *Pub. Health Rep.*, 1931, **46**, 5.

[7] Schwetz, J., *Ann. Soc. Belge de Med. Trop.*, 1933, **13**, 43.

nephthalein are ineffective, while o-cresolsulphonephthalein is active in concentrations of 0.01%. Again tetrabromphenolsulphonephthalein, dibromthymolsulphonephthalein and dibromcresolsulphonephthalein are not active

The *second* group of substances studied were metallic organic compounds most of which have been used with varying success on human leprous subjects.[8] The more potent leprocidal drugs of this group are represented in Table I, where it may be noted that Hg, Sb, Au, Ag and Cu organic compounds have high activity. Pentavalent arsenical compounds were without effect, atoxyl, carbarsone, thiocarbaminophenyl arsonic acid, acetarsone, etharsenol, proparsenol and tryparsamide being taken as examples. Activity among the trivalent arsenicals is variable; while arsphenamine is highly active, arsenious trithiosalicylic acid and arsenious trithiophenylsulfonic acid are ineffective. Na or K iodides were active only in a concentration of 1%, as is iodoform also.

The *third* group includes a miscellaneous series selected for properties which in the chaulmoogric acid derivatives have been held to cause the peculiar activity of the molecule. Surface tension lowering agents, enzymes, highly unsaturated compounds, compounds containing 16-18 carbon atoms, specific and general protoplasmic poisons were tested. The highly effective compounds in this series may be found in Table I, while some of the more interesting of the ineffective compounds were: papain, saponin, p-phenylenediamine, pyridine, benzaldehyde, p-dimethylaminobenzaldehyde, cuprous cyanide, guaiacol, aniline, dimethyl aniline, diphenyl amine, benzidine, hydroxylamine HCl, Bayer 205, ammonium iodoxybenzoate, hydroquinone, monobromcamphor, menthol, emetine, quinine dihydrochloride, optochin, berberine, cinchophen, vioform and acetonitrile. Alcohols were particularly ineffective, for growth occurred in 10% solutions of methanol, while growth in 1% solutions occurred in the series up to iso-butanol, and in 0.1% up to capryl. Allyl alcohol and tribromethanol also were inactive. It appears that in some respects ethanol is a poor antiseptic for use in leprosaria. While resorcinol itself was inactive, hexyl to octyl resorcinols were markedly effective, as shown in Table I.

A *fourth* group of compounds considered were certain fatty acids

[8] Eubanas, F. C., *J. Philipp. Is. Med. Ass.*, 1927, **7**, 319; Hoffmann, W. H., *Arch. f. Schiffs.-u. Tropen Hyg.*, 1927, **31**, 139; Paldrock, A., *Arch. f. Schiffs.-u. Tropen Hyg.*, 1927, **31**, 459; *Ibid.*, 1932, **36**, 136; *Dermatol. Wochenschr.*, 1931, **92**, 273; Feldt, A., *Klin. Wochenschr.*, 1928, **7**, 73; Palmer, F. J., *Brit. Med. J.*, 1925, **2**, 96; Koga, G., *Jap. Med. Lit.*, 1917, **2**:**3**, 4.

from sources other than chaulmoogra. Our findings on the action of 13 chaulmoogric acid derivatives have been reported elsewhere.[9] The plating method was used to evaluate agents of this group, and the tabulated results in Table II represent the mean diameter of the circle of inhibited growth about a 0.05 cc. drop of the test substance placed in the center of an inoculated 15 cc. plated dish of 5% glycerol agar, after 144 hours of growth. In regard to ricinoleic acid,‡ it may be noted that Adams[1] found inhibition in dilutions below 0.002% for "total castor oil acids" but nevertheless considered them inactive.

TABLE II.
Leprocidal Activity of Some Fatty Acids and Related Compounds by a Plating Technique.

Test Substance	Diameter of Inhibited Area
	cm.
Total Hydnocarpus glycerides	2.0
" Chaulmoogra glycerides	1.5
" Calophyllum glycerides	0
" Castor oil glycerides	0
" chaulmoogra acids	2.5
Chaulmoogric acid	1.5
Total castor oil acids	3.5
Ricinoleic acid	3.0
Cinnamyl chaulmoograte	1.0
Ethyl chaulmoograte	0
" clupanodonate	0
" ricinoleate	4.0
Butyl ricinoleate	0
Methyl ricinoleate	0
" undecylineate	2.0
Undecylinic acid	2.5
Ethyl acetylricinoleate	0
Acetyl ricinoleic acid	3.0
Ethyl polymerized ricinoleates	3.0
Polymerized ricinoleic acids	4.5
Ethyl acetyl polymerized ricinoleates	0
Acetylated polymerized ricinoleic acids	2.0
"Chaulphosphate"	2.0
"Chaulphosphate" saturated aqueous solution	3.5
Na di-n-heptyl acetate saturated aqueous solution	7.5

Tests of these 4 groups of compounds were made in order to select promising drugs for experimental therapy in leprous rats. At present, several series of infected rats have been under treatment with merthiolate, gadusan, trypan blue, brilliant green, gentian violet and solganol for four months. A study of the toxicity of these com-

[9] Emerson, G. A., Anderson, H. H., and Leake, C. D., *Am. J. Trop. Med.*, in press; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 274.

‡ The ricinoleates and other castor oil derivatives tested in Table II were generously furnished by Mr. Kendall Marsh of the Baker Castor Oil Co., New York.

pounds, and the outcome of the experimental therapy will be reported shortly.

Summary. In a survey of the *in vitro* leprocidal effects of some 200 dyes, organic metallic compounds, fatty acids and miscellaneous compounds, high activity was found especially for merthiolate, metaphen, mercurochrome, solganol, triphenylmethane dyes, methylene blue, and octyl resorcinol. Trypan blue and the aliphatic alcohols were surprisingly inactive. While some correlation between therapeutic value and leprocidal activity may be noted, simple bactericidal tests are by no means a satisfactory excuse for clinical trial but are necessarily preliminary to the comprehensive pharmacological evaluation we have recommended.