with sterile distilled water. After all the excess stain had been removed they were centrifuged down once more and a large number added to broth. One was then able to watch the recovery of the organisms under the microscope. It was found that the bacteria gradually lost their stain, then after an interval of about 10 hours most of them had again regained their motility.

7649 C

Acute Toxicity of Ethyl Chaulmoograte.*

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At the suggestion of Dr. H. W. Wade we studied the toxicity in rats of single subcutaneous doses of a number of representative types of ethyl esters of the fatty acids of chaulmoogra oil. Preparations included were: (1) a purified ethyl chaulmoograte supplied by Dr. R. Wrenshall, fractionated by redistillation *in vacuo* at 10 mm. Hg., containing a small amount of ethyl hydnocarpate as indicated by the iodine number; (2) mixed ethyl esters of fatty acids of *Hydnocarpus wightiana* oil prepared at Culion, containing more than 80% ethyl chaulmoograte as indicated by polariscopic measurements; (3) a similar preparation containing 0.5% iodine, prepared by Cole's method[1]; (4) crude ethyl chaulmoograte containing 4% creosote, a preparation used in India, supplied by Dr. E. Muir from clinical supplies at Calcutta; and (5) "Chaulmestrol" (Winthrop), a proprietary preparation of mixed ethyl esters of fatty acids of chaulmoogra oil. The pertinent findings summarized in Table I agree with Martins' report[2] of 40 cc./kg. as being lethal for *Leptodactylus ocelatus*.

Examination of the tabulated data indicate that: (1) purified ethyl chaulmoograte preparations are more toxic than the ethyl esters of the total fatty acids of chaulmoogra oil, presumably because of

* Part of a cooperative study of the chemotherapy of leprosy conducted by the Pacific Institute of Tropical Medicine within the Hooper Foundation for Medical Research, and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

[1] Cole, H. I., *Philipp. J. Sci.*, 1929, **40**, 503.

[2] Martins, T., *Compt. rend. Soc. Biol.*, 1927, **96**, 474.

TABLE I.
Acute Subcutaneous Toxicity of Ethyl Chaulmoograte in Rats.

Substance	Iodine No.	Tolerated Dosage cc./Kg.	Mortality Ratio	Lethal Dosage cc./Kg.	Mortality Ratio	Time of Death	Cause of Death	Observations on Dying Animals
Ethyl chaulmoograte redistilled at 10 mm. Hg.	94.6	30	0/10	35-40	8/10	4 to 24 hr.	Pulmonary and gastro-enteric congestion	Irregular respiratory rate, followed by depression and opisthotonus before death in some animals
Crude ethyl chaulmoograte from Philippine Islands	87.6	35	0/10	40-50	5/15	12 hr. to 18 days	Pulmonary congestion	Symptoms less severe than with purified ethyl chaulmoograte
Crude ethyl chaulmoograte with ½% iodine from Philippine Islands	—	35	0/10	40-50	5/15	12 hr. to 14 days	Pulmonary congestion	Symptoms less severe than with purified ethyl chaulmoograte
"Chaulmestrol" (Winthrop Chemical Co.)	96.3	30	0/10	35-40	8/10	12-14 hr.	Pulmonary congestion	Symptoms less severe than with purified ethyl chaulmoograte
Crude ethyl chaulmoograte with 4% creosote from Calcutta	93.2	30	2/5	35-40	9/10	3-48 hr.	Pulmonary and gastro-enteric congestion	Markedly depressed with increased respiratory rate and fibrillary twitchings
Cottonseed oil with 4% creosote, control	—	30	2/5	35-40	7/10	1-24 hr.	Congested gastro-enteric tract	Markedly depressed with increased respiratory rate and fibrillary twitchings
Cottonseed oil, control	—	60	0/5	60	—	—	—	—

higher content of ethyl chaulmoograte; (2) iodizing ethyl chaulmoograte with 0.5% iodine has little effect on toxicity; (3) addition of 4% creosote increases the toxicity, commensurate with the amount of creosote present; (4) the degree of unsaturation of crude ethyl chaulmoograte is not a sufficiently sensitive index of toxicity to be reliable; and (5) if subcutaneously administered ethyl chaulmoograte exerts its toxic effect through liberation of Na chaulmoograte into the circulation, the summation of rates of hydrolysis and diffusion cannot much exceed that causing a disappearance of 2 millimols per Mol of the injected ester per hour, since Na chaulmoograte kills rats in intravenous acute doses[3] of 0.1-0.125 gm./kg.

Intensive treatment of leprous humans with ethyl chaulmoograte may give rise to a sterile tetanus in rare instances, perhaps related to the type of terminal convulsions seen in rats after administration of a single lethal dose. Conceivably, then, from this observation and the autopsy findings reported in Table I, death may occur from respiratory failure caused either by multiple emboli in the lungs or by prolonged central depression following a relatively short period of excitation, or from reduction of diffusible calcium in the plasma through formation of Ca dichaulmoograte, leading to tetany. No conclusions can be drawn here as to the importance of these and other factors in producing types of injury seen on chronic administration of chaulmoograte[4] nor as to the possible relative therapeutic rank of the different preparations, aside from an application of this acute toxicity data as a rough index of the absolute amount of ethyl chaulmoograte present.

7650 C

Attempts to Isolate Dihydroxy-pyrrol-Alanine from Gelatin Hydrolysates.

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Van Slyke and Hiller[1] and Van Slyke and Robson[2] have reported the presence in gelatin of a new amino acid which is precipitable by phosphotungstic acid. On the basis of the elementary analysis of

[3] Emerson, G. A., Anderson, H. H., and Leake, C. D., *Arch. Internat. de Pharmacodyn. et de Therapie*, 1934, **48**, 247.

[4] Frazier, C. N., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 44.