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Effect of Chaulmoogric Acid Derivatives on Lipolytic Activity in vitro.*

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Following the demonstration by Shaw-Mackenzie[1] that sodium gynocardate specifically activates pancreatic lipase *in vitro* by some 100% under the experimental conditions employed, Rogers[2] advanced the hypothesis that the mode of action of chaulmoogrates in leprosy therapy consists of a similar activation *in vivo* which would facilitate humoral destruction of the fatty capsule of *Mycobacterium leprae*. Significant differences were found by Rogers in the ethyl-butyrase activity of sera, shipped on ice from India, of treated and untreated lepers, the former approximating normal. Neill and Dewar[3] have been unable, however, to confirm these observations in a more extensive study of fresh leprous sera.

We have previously studied[4] by Loevenhart's method[5] the *in vivo* effects of certain antileprotic drugs on the ethyl-butyrase activity of tissues of rats experimentally infected with a standard strain of rat leprosy supplied by Dr. E. L. Walker. No reversion of the lipolytic activity to normal was brought about by any of the chaulmoogrates studied. Preliminary *in vitro* ethyl-butyrase studies on liver bries, in which 1 cc. of a 1% solution of the test agent was added to the Loevenhart reaction mixture, revealed an inhibition rather than an activation, varying to some extent with the type of chaulmoograte used. For the following drugs† averages of 5 determinations ex-

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[1] Shaw-Mackenzie, J. A., *Med. Press and Circ.*, 1920, **2**, 122; *J. Trop. Med. and Hyg.*, 1921, **24**, 161.

[2] Rogers, L., *Brit. Med. J.*, 1923, **2**, 11; *Lancet*, 1924, **2**, 1297.

[3] Neill, M. H., and Dewar, M. M., *Pub. Health Bull.*, 1927, **168**, 1.

[4] Emerson, G., Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 150; 1933, **31**, 18.

[5] Kastle, J. H., and Loevenhart, A. S., *Am. Chem. J.*, 1900, **24**, 491; Loevenhart, A. S., *Am. J. Physiol.*, 1902, **6**, 331.

† All but the last drug were prepared and generously supplied to us by Dr. Richard Wrenshall, Department of Chemistry, University of Hawaii.

pressed as per cent of original activity lost, were: sodium gynocardate, 60%; alepol, 40%; sodium chaulmoogryl-p-phenetidine sulfonate, 90%; sodium dihydrochaulmoogryl-p-phenetidine sulfonate, 90%; sodium chaulmoogryl glycinate, 65%; hydroxymercuriethoxy chaulmoogric anhydride[6] (saturated), 10%; and sodium dichaulmoogryl-β-glycerophosphate, 15%.

Addition of an only slightly soluble salt of a weak acid (sodium gynocardate) to an enzyme-substrate system results in considerable inaccuracy in the determination of the true end-point on titration unless careful comparison with a color standard is made, but the magnitude of the activating effect observed by Shaw-Mackenzie indicated a more significant factor was involved. It occurred to us that since in Shaw-Mackenzie's experiments sodium gynocardate was added to a glycerol pancreatic extract and the hydrolysis of the olive oil substrate allowed to proceed for 18 to 24 hours before titration, considerable enzymatic synthesis of chaulmoogryl glycerides must also take place in this time, resulting in the removal of weaker acids (chaulmoogric and hydnocarpic) with replacement by stronger acids from the glycerides of the olive oil substrate, thus leading to an apparent higher acid value on titration. We have, therefore, repeated Shaw-Mackenzie's experiments using both an aqueous and a 60% glycerol extract of fresh dog pancreas. Inhibition was found in the case of the aqueous extract while only slight apparent activation was observed with the glycerol extract.

Summary. In addition to the previously demonstrated lack of *in vivo* lipase activation by the antileprotic chaulmoogrates, it is shown that the apparent *in vitro* activation is not real, but is more likely an artefact due to the disturbance of equilibria brought about by the introduction of a salt of a weak acid into an enzyme reaction system containing large amounts of free glycerol. If the chaulmoogrates exert any indirect action in leprosy therapy besides their direct bactericidal action, it is improbable that the fat-splitting ferment is involved.

[6] Dean, A. L., Wrenshall, R., and Fujimoto, G., *J. Am. Chem. Soc.*, 1925, **47**, 403.