

progressed, direct evidence of a relationship among three of the compounds has been shown. The essential facts are as follows. Rotenone upon mild oxidation yields dehydrorotenone $C_{23}H_{20}O_6$. This compound when boiled with alcoholic potassium hydroxide and zinc dust gives rise to a hydroxy acid² $C_{23}H_{24}O_8$, which, when oxidized with hydrogen peroxide, yields derric acid³ $C_{12}H_{14}O_7$. Derric acid contains the two methoxyl groups originally present in rotenone and represents one half of the rotenone molecule.

Upon oxidation with potassium ferrieyanide deguelin $C_{23}H_{22}O_6$, the light green compound melting at 171° which is found in derris and cubé roots, the leaves of *Cracca vogelii* and the roots of *Cracca toxicara*, gives dehydrodeguelin $C_{23}H_{20}O_6$. This substance, analogous to dehydrorotenone, yields on boiling with alcoholic potassium hydroxide a phenolic monocarboxylic acid $C_{23}H_{24}O_8$, which has been called deguelic acid. Deguelic acid when oxidized with hydrogen peroxide in the same manner as was the acid from dehydrorotenone also yields derric acid.

Tephrosin $C_{23}H_{22}O_7$ when treated with a mixture of sulphuric and acetic acids or with acetic anhydride loses the elements of water and forms dehydrodeguelin. Thus derric acid constitutes one half of the molecule of rotenone, of deguelin and of tephrosin. The evidence also shows that tephrosin is intimately related to deguelin, since the loss of one molecule of water from tephrosin gives dehydrodeguelin. Without further experimental evidence, it appears probable that tephrosin is a hydroxydeguelin. Detailed reports of this work will appear elsewhere.

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DISSOCIATION OF BACTERIUM GRANULOSIS NOGUCHI AND IDENTIFICATION OF THE ORGANISM BY MEANS OF RABBIT IMMUNE SERA

THE viability of *Bacterium granulosis* for periods of a year or more on the semisolid ("leptospira") medium of Noguchi has already been recorded.¹ Recently, on transfer to blood agar of a culture which had stood for 8 months on semisolid medium without transfer, and which had shrunk by evaporation from 8 cc to 2 cc or less, a growth was obtained of discrete, yellowish gray, opaque, dry, bead-like colonies, with rough surface, which were distinct from the semitransparent, grayish, mucoid, confluent colonies usu-

ally seen in young cultures of *B. granulosis*. Microscopic examination, however, showed a morphology typical of *B. granulosis*. The strain fermented the usual carbohydrates, and agglutination tests with immune sera prepared in rabbits by means of the ordinary type cultures yielded clearly positive results.

A few smooth mucoid colonies were found among the rough dry ones, and a pure smooth strain was readily isolated from one of these. Plating of rough colonies yielded a growth chiefly of the rough type, with gradual reversion to smooth within 3 or 4 days in those portions of the plate where the colonies were widely separated. Replating every 24 to 48 hours reduced the tendency to reversion until it has practically disappeared. The tendency to the formation of yellow pigment, which is ordinarily seen only in old cultures of *B. granulosis*, is much enhanced in the rough cultures and appears early. The dissociation has since been found in other strains of *B. granulosis*, the identity of which had been uncertain until they were found to be agglutinated by immune serum.

Agglutination tests have so far proved the most useful means of identifying unknown cultures, since fermentation tests may vary occasionally from strain to strain. The serum² is highly specific for *B. granulosis*. Fourteen known strains of the organism so far tested have been agglutinated in dilutions of 1:256 to 1:1024, while no agglutination takes place in the case of the common bacteria found in the conjunctival secretions or tissue of man or monkey (*M. albus*, *M. aureus*, *B. xerosis*, *B. influenzae*), or of numerous gram-negative bacteria from the same source.

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² A. Butenandt, *Ann. d. Chem.*, 464: 272, 1928.

³ F. B. LaForge and L. E. Smith, *Journal Am. Chem. Soc.*, 52: 1091, 1930.

¹ Tilden, E. B., and Tyler, J. R., *J. Exper. Med.*, 1930, 52, 617.

² The sera have been prepared by injecting rabbits intravenously at 5- to 6-day intervals with gradually increasing doses (1 cc to 5 cc) of heavy suspensions of *B. granulosis* grown on freshly prepared nutrient agar in Blake bottles.