Nondiphtheric corynebacterial toxins and their mode of action

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1. *Corynebacterium pyogenes hominis* was demonstrated to be identical with *C.haemolyticum* and was first isolated and designated *C.scarlatinoides*. It is a separate species different from *C.pyogenes bovis*. It is a human epiphyte and a pathogen frequently present in the respiratory tract. Its difference from commonly studied corynebacteria and relationship to lactobacilli is discussed. It produces a complex of toxic proteins, *i.e.* a hemolysin, and especially enzymatic components the nature of which is described in the following reports. By the activity of these newly discovered toxic components on substrate *C.pyogenes hominis* is similar to *C.ovis*, and *C.ulcerans* (which moreover produces diphtheria toxin), and forms in this respect a group of related corynebacteria.

2. In the toxic filtrate of strains of *C.pyogenes hominis* was demonstrated the presence of a soluble hemolysin neutralizable by specific antiserum. The hemolysin differs

immunologically and by its thermolability from the proper toxic protein. The toxic protein on intradermal injection to rabbits or guinea pigs produces necrosis with hemorrhage and oedema. This toxin is on intravenous injection lethal in the rabbit. It differs from the hemolysin antigenically, by the kinetics of its production and persistence in the cultivation medium. Toxic filtrate clarifies egg yolk with consequent precipitation, splits lecithin, lysolecithin, and Tween 20. Detailed investigations showed that toxic filtrate possesses at least three enzymatic activities of which one corresponds to that of phospholipase A and another to that of phospholipase D. According to neutralization, production and persistence, and comparison of direct titrations it can be concluded that phospholipase D is identical with toxic protein. The third enzyme splits Tween 20 and differs from the foregoing two. Its antigenicity was not demonstrated yet.

C.ovis, as is well known, produces a toxic protein which has the same effect in the experimental animal as the toxin of C.pyogenes hominis. It was demonstrated that this toxin possesses D phospholipase activity too.

C.ulcerans produces into cultivation media two soluble toxins. One of them by its effect and antigenicity is identical with diphtheria toxin. The second toxin possesses the same enzymatic activity as the toxin of C.ovis.

3. All the three described corynebacteria can be diagnosed by means of the "inverted" CAMP test. This test enabled the demonstration of a large number of human pyogenic corynebacteria in the human population. This test shows that these corynebacteria produce a substance which markedly inhibits the effect of beta-lysin of Staphylococcus pyogenes in sheep erythrocytes, and the effect of alpha-lysin. This inhibition of staphylococcal hemolysis can be demonstrated in the filtrate and determined quantitatively by inhibition of alpha-lysin of S.pyogenes in rabbit erythrocytes. Accor-

ding to the production curve and by immunologic analysis the identity of this inhibitor with the toxic protein was demonstrated. It was newly found that toxins of all three corynebacteria inhibit hemolysis by alpha-toxin of Clostridium perfringens. The mechanism of the inhibition of the effect of alpha- and beta-lysin of S.pyogenes, and alpha-toxin of Cl.perfringens is discussed. Extracts of erythrocytes after treatment with toxins of C.pyogenes hominis and C.ovis were analyzed chromatographically. Chromatography revealed that sfingomyelin is split from sheep, human, and rabbit erythrocytes.

4. Toxic components of filtrate and endoplasmic material of C.pyogenes hominis were separated by gel filtration and chromatography in DEAE cellulose. In Sephadex G-100 the component possessing dermonecrotic, adsorption, and "yolk" activities (component alpha) was separated from hemolysin (component beta) and the Tween 20-splitter (component gamma). The separation of component alpha lead to the elaboration of a purification procedure by which 130-fold purification was attained with a yield of about 50%.

From the endoplasmic material by a similar procedure another component of higher molecular weight possessing "yolk" and dermonecrotic activity was obtained. By separating concentrated filtrates both components were obtained together with another component with "yolk" activity. The yolk activity of filtrate and endoplasmic material was also separated into three components by DEAE cellulose chromatography. The molecular weight of these components was determined by gel filtration.

5. Summarizing the foregoing reports, the production of phospholipases (especially phospholipase D) as an hitherto unknown component of toxins of corynebacteria pathogenic for man and animals was demonstrated. The possible significance of these phospholipases for the metabolism of the bacteria under study was discussed and the mechanism of

their action on some superficial structures of mammalian cells and their utilization for the differential diagnosis and classification of corynebacteria was indicated. The hitherto unknown frequency of occurrence and significance of Corynebacterium pyogenes hominis in the pathogenesis of human disease was stressed.