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OBSAH — СОДЕРЖАНИЕ — TABLE DES MATIÈRES

BIOLOGICAL PROPERTIES OF CORYNEBACTERIUM PYOGENES VARIETAS HOMINIS AND ITS DIFFERENTIATION FROM OTHER PATHOGENIC CORYNEBACTERIA

F. Patočka, A. Souček and *A. Součková*

Department of Medical Microbiology and Immunology,
Charles University Medical Faculty, Prague

The atypical corynebacterium designated by Patočka (Čas. lék. čes. 94, 1323, 1955) *Corynebacterium pyogenes var. hominis*, in view of some similarities with *Corynebacterium pyogenes* (Glage) Ebersson, is a frequent pathogen found in man. Only in Czechoslovakia over 500 strains have been isolated since 1959. On the basis of many years' experience we have attempted to present the properties which distinguish this corynebacterium from other pathogenic corynebacteria without pronouncing, however, our final judgment whether or not it is a separate species.

In previous reports we have demonstrated that *Corynebacterium pyogenes var. hominis* is identical with *Cor. haemolyticum* described by American workers (McLean, Liebow, Rosenberg, J. Infect. Dis. 79, 69, 1946) and answers Müller's description of *Cor. scarlatinoides* (Zbl. Bakt. 40, 613, 1906), *Cor. necroticans* isolated by Lodenkämpere Zbl. Bakt. I. 152, 419, 1947 and Drescher ärztl. Wschr. 8, 573, 1953 which is likely to be identical with this bacterium too.

According to the morphology of the bacterial cell, its cell wall composition, and some biochemical properties, *Cor. pyogenes* var. *hominis* belongs to a group of "atypical corynebacteria" which differ from "typical" corybebacteria (e.g. *Cor. diphtheriae*, *Cor. pseudotuberculosis*, *Cor. ulcerans*).

Cor. pyogenes var. *hominis* is a coryneoid rod, its plasma is not homogeneous. and it does not as a rule contain metachromatic granules. Cummins and Harris pointed out differences in cell wall composition of *Cor. haemolyticum* and the typical corynebacteria. They demonstrated rhamnose and lysine in *Cor. haemolyticum* but not arabinose and diaminopimelic acid which are found in cell walls of typical corynebacteria. This finding shows a similarity of atypical corynebacteria with streptococci.

In culture *Cor. pyogenes* var. *hominis* has greater requirements than typical corynebacteria. Fresh serum is essential for growth. Similarly as the other atypical corynebacteria it is inhibited by *kalium tellurosum* and therefore does not grow in selective media used for detecting *Cor. diphtheriae*. Colonial growth on blood agar markedly differs from typical

corynebacteria. Colonies are small, surrounded by a zone of hemolysis which is especially pronounced under anaerobic conditions. As a whole under anaerobic conditions not only growth of bacterial mass but also production of active components is enhanced.

In its morphological and cultural characteristics *Cor. pyogenes* var. *hominis* substantially differs from all the typical corynebacteria. In the hands of a less experienced worker, however, it may be confused with *Cor. pyogenes*. It can be distinguished from most strains of *Cor. pyogenes* by its biochemical properties chiefly in that it does not split xylose, it does not liquefy clotted milk, and only rarely does some freshly isolated strain liquefy gelatine. Production of phospholipase and lipase is an important point in distinguishing *Cor. pyogenes* var. *hominis* from *Cor. pyogenes* as will be shown in an analysis of toxic antigens.

A test which is similar to the CAMP test in its design and is based on inhibition of staphylococcal hemolysis was described by Záhorová (Folia microbiol. 5, 57, 1960). It has been widely used in the diagnosis of human infections and is considered to be typical of *Cor. pyogenes* var. *hominis*. Detailed studies showed that staphylococcal alpha/beta hemolysin is also inhibited by *Cor. pseudotuberculosis* and *Cor. ulcerans*. *Cor. pyogenes* does not inhibit beta lysin of *Staphylococcus pyogenes*, but alpha lysin of strain WOOD is inhibited later. This has to be taken into account when this "inverted" CAMP test is evaluated.

In view of the frequency with which *Cor. pyogenes* var. *hominis* is isolated we devoted our attention chiefly to the production of toxic antigen. All the strains of *Cor. pyogenes* var. *hominis* we were able to study produced a factor which after adsorption onto sheep erythrocytes inhibited their lysis by staphylococcal alpha/beta hemolysin. By direct and indirect titrations with antisera we demonstrated that this factor is identical with dermonecrotoxin and differs from soluble hemolysin. Souček and Součková (J. Hyg. Epidemiol. Microbiol. 8, 199, 1964) have demonstrated that this toxin is enzymatically active, it splits purified lecithin, releasing choline. This event is useful in distinguishing *Cor. pyogenes* which does not possess this activity. This phospholipase can be demonstrated in a growing strain or in a filtrate of broth culture in plates with lecithin or egg-yolk where dissolution and later precipitation occurs. Some strains produce a lister of fatty acids on the surface of the medium, because they also produce lipase, which can be assayed in media with Tween 20. *Cor. pyogenes* produces neither phospholipase nor lipase. Specific antiserum neutralizes dermonecrotic activity, adsorption onto erythrocytes, splitting of lecithin and precipitates in agar with this toxic antigen. Hemolysin is a separate antigen, its antigenic similarity with hemolysin of *Cor. pyogenes* cannot be ruled out.

Dermonecrotic activity of broth culture or filtrate on intradermal

or subcutaneous injection in the rabbit or guinea pig can be distinguished from the effect of diphtheria toxin chiefly in that changes appear earlier, and that there is a pronounced oedema at the site of injection with a subsequent central necrosis surrounded by a hemorrhagic border. Later, an abscess develops. These changes cannot de distinguished from changes caused by toxin of *Cor. pseudotuberculosis* or *Cor. ulcerans*. The latter one also produce diphtheria toxin in low concentrations. Differentiation is possible by neutralization with specific antiserum because the toxins differ antigenically. *Cor. pyogenes* in broth culture after intradermal injection produces an abscess in the rabbit or guinea pig, filtrate, however, produces irregularly a barely discernable necrosis without oedema. This activity is further diminished after inactivation of hemolysin, while in *Cor. pyogenes* var. ***hominis*** the dermonecrotic activity does not decrease even after inactivation of hemolysin.

In conclusion, *Cor. pyogenes* var. ***hominis***, or *Cor. **haemolyticum***, or *Cor. scarlatinoides* is a bacterium which by its morphology, cultural and biochemical characteristics, and chiefly by its production of toxic antigens is distinguishable from *Cor. diphtheriae*, *Cor. pseudotuberculosis*, and *Cor. ulcerans* and from *Cor. pyogenes* with which it was grouped or even confused. The origin of the isolated bacterium cannot serve as a reliable criterion for its identification, although *Cor. pyogenes* var. ***hominis*** has so far been isolated from human infections and carriers only. In contrast, *Cor. pyogenes* is isolated from animals, isolations from humans that are precisely described are few and date back to the period before the soluble toxic antigen of *Cor. pyogenes* var. ***hominis*** was recognized.

The authors for the time being do not propose any definite designation for this very widely distributed human toxic corynebacterium. They wish to remind, however, that the first, and of course incomplete description of this bacterium, which is probably identical with the human strains studied by the authors in Czechoslovakia and with *Cor. **haemolyticum*** was given by Reiner Müller in 1906 under the name *Cor. **scarlatinoides***.