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NEW OBSERVATIONS ON BIOLOGICAL PROPERTIES AND TOXINOGENESIS OF ATYPICAL HAEMOLYTIC CORYNEBACTERIA ISOLATED FROM HUMANS

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ANNOTATION

In a report published in Časopis lékařů českých (1955)[1]) the authors described 12 strains of markedly haemolytic corynebacteria isolated from humans, either as the agent of suppuration in pure culture or mostly as secondary invaders of necrotic lesions. In an attempt to classify this species it was found that these microorganisms have many properties in common with animal strains of *Cor. pyogenes,* which have repeatedly been isolated from man by different authors. Attention has to be drawn to studies [Barksdale et al.[2]), Cummins and Harris[3])] in which an attempt was made to demonstrate a close relationship between this microbe and pyogenic sreptococci on the basis of the chemical analysis of the cell walls of these organisms and by precipitation reactions of some of their mutants with endoplasmatic polysaccharide (in one of these studies one of our strains was used). It does not seem to the very likely that there is a relationship between our strains and some other species of the genus Corynebacterium occurring rarely in man, especially *Cor. striatum,* or *Cor. pseudotuberculosis* (according to the description in Bergey's Manual 1957).[4]) In the same report it was concluded that strains isolated by us are most likely "humanized" animal microbes which through selection by interhuman passage may acquire new biological properties. Further, certain properties of haemolysin were described together with, as then was thought, a thermostable toxic component of these bacteria, which was partially purified and tested. According to our findings and from the bibliography at that time, we were of the opinion that this infection in man is relatively rare at least in this country.

In the period 1955—1958 another 10 strains of this microbe were isolated in our laboratory. These strains had similar properties but varying and rapidly disappearing toxicity. In 1958 Záhorová and Kubelka[5]) described the phenomenon of inhibition of staphylococcal haemolysis by certain products of this microbe. With the aid of this test it was then very easy to isolate this microbe from pathological products under conditions in which it would otherwise escape notice. After systematic implementation of this haemolysis inhibition test it was found,

to our great surprise, that this microbe can frequently be isolated from the nasopharynx and tonsils in humans. Thus, more than 80 strains were isolated and identified in two routine laboratories in Prague within one year.[6]) At the same time the relation of this microbe to human tonsillitis, similar to streptococcal infection and frequently chronic, was demonstrated with occasional severe metastatic suppurative complications. This led us to further and more detailed investigations of the biological properties of the toxic components and the inhibitor of staphylococcal haemolysis of this microbe. At the same time we are studying the similarities between this microbe and the animal *Cor. pyogenes* whose frequent occurrence in animals is the subject of observation of our veterinary research workers. The results of our present experiments are reported in brief.

A very toxic strain (K-29) isolated from a case of tonsillitis was selected for our studies. Massive diffuse growth was obtained by cultivation in Todt-Hewitt broth with neopeptone (without glucose) and serum and its dynamics was followed by nephelometry.

Such a broth culture was passed through a Seitz filter, and was found, from the beginning of the log phase, to contain, a toxin which, when injected intradermally (0.2 ml. diluted up to 1 : 64) produces necrosis with haemorrhage and massive oedema in rabbits.

Maximal production of toxin takes place at about 26 hours of cultivation, the concentration of toxin remains roughly the same during further cultivation for a period of three weeks.

Somewhat later in the log phase a not very active thermolabile haemolysin was produced by the microbe, but its concentration in culture rapidly diminished.

A filtrabile factor inhibiting alpha and beta staphylococcal haemolysis appeared in the culture at about the same time as the toxin and persisted for just as long.

It was attempted to purify the toxin by ammonium sulphate and methanol precipitation. Both methods were effective. Maximal precipitation took place between 25—50% saturation of ammonium sulphate. The toxin was found to be relatively thermolabile (practically destroyed when heated at 80^0 C for 30 min.) which together with salting out points to its probable protein character. The antihaemolytic factor could be salted out with the toxin and had the same thermoresistance. Both factors could be concentrated. Under certain circumstances the toxin is lethal for rabbits and guinea-pigs. It is possible that the dermonecrotizing-lethal toxin has some direct relationship to the heamolysis inhibition factor.

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