Bacterial Toxins and Selected Topics in Virology
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NEW DATA ON THE BIOLOGICAL PROPERTIES OF CORYNEBACTERIUM PYOGENES HOMINIS.

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P r a g u e

These introductory remarks are not intended to be a taxonomical study which would not fit into this program any way. Nevertheless, I consider them necessary because as a whole we are dealing with a not very well known problem the study of which is far from complete.

Of all the corynebacteria pathogenic for man the greatest attention is paid, of course, to the species C.diphtheriae. The others which are less important in the pathogenesis of human disease, or were observed only as occasional pathogens for man, were thus far so insufficiently investigated that, with the two exceptions of C.ulcerans (which is sometimes considered either a variant of C.diphtheriae or a distinctly separate species), (Bergey, 1957; Saxholm, 1951; Jebb, 1948) and the notorious zoopathogen C.ovis, none of them were proved to be producers of a potent and specifically active exotoxin.

Of course even in C.ulcerans and C.ovis the toxins are chiefly studied as to their lethal effect and antigenicity, while so far as we know, the physiology of their production and activity on specific substrate were not investigated in any great detail. C.ulcerans is in some respects an exception in that one of its two toxins is qualitatively identical with the toxin of C.diphtheriae.

During our studies over a period of many years we have found that among these nondiphtheric corynebacteria regularly pathogenic for man, or among zoopathogenic corynebacteria there is a species which hitherto is not designated as an independent species and which is strikingly similar to C.ulcerans and C.ovis by its production of toxins possessing identical biological

(substrate) activities.

This circumstance in our opinion justifies its classification as a separate group among the corynebacteria which we have until now designated as pathogenic atypical corynebacteria.

The greater part of our toxin investigations which will be referred to here is concerned with the species we have preliminarily designated C.pyogenes hominis. By this we stress its similarity and at the same time its species dissimilarity with C.pyogenes bovis. We found C.pyogenes hominis to be identical with C.haemolyticum described by McLean et al.(1946). From time to time it was isolated from human materials and received several other designations, such as C.necroticans (Lodenkämper,1947; Drescher, 1953). Further, according to the rather detailed description of C.scarlatinoides by Müller in 1906 C.pyogenes hominis is identical with it. In accordance with classification usage Müller's designation has a claim to priority.

C.pyogenes hominis was at first confused even by us, and by others to this day with C.pyogenes bovis (Lovell, 1937,1941, 1944). These two corynebacteria, however, can be distinguished by their cultivation properties, metabolic activities, antigenicity and especially by the production of specifically active toxin, the presence of which was first demonstrated in endoplasmic material of disrupted cells (Patočka,1955) and subsequently proved to be an exotoxin (Patočka et al., 1960,1962 a,b; Souček et al., 1962). The description of this toxin will be the subject of our present communications.

C.pyogenes hominis similarly as C.diphtheriae is spread by interhuman contact. The isolation of a thousand strains in a limited area in Czechoslovakia in the course of three years from various affections of the upper respiratory tract, most frequently tonsillitis (occasionally accompanied by pseudomembranes and exanthema), further from various wounds, especially after burns, from the genital tract, and rarely from very grave metastatic complications, just goes to show that in central Europe certainly, and perhaps even elsewhere in the world,this corynebacterium is a generally widespread toxic pathogen in humans (Hermann,1961).

The determination of the taxonomic position of C.pyogenes hominis is difficult for the present. We had a feeling that it is an intermediate species standing between the genus of Lactobacilli and Corynebacteria. According to its growth requirements and hemolysis it approximates the streptococci. Actually, by its production of lactic acid, it occupies the intermediate position between the typical corynebacteria and streptococci (Table 1).

In C.pyogenes hominis was demonstrated beyond doubt the presence of cytochrome b_1 with differential maxima at 430 and 560 nm (strains K-29 and 501). The peaks at 432 and 564 of differential spectra of C.diphtheriae correspond to Pappenheimer's results (Pappenheimer et al., 1962) done on several other strains of C.diphtheriae (Fig.1).

The substantial differences between C.pyogenes hominis (strain K-29) and C.pyogenes bovis (strain E-419) are outlined in Table 2 and Table 3.

By the classical methods using brain-heart infusion Difco agar (horse serum and yeast extract added) with 2% sodium chloride and penicillin, we succeeded in isolating L-forms of C.pyogenes hominis which were mostly stable, reversion occurring only sporadically. The cultivation of these L-forms in liquid medium was not successful so far. It has been repeatedly found that stable L-forms produce a weak hemolysin. In one pilot experiment L-forms isolated from agar and disrupted by freezing-thawing also were shown to produce a typical toxin (Fig.2).

C.ulcerans which resembles most C.diphtheriae, resembles C.pyogenes hominis in that its other insufficiently studied toxin possesses the same substrate activity.

For the same reason a third microbe C.pseudotuberculosis ovis (Preisz-Nocardi) was included in our investigation. It is a well known zoopathogenic microbe. As will be demonstrated later even the very activity and long familiar toxin of this corynebacterium in this action on substrate is analogous with the toxin of C.pyogenes hominis and the other component of the C.ulcerans - toxin.

In the next communications will be described the principle

of the toxins of these three corynebacteria and of the phenomenon caused in vitro by these toxins (Záhorová, Kubelka, 1960), which is the basis of an important diagnostic tool by which it was possible to demonstrate the widespread occurrence, the frequency and significance of C.pyogenes hominis in human pathology.

References

1. Drescher, R.
   Ärztl.Wschr. 8 : 573, 1953.

2. Herrmann, G.J.
   Amer.J.Med.Technology 61, 1961.

3. Jebb, W.H.H.
   J.Path.Bact. 60 : 403, 1948.

4. Kielstein, P., Kötsche, W.,
   Monatschrft.Veterinar med. 21 : 20, 1966.

5. Kusaka, L., Sato, R., Shoji, K.
   J.Bacteriol. 87 : 1383, 1964

6. McLean, P.D., Liebow, A.A., Rosenberg, A.A.
   J.Inf.Dis. 79 : 69, 1964.

7. Lodenkämper, H.
   Zbl.Bact.I.Abt.Orig. 152 : 419, 1947.

8. Lovell, R.R.
   J.Path.Bact. 45 : 339, 1937.

9. Lovell, R.R.
   J.Path.Bact. 52 : 295, 1941.

10. Lovell, R.R.
    J.Path.Bact. 56 : 525, 1944

11. Müller, R.
    Zbl.Bact.I.Orig. 40 : 613, 1906.

12. Novotný, P.
    Nature 202 : 364, 1964.

13. Pappenheimer, A.M.,Jr., Howland, J.L., Müller,P.A.
    Biochem.Biophys.Acta 64 : 229, 1962.

14. Patočka, F.
    Čas.lék.čes. 94 : 1323, 1955.

15. Patočka, F., Souček, A., Mára, M.
    J.Hyg.Epidem.Microbiol.Immunol. 4 : 307, 1960

16. Patočka, F., Mára, M., Souček, A., Součková, A.
J.Hyg.Epidem.Microbiol.Immunol. 6 : 1, 1962.

17. Patočka, F., Souček, A., Mára, M., Součková, A.
VIII. Internatl.Congr. for Microbiol. - Abstracts E 33B8, 115, 1962 b.

18. Saxholm, R.
J.Path.Bact. 63 : 303, 1951.

19. Souček, A., Součková, A., Mára, M., Patočka, F.
J.Hyg.Epidem.Microbiol.Immunol. 6 : 13, 1962.

20. Záhorová, L., Kubelka, V.
Folia Microbiol. 5 : 57, 1960.

Table 1.

Strains	Production of lactic acid %
Streptococcus pyogenes	
β - hemolyticus	
Richards	52,5
6071	51,5
6138	52,5
C. diphtheriae	24,8
C.pyogenes hominis	
1629	32,6
501	34,4
4033	37,3
4375	35,4
5063	34,0
5104	35,8
4459	31,6
4034	31,6
C.pyogenes bovis (Lovell)	28,9

The production of lactic acid by streptococci and some corynebacteria. Lactic acid was determined by increasing $NaDH_2$ content (Boehringer enzymatic test) measuring the optical density at 366 nm in culture filtrates (Todd-Hewitt broth with 2% bovine serum and 1% glucose) after 48 hours incubation. Expressed in percentages of utilized sugar converted to lactic acid. Glucose was determined by anthrone.

Table 2.

Morphology.

K - 29 : fine, nonhomogenous, polymorphic corynebacteria, gram-labile, tends to aggregate in broth.

E -419 : short, more homogenous, diphtheroid rods, little pleomorphism, distinctly gram+, no tendency to aggregate in broth.

Growth in blood agar.

K - 29 : initially similar to pyogenic streptococci, later colonies rounded, matt with pearl lustre.

E -419 : resemble colonies of pyogenic streptococci, appearance does not change after further growth.

Antigenicity (Kielstein and Kötsche, 1966).

C. pyogenes hominis - two antigenic groups totally different from C.pyogenes bovis.

C. pyogenes bovis - in precipitation reactions antigenically homogenous.

Table 3.

BIOCHEMICAL PROPERTIES.

Strain	K 29	E 419
Glucose	+	+
Xylose	-	+
Galactose	+	+
Arabinose	-	-
Lactose	+	+
Sucrose	-	-
Maltose	+	+
Trehalose	+	+
Starch	+	+
Mannitol	-	-
Inositol	+	+
Glycerol	+	-
Indole	-	-
Hydrogen - sulphide	+	-
Reduction of nitrates	-	-
Urease	-	-
Loeffler's medium	-	+
Gelatin liquefaction		
at 20°C	-	+
at 37°C	-	+
Gelatin + serum		
at 20°C	-	-
at 37°C	-	+
Litmus milk :		
acid production	+	+
coagulation	+	+
peptonization	-	+
Milk + bromcresol purple :		
acid production	+	+
coagulation	+	+
peptonization	-	+
Milk + methylene blue	-	-
Catalase	-	-
Cytochrome b_1	+	not done
Growth on tellurite media	-	-

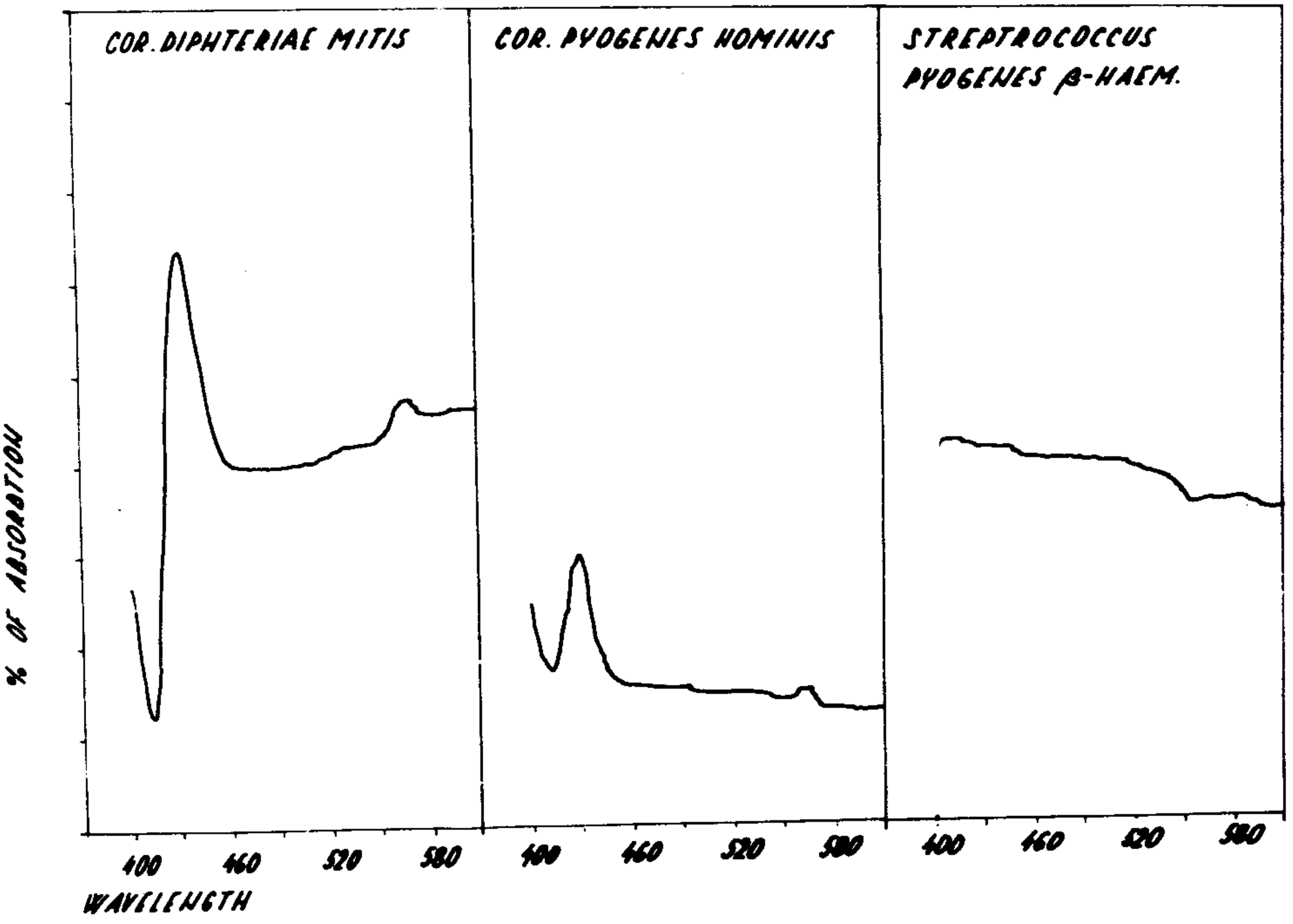

Figure 1.

The demonstration of cytochrome system in C.pyogenes hominis (strain K-29) in comparison with C.diphtheriae (strain 4489) and Streptococcus pyogenes β-hemolyticus (strain Richards). Aerobically grown bacteria were washed in saline, disintegrated mechanically with balotino (Novotný, 1964), and the analysed fraction of endoplasmic material was obtained by differential centrifugation at 10,000g and 100,000g respectively. Similar procedures were published by Pappenheimer et al.(1962) and by Kusaka et al.(1964). Differential spectrum was determined in resuspended sediment after final centrifugation using dithionite for reduction. Measured on a registration spectrophotometer Optica Milano . +

+ to be published in extenso with M.Mára and D.Kalvodová

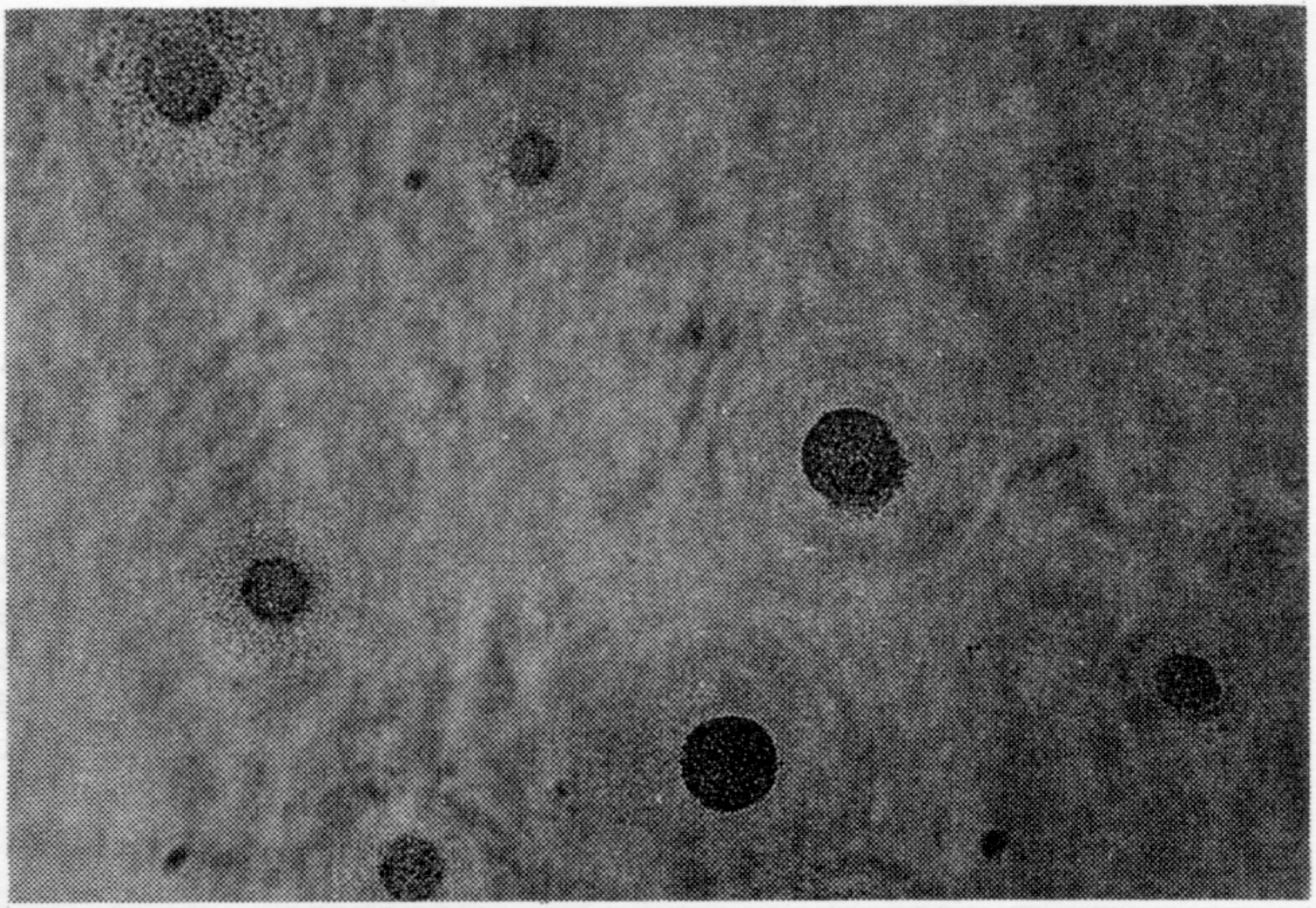

Figure 2.

Typical L - colonies of C.pyogenes hominis (60x).

Discussion

Ungar : Professor Patočka, do you think the Corynebacterium scarlatinoides described by Mendelbaum is related to the strains you isolated?

Patočka : As far as I know Mendelbaum did not describe either a specific toxin or a hemolysin in his Corynebacterium which he considered to be the aetiological agent of scarlet fever im humans. Therefore, I do not think that this Corynebacterium is either identical with, or related to C.pyogenes hominis which we are studying.

Pappenheimer : The cytochrome difference spectrum you showed for C.pyogenes hominis resembled that of PW 8 (but not wild type) C.diphtheriae strains: do your strains grow slowly compared with wild type as does PW 8?

Patočka : The strains of C.pyogenes K 29 and 501 in which we demonstrated cytochrome B, due to their fastidiousness, generally grow more slowly than strains of C.diphtheriae. We did not determine their exact generation time. Even if we did, it would not be possible to compare them with Pappenheimer's mutant PW 8 because both our strains after isolation from humans and passage in guinea pigs were subcultured in blood agar, or Todd-Hewitt broth only several times. They are in fact wild strains, not selected mutants.