JOURNAL OF HYGIENE, EPIDEMIOLOGY, MICROBIOLOGY AND IMMUNOLOGY 1962 — VI — 1

OBSERVATIONS ON THE BIOLOGICAL PROPERTIES OF ATYPICAL HAEMOLYTIC CORYNEBACTERIA ISOLATED FROM MAN AS COMPARED WITH COR. HAEMOLYTICUM, COR. PYOGENES BOVIS AND COR. OVIS

I. In vivo investigations

F. PATOČKA, M. MÁRA, A. SOUČEK, A. SOUČKOVÁ
Technical assistance: MIROSLAVA ŠKVOROVÁ and VĚRA SAHULOVÁ

Department for Medical Microbiology and Immunology, Charles University, Prague

Our previous investigations on human haemolytic corynebacteria brought to light many new findings (8—14).

Primarily, in a folow-up over a period of many years it was determined that these bacteria are a frequent parasite in man and an important agent for the pathogenesis of disease. Secondly, significant differences were demonstrated between the human corynebacteria and typical *Corynebacterium pyogenes bovis* with which the human strains have been identified so far. It is therefore highly probable that the human haemolysing corynebacterium belongs to a distinct species hitherto unrecognized. Lastly, it has been determined that the human haemolysing corynebacterium produces a toxic substance during the exponential phase of growth, also capable of inhibiting α-β haemolysis of pyogenic staphylococci.

These findings, described in previous reports, have led us to more detailed investigations of the human strains and their comparison with pathogenic animal corynebacteria. This report deals with the morphology, pathogenicity and biochemical properties of a selected human strain as compared with a strain of *Corynebacterium haemolyticum* (4, 6) and three animal strains. In all cases special attention is paid to the production of toxic substances.

MATERIAL AND METHODS

1. Strains

K 29 — Corynebacterium pyogenes var. hominis, isolated from a case of acute tonsillitis by Dr. Záhorová at the City Microbiological Laboratory, Prague.

73/61 — Cor. pyogenes bovis, isolated from cow's milk at the State Veterinary Institute, Prague.

E 419 — Cor. pyogenes bovis, isolated from a bovine foetus at the State Veterinary Institute, Prague.

KC 472 — Cor. ovis, isolated from a case of equine lymphangitis, kindly supplied from the collection of Communicable Disease Center, Atlanta, U. S. A.

ATCC 9345 — Cor. haemolyticum, also from the Communicable Disease Center in Atlanta.

2. Media

Blood agar: meat peptone broth with 2% agar and 10% citrated sheep blood.

Tellurite agar: Blood agar (supra) with 33 mg% K_2TeO_3.

Todd-Hewitt broth (7): with Neopeptone (Difco), without glucose; pH adjusted to 7.2, calf serum added to 10% concentration prior to inoculation.

3. Cultivation

Two litre Erlenmayer flasks with 400 ml. nutrient medium were incubated 48 hrs at 37^0 C. For determination of growth and production curves, calibrated tubes of Hilger's absorptiometer were used; broth added in 10 ml. amounts was concurrently agitated.

4. Morphology

Smears were stained by Gram's mehod in Hucker's modification (7) and by Albert's method (7).

5. Pathogenicity

Broth culture (BC) was prepared in Todd-Hewitt's broth with 10% calf serum incubated for 48 hours.

Filtrate (F) was first obtained by centrifugation and filtration througt Seitz EK filter. Since it was found that EK pads retained a part of the active components, Sartorius 3 membrane filters were used in the further work.

Washed bacterial bodies (WBB) were prepared by washing bacterial cells three times in saline and resuspending in saline to the original volume of BC.

Supernatant of disrupted cells (S). Washed cells were disrupted with balotin (No. 16) on a mechanical shaker (300 oscillations per min., amplitude 17 cm.) for 48 hrs. The degree of disruption was controlled under the microscope. After centrifugation (60 min. at 3000 r.p.m.) dry weight was determined in the supernatant and diluted in a volume proportional to dry weight of WBB.

6. Experiments on animals

Activity of BC, F, WBB and S was assayed in rabbits, guinea pigs and white mice.

Intravenous lethality of *filtrate (F)* was determined in *rabbits* (2 to 2.5 kg.) — MLD = death of 66% inoculated animals. The diameter of necrosis and oedema after 48 hours under standard conditions of injection (0.25 ml.) was taken for evaluation of the intradermal reaction. The dermal reaction can be confirmed and made more distinct by the intravenous injection of 2 ml. of 1% trypan blue 1 to 2 hours following intradermal inoculation of *F.* In determining production of toxic substances 0.25 ml. of sterile *filtrate* was injected intradermally and the minimal reactive dose (MRD) was determined as the highest dilution producing palpable oedema 0.5 cm. in diameter after 48 hrs. This figures in graphs as the limiting dilution of *filtrate.* 20 to 40 intradermal injections were made in one rabbit. In determining MRD the *filtrate* was diluted with T-H broth just prior to injection in two-fold dilutions. For determination of the limiting dilution further titration was performed at 20% levels.

Guinea pigs (300 g.) were injected with 1 ml. of BC, WBB, and F subcutaneously and 0.25 ml. of F intracutaneously.

White mice (30 g.) were inoculated intravenously with 0.5 ml. of F.

7. Growth and production curves

The number of viable cells was determined by the plate method on blood agar. Decimal dilutions in buffered physiological saline (pH 7.2) were inoculated in 0.1 ml. amounts. Three plates were used for each dilution. Plates were read at 48 hrs, the variation coefficient did not exceed 20% in 4 strains, in Cor. ovis 37%. The resulting number

was calculated per 1 ml. in millions and expressed in $\log_2$. The initial inoculum of each strain was adjusted to approximately 1 million viable cells per ml.

Bacterial density was derived for each strain from calibration curves which express the relationship between optical density and dry weight (Fig. 1).

Optical density was measured in Hilger's absorptiometer without a filter. The average of 10 tubes was used for calculations.

Dry weight was determined by the usual methods after washing bacterial cells three times in distilled water, starting with 20 ml. of culture.

Specimens for the determination of active components were taken at specified intervals, centrifuged and then filtered through membrane filters and stored at 4° C.

Biochemical properties

Strain	`K 29	73/61	E 419	KC 472	ATCC 9345
Glucose	+	+	+	+	+
Xylose	−	−	+	−	−
Galactose	+	+	+	+	+
Arabinose	−	−	−	−	−
Lactose	+	−	+	−	+
Sucrose	−	−	−	−	−
Maltose	+	+	+	+	+
Trehalose	+	+	+	+	+
Starch	+	−	+	−	+
Mannitol	−	−	−	−	−
Inositol	+	+	+	−	+
Glycerol	+	+	−	−	−
Indole	−	−	−	−	−
Hydrogen-su'phide	+	−	−	+	+
Reduction of nitrates	−	−	−	+	−
Urease	−	−	−	+	−
Loeffler's medium	−	+	+	−	−
Gelatinu 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	−	−	−	+	−

8. Preparation of immune serum

Rabbits were immunized with the *filtrate* and purified preparation (12, 13) intravenously with increasing doses at four day intervals, and bled after 1 to 2 months.

9. Biochemical properties

For fermentation reactions 10% peptone water (Proteosepeptone 3 - Difco) with calf serum and 1% sugar, pH 7.2, were used. With the exception of starch and glycerol, which were sterilized by fractionation, all other sugars were sterilized by filtration. Brom cresol purple, used as indicator, was added prior to inoculation. The production of indole, re-

duction of nitrates and the presence of urease were demonstrated by standard methods (7) in media with 5% calf serum. H_2S was detected in the medium with thiopeptone and 5% calf serum by blackening of lead acetate paper. Calf serum was used in preparing Loeffler's medium. Gelatin (DGF Weiss Gold) was dissolved in T-H broth to 12% concentration. To gelatin with serum calf serum was added to 5% concentration. Liver pieces were added to litmus milk with brom cresol purple. Mythylene blue was added to milk in 0.1% concentration. Catalase was demonstrated on agar with 10% serum with the aid of 3% H_2O_2. Media were inoculated with one loopful of 48 hr blood agar cultures and the results recorded concurrently up to 30 days. Cultures were incubated at 37^0 C.

RESULTS

Morphological and staining properties of studied strains.

Strain K 29 — generally characteristic corynebacteria, fine, frequently not homogenous, polymorphic, Gram-labile; formation of filamentous forms with pseudoramifications. In broth cultures besides single rods, compact aggregates similar to actinomycotic granules, frequently in chains and, rarely, small granules analogous to metachromatic bodies, were to be found.

Strain 73/61 — short Gram-labile rods with a predominance of Gram-negative forms, sometimes clubbed. Usually similar to brevibacteria. Homogenous. Picture identical in broth cultures.

Strain E 419 — homogenous short diphtheroid rods, abundant navicular forms. Strongly Gram-positive, metachromatic bodies absent. No sign of pleomorphism. In broth cultures short forms, rather coccoid or diphtheroid without typical arrangement.

Strain KC 472 — on blood agar thick clubbed and navicular rods similar to *Cor. pseudodiphtheriae.* Markedly Gram-positive. In broth culture predominance of navicular forms, less frequently clubbed forms. Homogenous. With Albert's stain certain rods possessed more markedly stained ends.

Strain ATCC 9345 — on agar and in broth cultures this strain in respect to shape and staining was almost identical with strain K 29. In 24-hour cultures stained by Albert's method small distinct metachromatic granules were demonstrable which disappeared in 48-hour cultures.

Growth characteristics

Strain K 29: On blood agar colonies 1—2 mm. in diameter appeared in 48 hrs; round even edges, opaque, matt, adherent colonies surrounded by distinct zone of β haemolysis. On plain agar growth hardly apparent. No growth on blood agar with K_2TeO_3 (33 mg%). In broth culture diffuse growth with easily dispersed deposit.

Strain 73/61: On blood agar very small colonies appeared in 48 hrs Whitish, opaque, round, matt colonies with a very well defined, up to 3 mm. wide zone of β haemolysis. In broth culture diffuse growth with deposit. Poor growth on plain agar, no growth on medium with K_2TeO_3.

Strain E 419: Blood agar: in 48 hrs small whitish opaque colonies up to 1 mm. in diameter; besides typical colonies, fine, barely visible colonies appeared. Zone of β haemolysis wider than 2 mm. Growth on all other media as in above strains.

Strain 472: Blood agar: in 24 hrs. well defined colonies which at 48 hrs. attained 2—3 mm. in diameter. Whitish, opaque, matt pearly gloss with elevated centre and uneven edges; fragile, could be moved about as a whole. β haemolysis under the colony, but around it rare and indistinct. General appearance similar to *Cor. pseudodiphtheriae.* Good growth on plain agar though less abundant than on blood agar. Good growth in presence of K_2TeO_3. In broth culture fine granules were formed on sides of tubes which fell to the bottom after 48 hrs. and formed a deposit not readily dispersible. A thin pellicle formed on the surface.

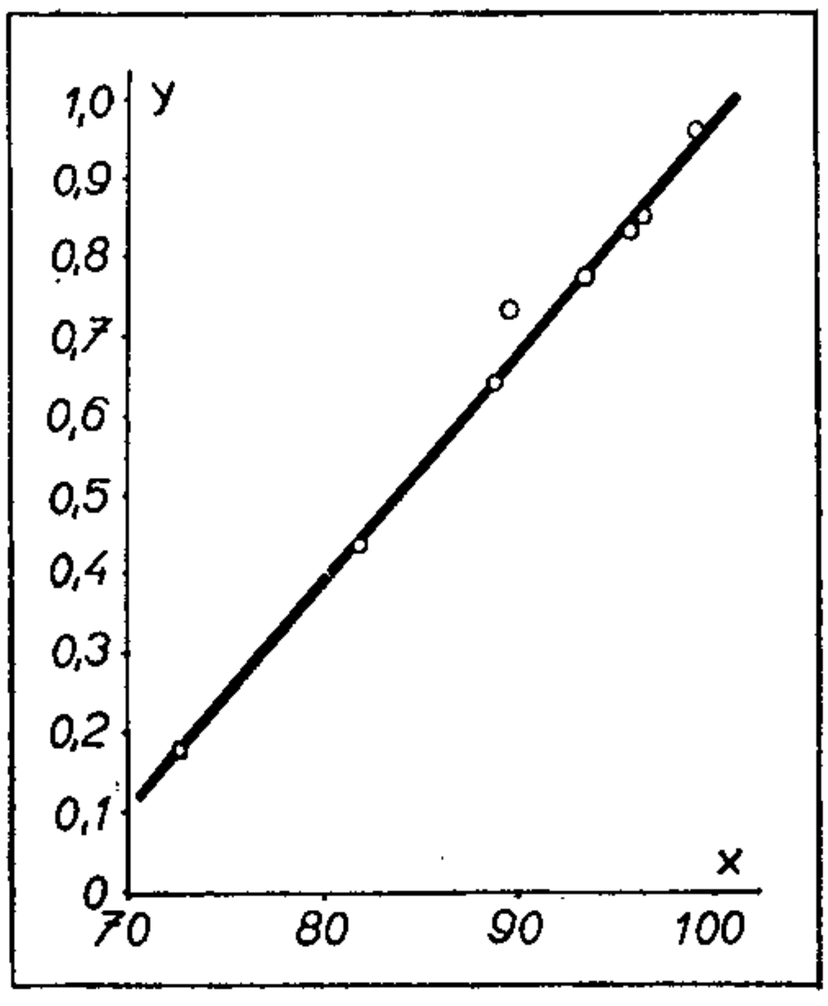

Fig. 1.

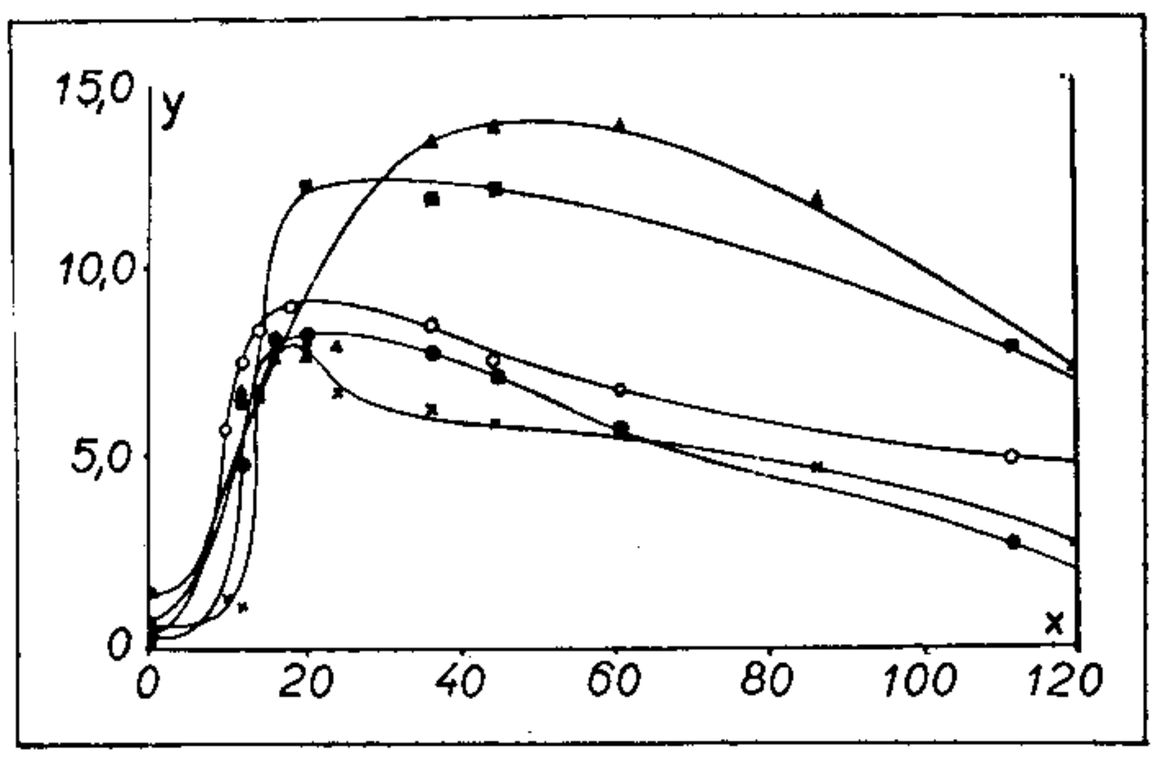

Fig. 2.

Fig. 1. Calibration curve for determination of bacterial density derived from optical density of strain K 29. y = optical density; x = $\log_2$ of bacterial density; values on axis x should be expressed in decimal factions). Fig. 2. Crowth curve of studied strains expressed in number of viable cells. ● K 29; ■ E 419; ▲ 73/61; ○ KC 472; × ATCC 9345. y = $\log_2$ of viable cells mil./ml.; x = time in hours.

Strain ATCC 9345: Generally, shape and nature of colonies on blood agar as in strain K 29, only zone of haemolysis was wider and far more distinct. Other cultural properties as in strain K 29.

All strains were cultivated under aerobic conditions. In their growth they are typical facultative anaerobes. It is evident that strains K 29 and ATCC 9345 are morphologically and culturally identical. Strains 73/61 and E 419 differ from them in all respects. Strain KC 472 *(Cor. ovis)* differs from the rest most markedly and in its morphological and growth characteristics is closest to *Cor. diphtheriae.*

Biochemical properties

An outline of the most important biochemical properties of the investigated strains is given in Figure 1. The graph illustrates the almost complete identity of biochemical properties of strains K 29 and ATCC 9345 (with the exception of glycerol fermentation of strain K 29 (4). Strains 73/61 and E 419 differed between themselves less (73/61 did not ferment xylose and lactose, did not acidify milk

or peptonize it), but differed more from the foregoing two strains, especially in their liquefaction of gelatin and Loeffler's medium, and in their acidification and clotting of milk. Strain E 419 in all respects answers the description of typical strains of *Cor. pyogenes* bovis, even in that gelatinolysis was slowed down by the addition of serum (15). Strain 73/61 is atypical in that it did not fermented xylose and lactose. Strain KC 472, a typical *Cor. ovis, was absolutely different,* e. g., in the transfer of hydrogen ions (reduction of nitrates, production of catalase); production of urease is an important feature distinguishing it from the

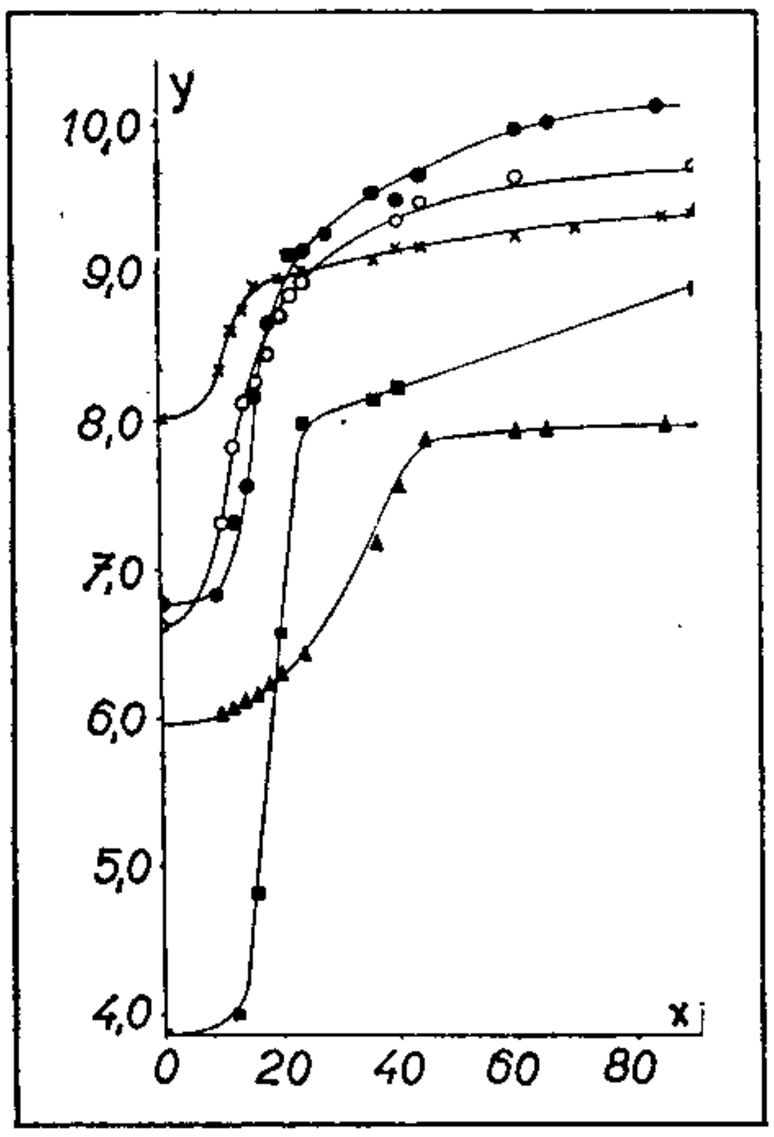

Fig. 3.

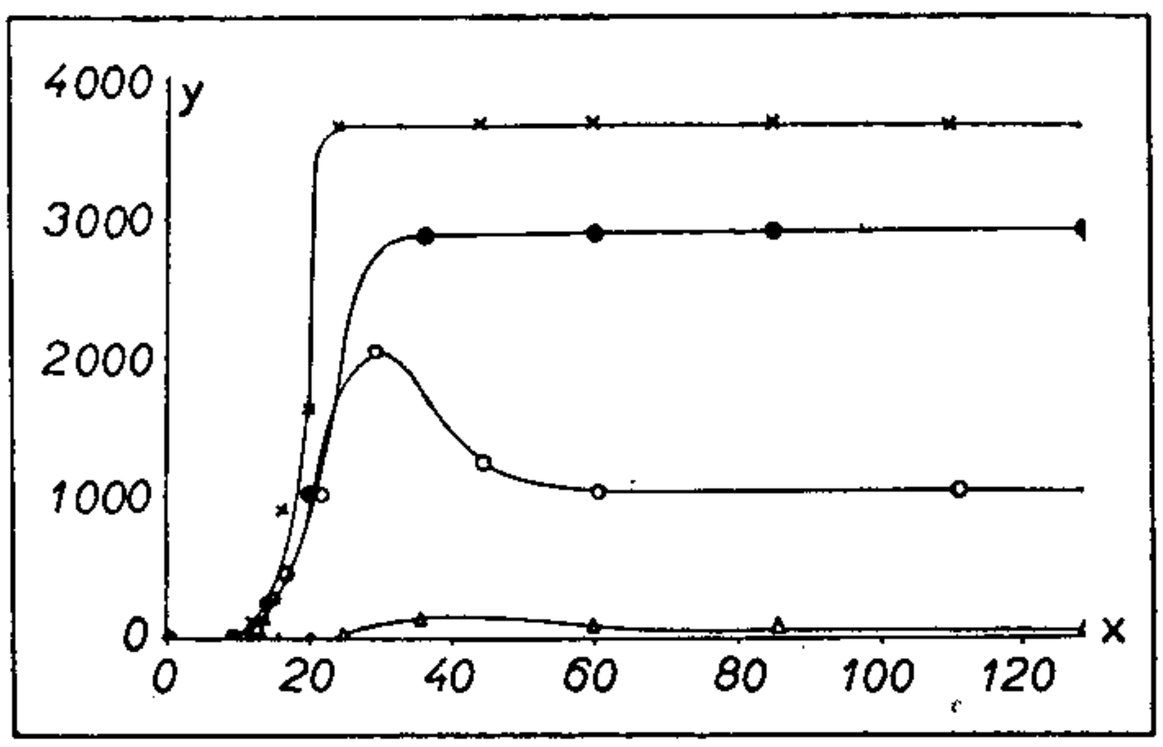

Fig. 4.

Fig. 3. Crowth curve of studied strains expressed by bacterial density. ● K 29; ■ E 419; ▲ 73/61; ○ KC 427; × ATCC 9345. y = log cf bacterial density; x = time in hours.
Fig. 4. Production curves of dermonecrotic components of filtrates expressed by limiting dilution of filtrate per 1 ml. ● K 29; ▲ 73/61; ○ KC 427; × ATCC 9345. y = limiting dilution of filtrate; x = time in hours.

other strains under investigation. In general, the described biochemical similarities and differences reflect morphological and cultural characteristics.

Dynamics of growth (growth curves)

Dynamics of growth in the bacterial populations have been investigated, especially in view of production and persistence of physiologically active components of bacterial strains. Growth curves were plotted by determination of number of viable cells, and by determining the dry weight and optical density of cultures. Figures 2 and 3 show uneven growth and a indirect relationship between the number of viable cells and bacterial density. Both animal strains (E 419 and 73/61) attained much higher numbers of viable cells while bacterial density was lower than in other strains. Cells of both these strains, therefore, weigh less than those of others. Logarithmic phase of growth in four investigated strains lasted from the 9th to the 20th hour of cultivation. In strain 73/61 the log phase was somewhat prolonged.

Pathogenicity

Strain K 29: A 48-hr. broth culture injected subcutaneously into *guinea pigs* in 1 ml. amounts regularly killed them within 4 days. Massive oedema appeared at the site of inoculation, at its highest point necrosis with haemorrhagic border became discernible. At autopsy slightly haemorrhagic fluid was found with distinct pseudomembrane at the base. The viscera were hyperaemic, suprarenals (as against diphtheria) were never haemorrhagic or enlarged. The same amount of *filtrate* (*F*) produced oedema and the same sequence of subcutaneous

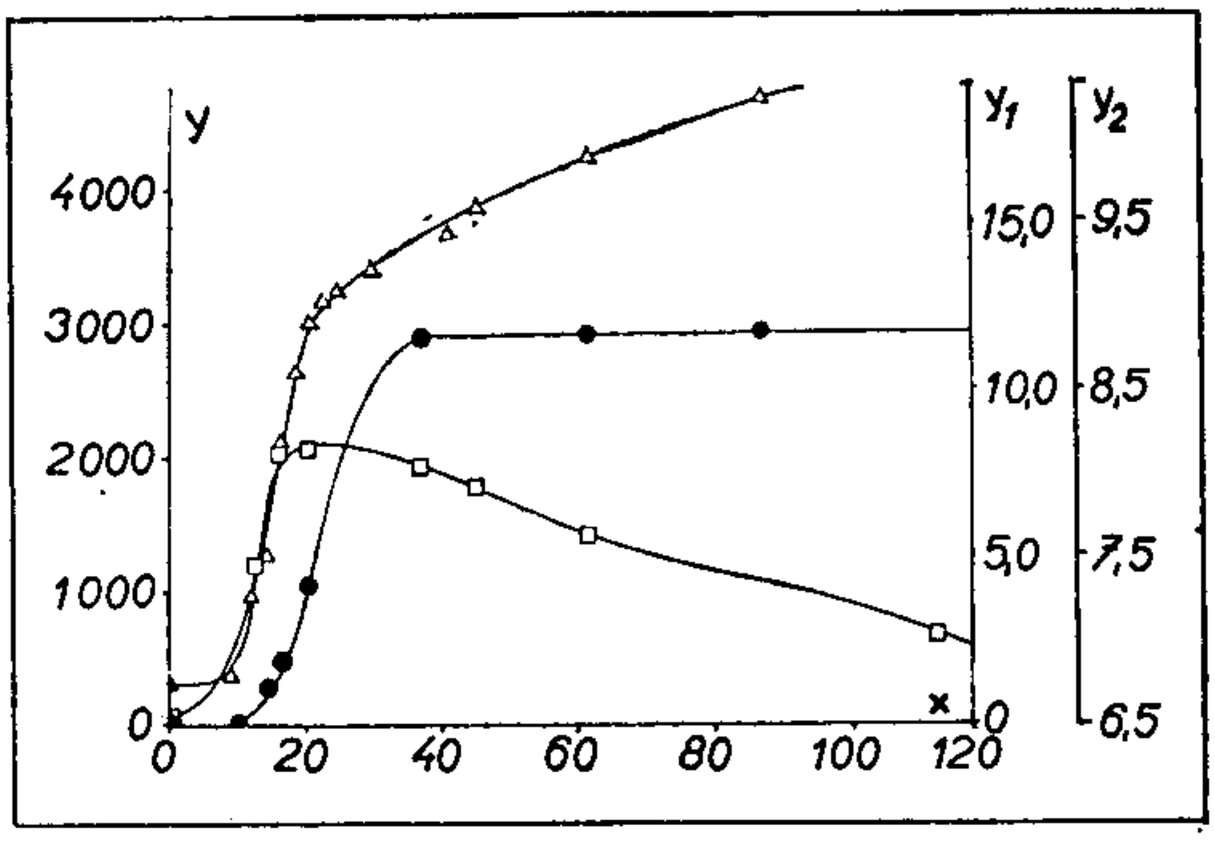

Fig. 5. Comparison of growth curves with production of dermonecrotic component of strain K 29. △ bacterial density; □ viable cells; ● dermonecrotic component. $y_1 = \log_2$ of viable cells mil./ml.; $y_2 = \log_2$ of bacterial density.

changes in guinea pigs but without a lethal outcome. WBB produced similar changes at first, later on pus accumulated, death did not follow. S produced the same reaction as (*F*) but to a lesser degree. (*F*) injected intradermally produced necrosis with a haemorrhagic border at the site of inoculation.

Rabbits proved to be the most sensitive animals in our skin-test experiments. BC injected intradermally in 0.25 ml. amounts produced marked necrosis with a haemorrhagic border in 48 hrs. and oedema 3—6 cm. in diameter. *F* injected intradermally (0.25 ml.) produced, after 4 hrs., oedema which gradually enlarged up to 3 cm. Necrosis with a haemorrhagic border appeared in its centre. WBB and S produced a quantitatively less intensive reaction. Massive suppuration (abscesses) appeared in the later stages of all intradermal necroses. Sublethal doses of BC injected intravenously in a rabbit produced septic dissemination which was also apparent in the formation of small abscesses in the vicinity of some blood vessels in the skin, especially of the ears; all rabbits recovered. MLD varied from 0.3 to 3.0 ml. of *F*, according to batch of *F*. At autopsy no significant macroscopic changes were apparent.

White mice repeatedly, did not react to intravenous injection of up to 0.5 ml. of *F*.

Strain 73/61: *Guinea pigs* inoculated with 1 ml. of BC died within 2 days with massive swelling of the abdomen, the exudate was haemorrhagic, viscera hyperaemic.

Rabbit: 0.25 ml. of BC inoculated intradermally produced swelling with haemorrhagic and central necrosis. *F* in 0.25 ml. intradermally, as against K 29, gradually produced a flat livid oedema with an hyperaemic border, small central necrosis without haemorrhage appeared just as gradually. This phenomenon appears to differ from the massive bulging oedema rapidly developing after the injection of *F* of strain K 29. WBB produced identical changes as BC but to a lesser degree. S injected intradermally did not produce any changes. *Intra*-venous injection of 0.3 ml of *F* was usually lethal to white mice within 30 min. when *F* contained a high titre of haemolysin.

Photo. Titration of filtrate of K 29 in rabbit skin — 48 hrs after intradermal injection.

S t r a i n E 419: *Guinea pig:* BC produced oedema with hardly discernible necrosis, the animal survived. *F* did not produce subcutaneous changes. WBB produced hardly discernible oedema.

Rabbit: Intradermal injection of BC produced an abscess which healed within a week. *F* was without effect. WBB produced hardly discernible oedema. *S* was without effect.

White mouse: F was not lethal on injection.

S t r a i n KC 472: *Guinea pig:* 1 ml. of BC subcutaneously killed in 2 days, oedema developed with central necrosis and haemorrhage, and spread from the site of inoculation under the skin of the abdomen. 1 ml. of *F* subcutaneously produced oedema without a lethal outcome. WBB produced the same process as BC, only more gradual in development.

Rabbit: BC intradermally produced oedema with haemorrhage and necrosis which later demarcated. *F* produced the same reaction which was of slower progression and lesser intensity. WBB and S were still less active.

S t r a i n ATCC 9345: *Guinea pig & rabbit:* quality and sequence of changes after subcutaneous and intradermal injection of all tested preparations was ge-

nerally the same as in strain K 29. *F* injected intravenously was also not lethal to *white mice.*

Histology

Filtrate of strain K 29 after intracutaneous injection produced not only necrosis of the epidermis and cutis but also marked inflammatory vascular changes. Appearance of angiitic changes preceded necrosis and even later on exceeded simple demarcation of necrotic foci. Appearance of histologically discernible necrosis was so early that it is not possible to explain it by simple ischemia as a result of inflammatory occlusion of the vessels. This suggests that the *filtrate* contained a capillaritoxic and necrotoxic component. Changes produced by *F* of strain 73/61 were similar but less intense.

Experiments in animals clearly demonstrated the presence of toxic components produced into broth during growth of strains K 29, 73/61, KC 472 and ATCC 9345. The quality of macroscopic changes points to the identity of strains K 29 and ATCC 9345. The typical *Cor. pyogenes* bovis strain E 419 is, in our experiments, practically non-toxic.

Strain 73/61 produced toxic substances somewhat different from those of both human strains. Their activity was very similar to that described by Lovell (5) in his bovine strain of *Cor. pyogenes.* Toxicity of the *filtrate* of *Cor. ovis* is well known and has been repeatedly reported on (2, 3); our findings are in accordance with those of veterinary workers. Although the toxic product has the same, though more intensive, effect in experimental animals this microbe is undoubtedly more virulent than human strains, since washed bacterial cells, freed of toxins, produce an infection with a lethal outcome.

In human strains of pyogenic corynebacteria or of *Cor. haemolyticum,* which in our opinion is identical, the production of toxin is a new, hitherto, undescribed finding. Both groups of American workers (4, 6) state in their reports that filtrates of cultures do not contain toxic components. This discrepancy between these and our findings can be explained by assuming that we employed a culture medium in which the toxic agents are produced by the microbes in quantities that can be demonstrated in the filtrates. Strain ATCC 9345 of *Cor. haemolyticum* is the same as that studied by the American workers.

The dermonecrotic effect of toxin in rabbits proved to be the most sensitive in vivo reaction in the investigation of the activity and properties of filtrates of human strains and their comparison with animal strains (Plate I.).

Production of dermonecrotic components

The production of dermonecrotic components by four active strains is illustrated in Figure 4. The shape of curves again points to the identity of strains K 29 and ATCC 9345. Strain 73/61, vide supra, produces a dermonecrotoxin of different activity.

The production of newly discovered dermonecrotoxin by strain K 29 commences with the exponential phase of growth and persists in broth culture at 37^0 C with practically undiminished activity for a period of one month.

DISCUSSION

The findings described in detail in this paper confirm our views already presented (11, 12), concerning the nature of substances produced by strains K 29 and ATCC 9345. Both of these strains and practically all human strains of pyogenic corynebacteria isolated by us produce a toxic substance of dermonecrotic and lethal activity for rabbits. This toxic product, especially its lethal effect, is substantially less active than the toxic protein of diphtheria. Experiments not yet terminated, which will be the subject of another communication, show that this substance is macromolecular (non-dialyzing), thermolabile and of protein nature. The protein character is further demonstrated by precipitation with ammonium sulphate and methanol in broth culture filtrates (1 : 1).

This substance isolated by us is antigenic and induces specific antibody formation in rabbits. The antibodies neutralize both the dermonecrotic and the lethal effect in rabbits. (Unit of antitoxin not yet determined. In one batch of *filtrate* of strain K 29 the MRD was 0.00048 ml., and 400 MRD was completely neutralized by 0.05 ml. of immune serum.) In another experiment the antigenic relationship of toxic substances of both human strains under investigation was demonstrated.

Two strains (K 29, ATCC 9345 — *Cor. haemolyticum)* of identical properties, in accordance with foreign workers, have quite frequently been isolated by us from man, usually from the tonsils with lesions of varying gravity. During the past two years we and our collaborators have succeeded in isolating almost 200 strains. According to our experience, we consider them as obligatory parasites and occasional pathogens in man.

One of the intentions of this communication was to determine the taxonomic position of human pyogenic corynebacteria by comparing them with two freshly isolated animal strains of *Cor. pyogenes* bovis, and with a toxic prototype strain of *Cor. ovis.* It follows from our investigations that the Czechoslovak strain K 29 is identical with the *Cor. haemolyticum* strain ATCC 9345, isolated from a native of the Pacific. Similar strains are being isolated from man in the United States. Both of these human strains differ in many points from animal strains of *Cor. pyogenes. Cor. ovis* is an absolutely distinct species.

SUMMARY

1. The morphology, biochemical properties and growth characteristics of five strains: two human strains *(Cor. pyogenes* var. hominis, and *Cor. haemolyticum),* two strains of *Cor. pyogenes* bovis, and *Cor. ovis* are described.

2. Their pathogenic activity was assayed in the guinea pig, rabbit and white mouse.

3. Both human strains were found to be identical; differences between them and animals strains, especially *Cor. ovis,* are pointed out.

4. A new toxic antigen in filtrates of broth cultures of strains K 29 and ATCC 9345 is described. The substance has marked dermonecrotic activity in the rabbit and guinea pig.

5. The toxic antigen of human strains is similar to the toxin of *Cor. ovis* in biological effect.

6. The toxic substances of strains K 29 and ATCC 9345 were neutralized by immune rabbit serum in orientation experiments.

RÉSUMÉ

1. On a décrit la morphologie et des proprietés biochimiques et de la croissence de 5 souches, à savoir de deux souches humaines (Cor. pyogenes var. hominis et Cor. haemolyticum), de deux souches du Cor. pyogenes bovis et d'un du Cor. ovis.

2. C'est par l'expérience sur le cobaye, le lapin et sur la souris que l'on a évalué leurs qualités pathogènes.

3. On a trouvé la conformité totale de toutes les deux souches humaines étudiées et a tracé des différences de souches animales notamment de la souche du Cor. ovis.

4. C'est dans les filtrates des cultures de bouillon des souches K 29 et ATCC 9345 que l'on a determiné un antigène toxique possedant une proprité dermonecrotique accentuée pour le lapin et le cobaye.

5. C'est par son activité biologique que l'antigène toxique des souches humaines est similaire à toxin du Cor. ovis.

6. Par les épreuves d'orientation on a pu neutraliser des substances toxiques des souches K 29 et ATCC 9345 par le sérum réfracteur de lapin.

ZUSAMMENFASSUNG

1. Man beschreibt die Morphologie, Wuchs- und biochemischen Eigenschaften von 5 Stämmen, und zwar von zwei menschlichen (so genannte Cor. pyogenes var. hominis und Cor. haemolyticum), weiter von zwei tierischen Stämmen Cor. pyogenes bovis und von einem Stamm des Cor. ovis.

2. Man beurteilt ihre pathogenischen Eigenschaften mit Hilfe der Experimente am Meerschweinchen, Kaninchen und an einer Maus.

3. Man fand volle Identität beider untersuchten menschlichen Stämme und stellte die Unterschiede von den tierischen Stämmen, namentlich von dem Stamm Cor. ovis fest.

4. Neu hat man in den Filtraten der Bouillonkulturen der Stämme K 29 und ATCC 9345 ein toxisches Antigen mit einer ausgesprochenen dermonekrotischen Eigenschaft für das Kaninchen und das Meerschweinchen.

5. Was die biologische Wirkung anbelangt, ist das tierische Antigen der menschlichen Stämme ähnlich dem Toxin des Cor. ovis.

6. Zu Orientierungszwecken hat man die Neutralisation der toxischen Substanzen der Stämme K 29 und ATCC mit Hilfe des immunen Kaninchenserums bewiesen.

RESUMEN

1. Se describen la morfología y las cualidades bioquimicas y de la proliferación de cinco cepas, a saber de dos cepas humanas (Cor. pyogenes var. hominis y Cor. haemolyticum), de dos cepas de Cor. pyogenes bovis y de una cepa de Cor. ovis.

2. Por experimentos sobre una cobaya, un conejo y un ratón se valorizan las cualidades patogenas de estas cepas.

3. Se descubrió la concordancia des dos cepas humanas estudiadas y se trazan las diferencias de las cepas animales especialmente de la cepa de Cor. ovis.

4. Se descubrió un antígeno tónico en los filtrados de los cultivos de caldo de las cepas K 29 y ATCC 9345 poseente una capacidad expresiva dermonecrotica para el conejo y la cobaya.

5. Por su efecto biologico el antígeno toxico de las cepas humanas es semejante a los del toxino de Cor. ovis.

Con objeto de descubrir una orientación práctica se demostró la neutralización de las substancias toxicas de las cepas K 29 y ATCC 9345 con ayuda del suero inmune de conejo.

REFERENCES

1. Bergey's Manual of Determinative Bacteriology, 7th edition, 1957. — 2. Carne H. R.: J. Path. Bact. 49, 313—328, 1939. — 3. Carne H. R.: J. Path. Bact. 51, 199—212, 1940. — 4. Hermann G. J.: The American Journal of Medical Technology 61—66, 1961. — 5. Lowell

R.: J. Path. Bact. 56, 525, 1944. — 6. Mc Lean P. D., Liebow A. A., Rosenberg A. A.: J. Inf. Dis. 79, 69—90, 1946. — 7. Mikrobiologické vyšetřovací metody, Praha 1957. — 8. Patočka F.: Čas. lék. čes. 94, 1323, 1955. — 9. Patočka F.: Antropozoonosy 1—12, 1956. — 10. Patočka F., Schindler J.: Čs. Epidemiol., Mikrobiol., Imunol. 5, 268, 1956. — 11. Patočka F., Souček A., Mára M., Záhorová L., Jedličková A.: WHO symposium on Laboratory and Exp. Studies of Streptococcal Infections, Praha 160. — 12. Patočka F., Souček A., Mára M.: Journal of Hygiene, Epidemiology, Microbiology and Immunology 4, 307—308, 1960. — 13. Patočka F., Souček A., Mára M., Jedličková A., Záhorová L.: Čs. Epidemiol., Mikrobiol., Imunol. 10, 184—191, 1961. — 14. Schön E., Záhorová L., Souček A.: Čs. Epidemiol., Imunol. 9, 69—77, 1960. — 15. Verge J., Richen R., Quinchon Cl.: Comp. Rend. 151, 1726—1727, 1957.

Received November 16, 1961.

F. Patočka, Ústav pro lékařskou mikrobiologii, Praha 2, Studničkova 7, Czechoslovakia.