## The Effect of Penicillin on *Pasteurella pseudotuberculosis* Morphology

The present investigation arose from the finding of long filamentous cells of *Pasteurella pseudotuberculosis* in the microscopic preparations of small colonies grown at the border of the inhibitory zone on the blood agar medium around the disc soaked with penicillin used in the antibiogram test.

It is well known that Proteus and many other Gram-negative bacilli produce filaments, bulbous cells and spheroplasts when they are exposed to penicillin[1]. As far as we are informed, a similar action of penicillin on the microscopic morphology of *Pasteurella pseudotuberculosis* has not yet been described. We therefore systematically investigated the effect of penicillin and other antibiotics on the morphology of *Pasteurella pseudotuberculosis*.

*Materials and Methods.* Strains: Twelve strains of *Pasteurella pseudotuberculosis* were used for this investigation. Two strains of type 1 were isolated from the mesenteric lymphnodes taken during operation of cases of suspected acute or subacute appendicitis but found to be cases of lymphadenitis mesenterialis reticulocytaria abscedens caused by *Pasteurella pseudotuberculosis*. Five strains, type 1 to 5, were obtained from Prof. D. W. KNAPP (Tübingen) and five, type 1 to 5, from D. H. MOLLARET (Paris).

Media: Nutrient agar and 5% blood agar, both with and without peptone, were used.

Method: Whatman No. 2 paper discs, diameter 0.5 cm, were soaked with the solutions of antibiotics, the concentrations of which were as follows: penicillin 50 and 500 U/ml, terramycin, streptomycin, neomycin and ilotycin 0.5 mg/ml, chloramphenicol 0.7 mg/ml, and rovamycin 1 mg/ml. The discs were placed onto the media immediately after the inoculation, and the plates to be incubated at 37°C put into the incubator 15 min later.

Incubation was at 37°C and at room temperature (about 22°C) for 24 h.

The plates were inspected under a low power objective (magnification about 100×) after 24 h of incubation at either temperature. Smears were prepared from the growth taken from the border of the inhibitory zone around each disc soaked with antibiotics. They were fixed in the flame and stained by Gram's method.

The 24 h colonies taken from the border of the inhibitory zone were subcultured onto nutrient agar without antibiotics and incubated for 24 h at 37°C and at room temperature, respectively.

*Results.* When the field around the border of the inhibitory zone of penicillin 500 U/ml was inspected under a magnification of about 100× the picture shown in Figure 1 was seen. Along the borderline between the bactericidal and the bacteriostatic zone single elongated and slightly enlarged cells, some of them curled up, were seen. Deeper within the bacteriostatic zone bundles of elongated and enlarged cells were observed. In some cases these formed more or less regular stars. Still deeper within the bacteriostatic zone irregular colonies were found (Fig. 2). At a still greater distance from the borderline the colonies were larger and also more regular. No typical L-type colony picture was detected, not even with many repeated experiments. The picture, however, was similar to that of *Bacterium anitratum* exposed to sulphathiazol.

[1] K. MCQUILLEN, Fourth International Congress of Biochemistry, vol. XIII, Colloquia (Pergamon Press, London).

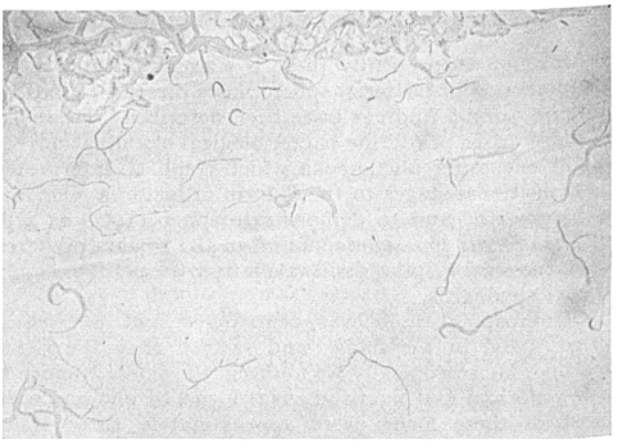

Fig. 1. Elongated and curved cells of *Pasteurella pseudotuberculosis*, single (nearer to the disc soaked with penicillin) and in bundles. Unstained. Magnification ca. 100 ×. (Nutrient agar used in the antibiogram test.)

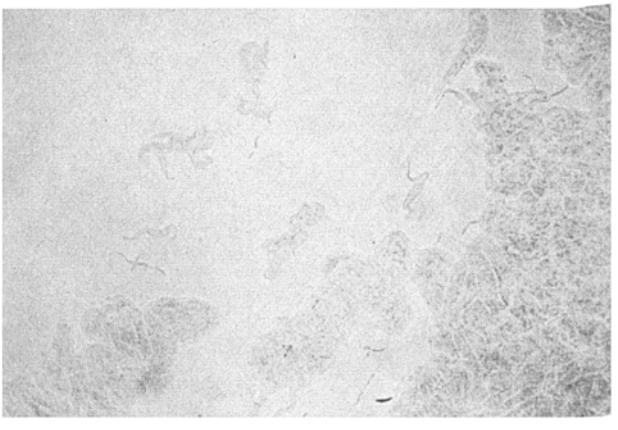

Fig. 2. Bundles of elongated cells of *Pasteurella pseudotuberculosis* and the irregular colonies of the same organisms. Unstained. Magnification ca. 100 ×. (Nutrient agar used in the antibiogram test.)

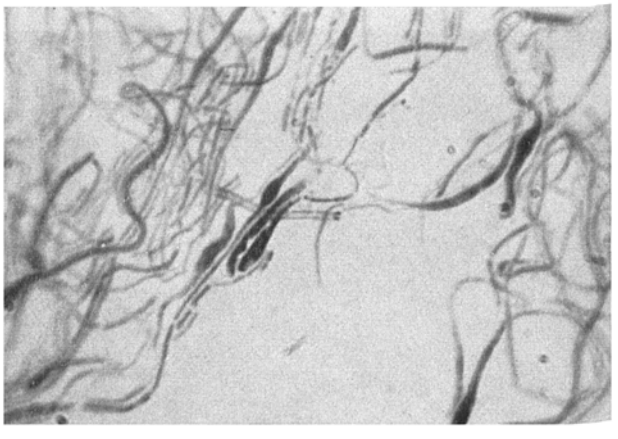

Fig. 3. Elongated, fusiform or irregularly enlarged cells of *Pasteurella pseudotuberculosis*. Stained by Gram's method. Magnification ca. 900 ×.

In the smears prepared from the growth taken around the disc containing penicillin (500 U/ml), elongated and irregularly enlarged (some spindle-shaped) cells were seen (Figure 3). Stained by Gram's method, the enlargements showed the tendency to retain methylviolet. Some of the filaments were enlarged at the end in the form of a pear. Some of the enlargements were constricted in the middle as though beginning to divide. Among the filaments some rare round cells were observed which might be considered to represent spheroplasts of *Pasteurella pseudotuberculosis*. Exceptionally some triangular or ramificated shapes were present. The changes around the disc of penicillin 50 U/ml were of a minor grade.

Transplanted to nutrient agar without antibiotics the microorganisms reversed to their normal morphological picture within 24 h of growth at 37°C or at room temperature.

The pattern of antibiotic sensitivity of the investigated strains of *Pasteurella pseudotuberculosis* was as follows: All strains were sensitive to penicillin 500 U/ml, to streptomycin, tetramycin, chloramphenicol and to neomycin, resistant to penicillin 50 U/ml, rovamycin and to ilotycin.

Neither the smears prepared from the colonies nor the plates inspected under a low power objective showed many abnormalities around the discs soaked with other antibiotics. Some medium-sized filaments were encountered in the colonies of *Pasteurella pseudotuberculosis* exposed to the action of streptomycin, chloramphenicol or terramycin. No changes were found around the discs soaked with rovamycin and ilotycin.

*Discussion.* The shapes of *Pasteurella pseudotuberculosis* exposed to the action of penicillin are mainly similar to those produced by the same agent on other Gram-negative bacteria. However, slight differences are encountered in the form of the enlargements and in other details. These, and also the consistent reaction of the studied microorganisms to the action of penicillin, might be used as a help in cases of difficult bacteriological diagnosis of *Pasteurella pseudotuberculosis*[2].

*Zusammenfassung.* Die morphologischen Veränderungen bei *Pasteurella pseudotuberculosis* unter dem Einfluss von Penicillin werden beschrieben.

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## Periodicity of $S^{35}$ Uptake in Rat Femurs[1]

Studies using the colchicine method to achieve metaphase arrest[2] and tritiated thymidine as a marker for DNA synthesis[3] have shown that events related to cell division in epiphyseal cartilage are subject to diurnal variations. This paper describes a preliminary experiment designed to detect daily changes in the functional activity of cartilage cells by the use of $S^{35}$ sulfate as an index of chondroitin sulfate synthesis. In view of definitive evidence[4,5] that chondrocytes are the loci in which chondroitin sulfate is synthesized and subsequently elaborated from the cells as a constituent of the ground substance of cartilage matrix, the possibility existed that differences in the amount of injected $S^{35}$ concentrated in the tissue might occur over a 24 h period.

*Method.* Albino rats (Sprague Dawley strain), 45–50 g, were singly housed and maintained for two weeks under a photoperiod of 12 h (09:00–21:00) alternating with 12 h of darkness (21:00–09:00) and at a temperature of 73–75°F. Food and water were supplied *ad libitum*. The animals were weighed 24 h prior to experimentation and groups and subgroups were established according to their weight gain performance during the two week conditioning period. Nineteen rats (175–200 g) were injected with 30 μc $S^{35}$-sulfate/100 g body weight in 0.1 ml distilled water *via* tail vein between 09:00–11:00 (day group); a second series (night group) was injected between 21:00–22:30. Subgroups of 4–5 rats were sacrificed by decapitation at 2–3, 4, 8 and 12 h after receiving the tracer dose. The left femora obtained at autopsy were fixed in 95% alcohol[6], embedded undecalcified in methyl methacrylate, and sectioned at 100 μ on a high speed rotary saw. The sections were exposed for three weeks in a freezer on Kodak Type A Autoradiographic Plates. The darkening of the developed images on the film was measured at 5 sites on the distal epiphyseal and articular cartilages, and epiphyseal marrow space by scanning these areas parallel to the long axis of the femur with a microscope-densitometer (30 μ aperture)-Brown Recorder combination. Transverse scans across the metaphysis and mid-shaft cortex and marrow were also performed. The values were corrected for background.

*Results.* The data (Figure) reveal that differences in the pattern of $S^{35}$ incorporation occur only in the distal growth cartilage of the femur. The uptake of the tracer is very uniform over the first four hours after injection in the day-night groups, but the cartilages of the *day* rats clearly retain (ca. 20%) more $S^{35}$ after 8–12 h. The retention of $S^{35}$ in articular cartilages is very constant. The curves of the metaphysis, diaphyseal shaft and marrow are nearly identical, and the intensities of the autoradiographic images remain relatively constant after an initial small decrease from 2–3 h.

*Discussion.* Exchange phenomena between tissue chondroitin sulphuric acid and blood within the first 4 h after $S^{35}$ administration probably account for the early apparent absence of day-night differences in the tracer con-

[1] This work was performed under the auspices of the Atomic Energy Commission.

[2] D. J. Simmons, Nature *195*, 82 (1962).
[3] D. J. Simmons, Clinical Orthopaedics No. 26, 176 (1963).
[4] D. D. Dziewiatkowski, J. cell. Biol. *13*, 359 (1962).
[5] R. D. Campo and D. D. Dziewiatkowski, J. biol. Chem. *237*, 2729 (1962).
[6] R. D. Campo and D. D. Dziewiatkowski, J. Biophys. Biochem. Cytol. *9*, 401 (1961).