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-

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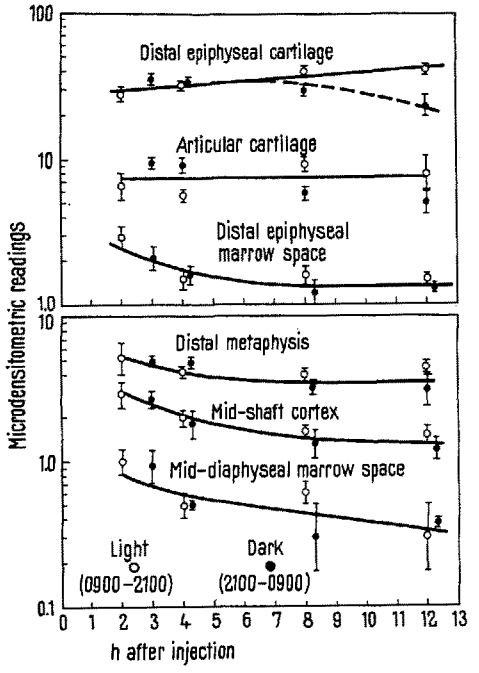
tent of epiphyseal cartilage, shaft and marrow[7]. Since high resolution autoradiography of mineralized and demineralized preparations have shown intense labelling of newly synthesized chondroitin sulfate in bone and in the cells of cartilage for at least 24 h[7,8] despite a rapid depletion of S^{35} in serum dialysates[9], the labelling after 4 h in epiphyseal cartilage at least suggests a true variability in cellular activity. Although, in these undecalcified preparations, changes in the amount of ground substance elaborated by very active metaphyseal bone cells may be masked by the presence of S^{35} associated with the mineral phase[7], it is probable that the uniform labelling of articular cartilage and mid-shaft cortical bone in both time series accurately reflects the relatively quiescent populations of cells in these tissues.



The patterns of S^{35} sulfate uptake in the femurs of rats injected at different times (09:00 and 21:00) within a 24 h period.

Résumé. Du radio-soufre a été administré intraveineusement à des rats à 09.00 h et à d'autres à 21.00 h, et ils ont été sacrifiés 2–3, 4, 8 et 12 h après l'injection. L'analyse microdensitométrique d'autoradiographies des fémurs a indiqué que la concentration et la rétention du traceur dans le cartilage de conjugaison étaient plus grandes dans les rats traités à 09.00 h. Les tissus non-cartilagineux n'ont pas montré de changements pareils.

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Radiological Physics Division, Argonne National Laboratory, Argonne (Illinois, U.S.A.),
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Hämoglobin vom «Alexandra-Typus» im ersten Lebensjahr

Bei Neugeborenen und Säuglingen lassen sich oft kleinste Mengen eines anomalen Hämoglobins (Hb) nachweisen, das die elektrophoretische Wanderungsgeschwindigkeit des Hb Alexandra besitzt. In einer früheren Arbeit wurde mitgeteilt, dass die anomale Fraktion im ersten Lebensjahr in 49 von 109 untersuchten Fällen und im 2. Lebensjahr bei 1 von 17 untersuchten Kindern gefunden wurde[1]. Die grösste Häufigkeit war im 2. und 3. Lebensmonat zu beobachten, im Zeitpunkt, da Hb F rasch zurückgeht und Hb A_2 in Erscheinung tritt. Die anomale Hb-Fraktion war in allen Fällen in der Elektrophorese erst nach Benzidinfärbung sichtbar. Die jetzige Mitteilung berichtet über die Fortsetzung der Untersuchungsserie und über erste spektrophotometrische Messungen der anomalen Fraktion.

Material und Methodik. Von 163 Neugeborenen und Säuglingen des ersten Lebensjahres wurden Blutproben untersucht, welche aus verschiedenen Kinderkliniken zur Bestimmung der Hämoglobine und der Glucose-6-phosphatdehydrogenase eingesandt wurden. Es handelte sich um Citratblut, vorwiegend von Fällen mit Icterus oder Anämien diverser Genese unter Ausschluss aller Hämoglobinopathien. Die Herstellung des Hämolysates aus gewaschenen Erythrocyten, die Technik der Stärkeblock-Elektrophorese und die Hb F-Bestimmung mittels Alkalidenaturierung sind andernorts ausführlich beschrieben[2]. Für die Elektrophorese wurden Proben zu 0,05 ml einer ca. 15 g-prozentigen Hb-Lösung aufgetragen. Zur spektrophotometrischen Untersuchung wurden 7 anomale Fraktionen mit 3 ml *Aqua. dest.* eluiert. Die Messung des Hb-Spektrums erfolgte mit einem selbstregistrierenden Beckman-DB-Gerät.

Ergebnisse. Hämolysate ohne nachweisbare Hb A_2-Fraktion wiesen nie ein anomales Hb auf (Figur 1). Hingegen enthielten Hämolysate mit sehr geringen Mengen Hb A_2, dessen Fraktion erst nach Benzidinreaktion sichtbar wurde, sehr oft auch kleinste Mengen eines anomalen Hb (Figur 2) mit der Wanderungsgeschwindigkeit von Hb Alexandra[3,4]. Die Häufigkeit dieses anomalen Hb ist

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