nucleus, inducing it to behave malignantly as well as forcing it to replicate new NN molecules.

If this hypothesis should be verified by biochemical methods, human carcinoma would assume the aspect of a virus disease, although the origin of that virus remains, for the time being, unknown.

Zusammenfassung. Veränderungen der Reaktion gegenüber Silber und Acridin-Orange-Fluorescenz im Parenchym, um eine metastatische Geschwulst, gestatten die Annahme eines Virus-ähnlichen Mechanismus bei der Übertragung carcinogener Eigenschaften von Zelle zu Zelle.

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Spores of Microorganisms Penicillin-Induced Destruction of Sporulating Cells of *Bacillus cereus*

Duguid's[1] primary hypothesis, that penicillin specifically interfers with the formation of rigid cell wall component, was verified by Park[2-4], who found accumulation of uridine nucleotides in penicillin-treated Gram-positive bacteria. These substances are bound with hexosamine, acetyl-muramic acid and specific peptides[5,6]. The authors presume that penicillin inhibits the transfer of these hexosamin-peptidic formations from nucleotide to the cell wall. Penicillin also interfers with the cell wall synthesis in Gram-negative bacteria, where incorporation of the compounds like diaminopimelic acid into nucleotide precursors of the cell wall is affected[7,8]. Interference of penicillin with the cell wall synthesis causes the formation of osmotically labile forms partially or completely deprived of the rigid layer of the cell wall, i.e. 'protoplasts'[9,10] or 'spheroplasts'[11]. Penicillin seems to be a relatively specific inhibitor of the synthesis of 'basal structure' of the cell wall in both types of microorganisms[12].

Cell wall synthesis is always linked with the cell division, so that penicillin can inhibit mainly the growing culture. Földes and Merétey[13] have described lysis (or protoplasts formation in hypertonic medium) of the growing cells of penicillinase-producing strain of *Bacillus cereus* by penicillin-treatment, while after the end of growth penicillin has no effect on the cells.

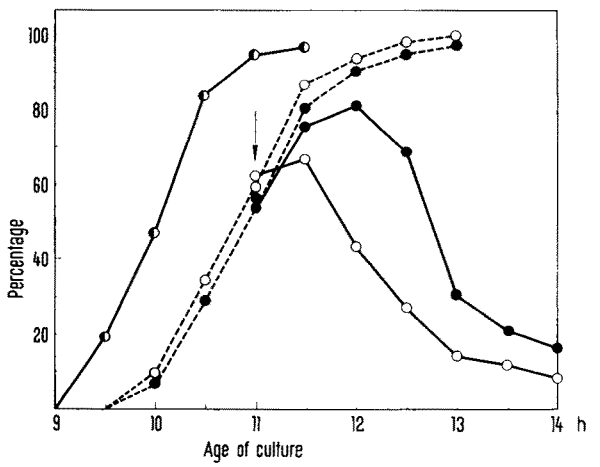

Fig. 1. Release of typical spore constituents after addition of penicillin to sporulating cells of *Bacillus cereus*. Abscisse; age of culture (h); ordinate: percentage. All the values are expressed in percentage, values of control culture at 14 h of cultivation being taken as 100%. (They were for number of spores 96%, for ^{45}calcium content 7300 cpm/mg of dry weight of sporangia and for dipicolinic acid content 30 µg/mg dry weight.) Dashed lines: control culture; full lines: penicillin-treated culture (addition of penicillin designed by arrow); o——o spore formation; ●——● dipicolinic acid content; o---o ^{45}calcium content.

Sporulation of bacilli is a special process, by which a new form of cell-existence spore is formed in the mother cell after the end of growth, equipped with complex protective mechanisms including extremely rigid envelopes. This interesting and curious analogy of the cell division attracts attention with regard to the possible effect of penicillin.

In examining the effect of this antibiotic on sporogenesis, the strain of *Bacillus cereus* (NCIB 8122) was used. It was cultivated in liquid medium containing bactopeptone (0.3%), glucose (0.1%), phosphates and trace elements[14] at 30°C, the culture being aerated by shaking. Three criteria were used for testing the effect of penicillin on sporulating cells:

(1) Synthesis of the component typical for spores of bacilli-dipicolinic acid, tested by the method of Janssen, Lund, and Anderson[15].

(2) Incorporation of ^{45}calcium ($^{45}CaCl_2$ was added to the culture after the end of growth, 0.166 µC/ml, final concentration 2.10^{-4} *M*). Methods of washing of cells by 0,001 *N* hydrochloric acid and determination of radioactivity were the same as in recent work[16].

(3) Morphology of developing spores and their final appearance after release from sporangia.

Penicillin was added in high amounts (1.000 units/ml) to the culture, in which 95% of cells had contained more or less refractive prespores (Figure 1), and during the thermostabilizing process characterized by ^{45}calcium accumulation in cells and dipicolinic acid synthesis (Figure 2) started to pass through. After addition of penicillin, dipicolinic acid continued to synthetise; but,

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during further cultivation, its content in the cells started to decrease very rapidly. Similarly, the content of ^{45}calcium slightly increased after addition of penicillin, though in a lower degree than dipicolinic acid content. While in the control culture the continual increase of dipicolinic acid and ^{45}calcium content occurred, the content of both compounds was highly reduced during further cultivation of penicillin-treated culture. Even during the decrease, there remained more dipicolinic acid than calcium in the cells. The major part of the cells did not show any morphological changes, only about 20% of cells did not increase their refractility, or, on the contrary, became transparent. These affected forms were often more rounded.

High decrease of dipicolinic acid, and especially ^{45}calcium content, shows that the whole culture is affected, even the sporangia without outstanding morphological changes. High affection of sporogenesis becomes mostly evident in later autolytic phase, when spores are released from sporangia. Besides a small number of relatively normal, fully refractile spores, a large number of empty envelope structures of spore-size without cytoplasmic content (Figure 3) appear in the culture. In the experiment mentioned, about 80% of these forms were present after autolysis of sporangia.

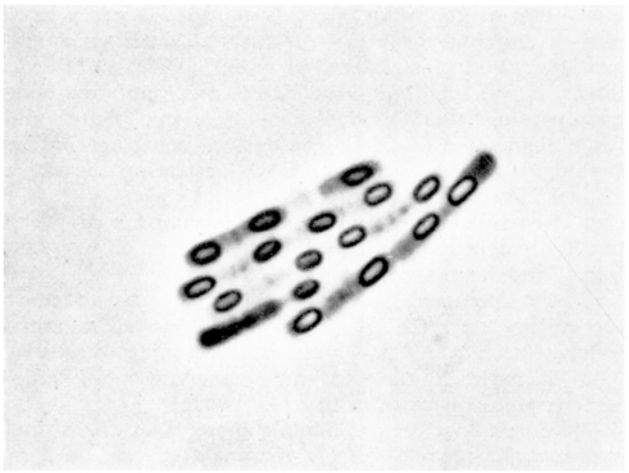

Fig. 2. Sporulating culture at the moment of addition of penicillin (phase contrast, magnification 1:3000, photographed by J. KUBEC).

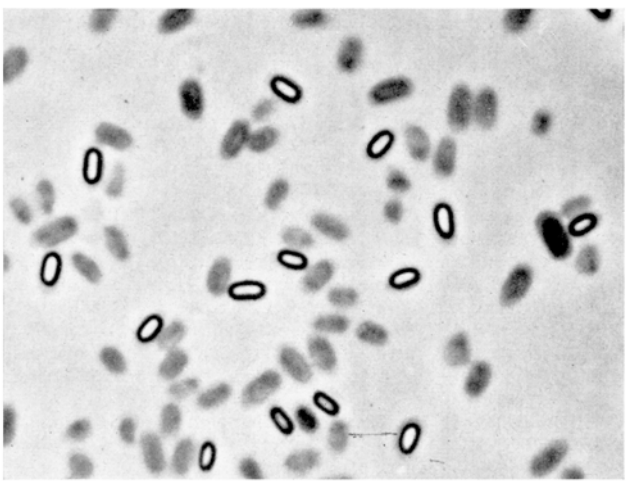

Fig. 3. Penicillin-treated culture after autolysis of sporangia (24th h of cultivation). Photographed by J. KUBEC.

It is obvious that, in the case of sporogenesis, penicillin showed its influence on the formation of rigid spore envelope. Spores of this strain of *Bacillus cereus* are equipped with three surface layers. The inner layer seems to be the thickest[17]. Although there is not yet any exact evidence where calcium and dipicolinic acid is located in the spore, spodographic studies show that most of mineral components are located in peripheral layer of spore[18,19], i.e. close to the inner envelope or in cortical layer. Affection of synthesis of a rigid layer of spore envelopes can thus lead to general desorganisation of spore structure, characterized by release of typical spore components, and in the phase of spore release to affection, presumably osmotic, of deficient rigid layer. Varying release of dipicolinic acid and ^{45}calcium during penicillin inhibition gives evidence of their different tenacity in intraspore structure revealed by means of washing procedures.

As regards the proper synthesis of spore envelopes during sporogenesis, the data from the literature are very incomplete. STRANGE and POWEL[20] have found, in studying the disintegrated cells of bacilli, that peptides containing diaminopimelic acid and hexosamine pass into the soluble fraction in the course of sporogenesis. Similar peptides were found after disintegration of resting spores of *genus Bacillus* and in exsudate during spore germination. Nondialysable peptides containing diaminopimelic acid and hexosamines are present in spore envelopes[21]. Participation of preexisting hexosamine-peptides as precursors in the synthesis of spore envelopes is, however, dubious because they are released into the medium during sporogenesis[22]. It is probable that penicillin effects here in the main the mechanisms of synthesis of rigid layer of spores, either by preventing the utilization of precursors or by influencing the composition of these precursors.

Zusammenfassung. Der Zusatz von einer hohen Dosis Penicillin (1.000 E/ml) zu sporulierender Kultur von *Bacillus cereus* hemmt den ^{45}Calcium-Einbau und die Synthese der Dipicolinsäure. In der Folge wird der Gehalt dieser Komponenten erniedrigt und das Cytoplasma der Sporen ins Medium gelöst. In der Kultur findet man nach der Lösung der mit Penicillin behandelten Sporangia meistens die Mantelstrukturen. Auswaschung mit verdünnter Chlorwasserstoffsäure zeigt eine deutlich schnellere Auswaschung des Calciums als die der Dipicolinsäure aus den mit Penicillin behandelten Sporangia.

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