Spores of Microorganisms. Chloramphenicol-Sensitive and Penicillin-Resistant Incorporation of ^{14}C-Diaminopimelic Acid into Sporulating Cells of *Bacillus cereus*

Sporogenesis of bacilli is a remarkable form of the last cell division, at which one of the arising cells forms a number of effective protective mechanisms including the formation of very rigid envelopes, and the second one disappears during further development.

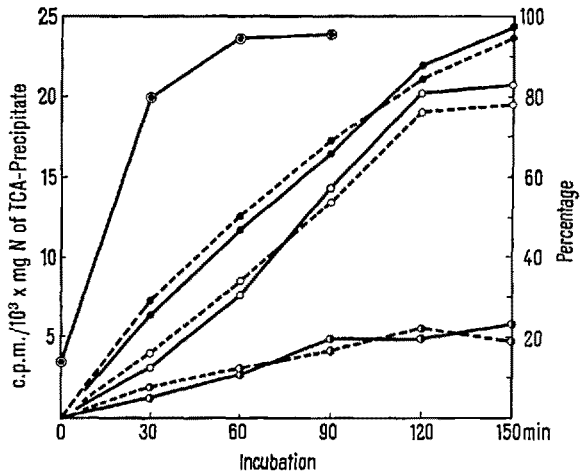
The surviving cell, the spore, is typical also in its new components, e.g. dipicolinic acid (DPA) and calcium. The synthesis of its proteins and polymerized peptidic structures during the sporogenesis is accompanied e.g. by considerably higher incorporation of ^{35}S-cysteine[1,2], ^{14}C-diaminopimelic acid and ^{14}C-lysine[3]. Diaminopimelic acid (DAP) is a typical component of the spore coats[4] and, according to recent findings, of the spore cortex as well[5–7]. Two periods of increased incorporation of ^{14}C-DAP into the hot-TCA-precipitate of cells can be distinguished during sporogenesis: the first approximately in the period when the prespore septum was formed and the second one after the formation of slightly refractile spores, i.e. during so-called 'whitening'[3]. While in the first period also ^{14}C-lysine resulting from ^{14}C-DAP decarboxylation is highly incorporated, in the second one during spore maturation practically only ^{14}C-DAP is being incorporated.

α,ε-diaminopimelic acid is a typical 'cell wall' amino acid. The incorporation of amino acids into the cell walls is inhibited by penicillin and not by chloramphenicol[8–10]. The penicillin inhibition of DAP-incorporation into the cell wall was found in a number of microorganisms[11,12].

When examining the effect of chloramphenicol (100 μg/ml) and of high doses of penicillin (1000 U/ml) on the sporogenesis, we have found that the incorporation of ^{14}C-DAP into hot-TCA-precipitate is not inhibited by penicillin during this process. Chloramphenicol, however, induces a considerable decrease of incorporation.

The strain NCIB 8122 of *Bacillus cereus* was grown in liquid bactopeptone medium[13] on a shaker at 30°C. Before the antibiotics and ^{14}C-DAP were added, the culture was divided into several series of flasks. Penicillin and chloramphenicol were added to the single series of flasks together with 2-^{14}C-α,α′-diaminopimelic acid (0.017 μc/ml, total DAP concentration 3.4 μg/ml) after about 15% of prespores had been formed. Therefore the culture was in contact with ^{14}C-DAP mostly at the time when no incorporation of ^{14}C-lysine resulting from DAP decarboxylation occurred. Nevertheless, the excess of ^{12}C-lysine ($1 \cdot 10^{-3} M$), which usually inhibited the incorporation of ^{14}C-lysine formed from ^{14}C-DAP during the prespore formation, was added to the parallel cultures[3]. In 30 min intervals the radioactivity was examined of cell hot-TCA-precipitate, washed and treated with fat solvents[14], by means of methane 2π flow counter. Radioactivity is expressed in counts/min per mg nitrogen of hot-TCA-precipitate. At the same time, the percentage of cells containing slightly refractile prespores was investigated. We have found (Figure) that chloramphenicol markedly inhibits the ^{14}C-DAP incorporation, while penicillin, especially in the first period of incubation, distinctly stimulates the incorporation of ^{14}C-DAP as compared to the control without antibiotics. Presence of excess of ^{12}C-lysine in no case influenced the rate of ^{14}C-DAP incorporation. Chromatography of hydrolyzed TCA-precipitate and its autoradiographic control showed that in all cases ^{14}C-radioactivity was localized in diaminopimelic acid only.

As for other effects of chloramphenicol on sporogenesis, this antibiotic considerably slows down the dipicolinic acid synthesis and calcium accumulation in sporangia. The arising spores are sometimes more round.



The incorporation of ^{14}C-diaminopimelic acid into proteins of sporulating cells of *Bacillus cereus*.
○—○ control culture
●—● penicillin-treated culture
◗—◗ chloramphenicol-treated culture
⊙—⊙ percentage of spores or spore-like forms
full lines: only ^{14}C-DAP added
dotted lines: ^{14}C-DAP plus excess of ^{12}C-lysine added.

It is not yet known why penicillin increases the ^{14}C-DAP incorporation at the beginning of incubation. In this phase penicillin promotes, within a short time, the reversion of prespores to transparent forms and to release of typical spore components, calcium and dipicolinic acid from the sporangia[15]. In later phases of penicillin inhibition, the marked release of ^{14}C-DAP from the cells together with accelerated autolysis of sporangia also takes place (VINTER, unpublished results). Our further experiments show that, while penicillin disturbs the cells during the whole period of DPA synthesis and calcium accumulation, the inhibitory effect of chloramphenicol decreases in this stage, being very effective at the beginning of prespore formation. Furthermore, we have found that chloramphenicol very strongly inhibits incorporation of ^{14}C-lysine, ^{35}S-cysteine, and ^{14}C-glutamic acid into hot-TCA-precipitate of the sporulating cells. The incorporation of these amino acids is very significant during sporogenesis.

The results of our experiment with chloramphenicol show that the mechanism of formation of DAP-containing

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structure, most probably of cortex, during sporogenesis is not identical with usual mechanisms of the cell wall synthesis during normal division.

The formation of some new enzymes during sporogenesis has already been described; and for the time being, it is not yet evident whether chloramphenicol specifically inhibits the formation of DAP-containing structure or the synthesis of some enzymes newly arising during sporulation and responsible or co-responsible for the synthesis of structures containing diaminopimelic acid.

Zusammenfassung. Während der Bildung der Praesporen wird der Einbau der ^{14}C-Diaminopimelinsäure durch Chloramphenicol (100 µg/ml) beträchtlich gehemmt und durch Penicillin (1000 E/ml) wenig erhöht.

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Mesencephalic Reticular Responses to Natural and to Repeated Sensory Stimuli

The purpose of this investigation was to evaluate the responsiveness of neurones in the Mesencephalic Reticular Formation to natural stimuli, and to study the effect of repetition of such stimuli.

Experiments were performed in cats either anesthetized with chloralose or immobilized with gallamine triethiodide. Spike activity of single neurones was recorded extracellularly with KCl-filled micropipettes placed stereotaxically. Natural stimuli of different modalities were tested: somatic, as light tapping of skin, brushing of hairs and clamping of toes; acoustic, as voices, clicks or claps; visual, as illumination with a weak flashlight or object movement in front of the eyes. More artificial stimuli as electrical shocks to subcutaneous tissues of pads, flashes and tones were used also. Stimuli were tested as frequencies between 0.05 and 10 c.p.s.

Findings can be summarized as follows: (i) A high percentage of reticular neurones responded well to natural stimuli (Figure 1). The set of natural excitations that influenced each unit varied greatly from cell to cell: variations involved the effective modalities (somatic only, acoustic only, somatic and visual, etc.); the most effective type within each modality (skin tapping, hair brushing, etc.) and, at least for the somatic sphere, the receptive field for each type (fields could be localized, widespread, or discontinuous).

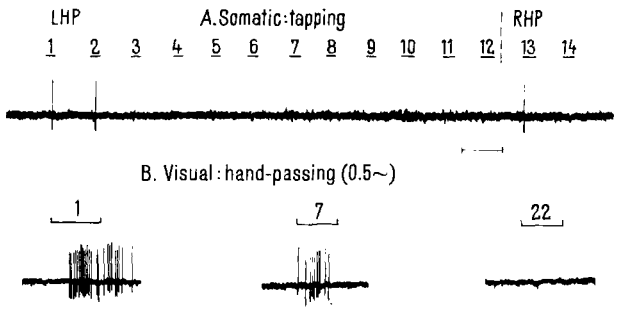

Fig. 1. *Convergence of natural stimuli and specific attenuation.* Unit activity in Mesencephalic Reticular Formation; lines above tracing indicate stimuli; numbers above bars indicate position of stimulus within the sequence. A, Taps. The unit responded to the initial (1, 2) but not to the subsequent (3–12) taps to the left hindpaw (LHP) (attenuation); tap 13 to the right hindpaw (RHP) was effective (specificity). B, hand passing in front of eyes. Initial effectiveness and subsequent attenuation.

(ii) Reticular units failed to 'follow' even low frequencies (i.e. failed to respond to every stimulus of a train) (Figure 2B): Figure 2A indicates that the maximal following rates for somatic excitations fell within the 0.2 to 10 c.p.s. These rates could not be forced outside the stated range, and in some cases could not even be modified by large increases in stimulus intensity. When a cell responded to different stimuli, as a tap to the skin and a click (or to different localization of the same stimulus, as a tap to the forepaw and to the hindpaw), the maximal following rates for each excitation (or for each placement) could be dissimilar but, in every case, remained within the same low frequency range (Figure 2B).

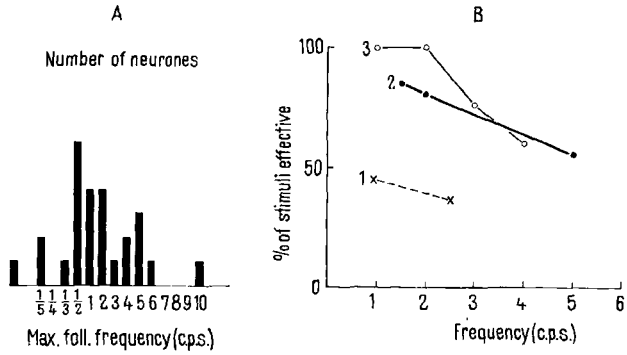

Fig. 2. A, *maximum following rates.* Histogram (bar on far left indicates one neurone). B, *following capacity in a single mesencephalic reticular unit.* On abscissae, stimulus frequency in c.p.s.; on ordinate, percentage of stimuli that were effective within a train of a given frequency for claps (curve 1), light taps to nose (curve 2) and shocks to pad of left hindpaw (curve 3).

(iii) The response of a high proportion of cells showed attenuation on repetitive application of a stimulus (natural or artificial): the term 'attenuation' implies that the effect of the first few shocks was clear but that of subsequent shocks was reduced and eventually perhaps absent (Figure 1). The response reappeared after a period without stimulation. The remaining cells did not attenuate, but equilibrated; in this case the proportion of stimuli evoking a response remained constant throughout a prolonged train. The time course of attenuation could be influenced by the modality, type, intensity and localization of the stimulus, but in most of the units even marked changes in these parameters did not convert attenuation into equilibration.

Attenuation was usually specific: after a cell had become unresponsive to a repeated stimulus, it would be unresponsive also to similar stimuli (generalization) but retain its reactivity to sufficiently different excitation (Figure 1). The degree of dissimilarity necessary to evoke a response varied: in some cells a shift of a few centimeters of the point of stimulation sufficed; in most, generalization was more marked and the response could be