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On the Synthesis of Protein and Polynucleotides in Spores of *Penicillium brevicompactum*

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Spores of *Penicillium brevicompactum* have been cultivated in submerged cultures, until, after 10 hours, the very first morphological signs showed a beginning development of mycelia.

In this system the net synthesis of ribonucleic acid (RNA) started during the first 30 minutes of cultivation and preceded the net synthesis of protein.

No measurable net synthesis of desoxyribonucleic acid (DNA) was found before 8 to 10 hours of cultivation. The amounts of DNA present in the spores is very low compared to animal and bacterial cells.

The net syntheses were determined by direct measurements of amounts and were investigated by tracer methods.

EXPERIMENTAL

Microbiological methods. Spores of *P. brevicompactum* were spread on Czapek-Dox maltose agar plates and grown two weeks at 25°C. After this period the plates were placed at room temperature for another three weeks. The spores were then removed by a soft sterilized brush. The pooled material was freed from contaminating mycelia by filtering a suspension in glass-distilled water at 0°C through Pyrex glass wool. No mycelia were found in the filtrate, as checked by means of phase contrast microscopy. In order to reduce the culture volume and to ascertain a rapid growth of the spores, the cells were kept submerged by continuous shaking and aeration. The medium used was the salt-glucose medium devised by Czapek-Dox. The glucose concentration, however, was only one tenth (0.4 %) of the one commonly used in their medium. The glucose was labeled in all carbon atoms with ^{14}C. The specific activity was 190 c.p.m. per μg of glucose carbon.

The culture volume was 10 litres. Inoculation was made with approximately 5 g of spores calculated as dry weight. The culture was aerated at a rate of 1.5 litres per min and litre of medium. The air was dispersed by a sintered glass filter.

In order to check if the spores would develop into mycelia in this medium, a 1 litre culture had previously been inoculated with about 5 mg of spores. After 3 days 700 mg of dry mycelium were harvested. The first sign of morphological changes were found between 10 and 12 h after inoculation in the form of a small bud on some cells. The spores in the main experiment were controlled in this way too, and the buds after 10 h were found to be extremely rare.

Chemical methods. The cells were disintegrated in a Griffith shaker with glass beads (0.28 mm in diameter) in 30 % ethanol (v/v) at 0°C. After 24 h no spores remained, and the disintegration seemed to be complete as checked by phase contrast microscopy. The poly-

nucleotides were isolated from samples of about 500 to 800 mg of dry spores according to Hammarsten [1]. RNA was degraded to mononucleotides by alkaline hydrolysis. The hydrolysates of RNA were chromatographed on Dowex 2 resin according to Cohn [2] and Hurlbert *et al.*[3] with 4 N formic acid-0.2 M ammoniumformate for elution. DNA was degraded enzymatically according to the method of Hurst *et al.*[4] as modified by Cohn [5]. Elution from Dowex 2 resin was accomplished according to Hurlbert and Potter [3] with 1 M ammonium formate-formic acid (pH = 4.8). Guanine was isolated after acid hydrolysis of guanylic acid (Dowex 50). Adenine from RNA and the pyrimidine nucleotides from RNA and DNA were only so far purified that it could be shown that their labeling did not significantly deviate from that of guanine. Adenine from DNA could not be isolated on account of the minute amounts of DNA in our material. The labeling of purines and polynucleotides followed the pattern found for *Escherichia coli B* in the lag-phase [6].

The activity of guanine was determined by measuring the activity at "infinite" thinness on lead planchets in a windowless gas flow counter. The amount of guanine was estimated from spectrophotometric data.

The protein fraction was isolated by the trichloracetic acid method of Schneider after mechanical disintegration of the cells in the way mentioned. The precipitate was boiled in 6 N hydrochloric acid for 18 h. The hydrolysate was filtered through Pyrex glass wool and the hydrochloric acid removed *in vacuo*. The nitrogen to carbon ratio was determined in four samples and found to be 0.26 ± 0.03.

All samples with a low labeling were counted to an accuracy of 10 % and the others to 5 %.

RESULTS AND DISCUSSION

It is shown in Table 1 that the RNA-phosphorus increased rapidly during the first half hour after inoculation. Since there was no corresponding high activity in the RNA-guanine, it is possible that the net synthesis to a large part derived from unlabeled procursors within the cells and only to a smaller part from the medium. Another possibility is the formation of phosphopentose backbone before incorporation of guanine. In recent work on *E. coli* in the lagphase [6] the cells had been labeled with ^{13}C before the actual culti-

Table 1.

Cultivation time, h	mg dry spores per litre of culture	mg phosphorus in RNA per litre of culture	mg phosphorus in DNA per litre of culture	mg nitrogen in protein per litre of culture	Counts per min per μg carbon in:		
					RNA guanine	DNA guanine	Protein
0	598	1.78	0.010	28.1	—	—	—
1/2	596	2.47	0.010	33.4	0.7	0.4	0
1	592	2.56	0.013	33.5	1.6	0.4	0
2	622	2.74	0.008	37.2	5.7	0.4	0.9
4	636	2.96	0.006	37.7	8.2	1.2	3.6
6	693	3.44	0.007	33.3	18.0	1.0	13.1
8	801	4.43	0.016	46.2	43.2	0.5	21.5
10	944	5.41	0.015	61.0	68.5	6.8	38.1

vation experiment. It was found in that work that the syntheses of polynucleotides and also of proteins used material from cell precursors in the earliest stages of growth in the lagphase.

During the period that followed, the increase per hour in RNA-phosphorus in the spores was between 7 and 12 %. Owing to the very small amounts of DNA present in the spores no very exact estimates could be made of the DNA-phosphorus. In contrast to the rapid incorporation of ^{14}C into RNA, the DNA did not show any appreciable incorporation.

The proteins did not incorporate ^{14}C during the first hour of growth. Compared to the RNA the specific activity of the protein carbon remains at low values during the first 4 to 5 h. Thereafter the incorporation of ^{14}C had still a lower gradient than the incorporation into RNA. In view of the incorporation and protein synthesis from labeled precursors within the cells found in *E. coli* [6] it is quite probable that some protein in the spores has been synthesized from precursors within the cells during the period when proteins remain unlabeled. If the protein had derived all its carbon from medium carbon exclusively, the specific activity of the protein carbon at 10 h after inoculation would have been approximately half that of the carbon in the medium glucose. It is, however, only 20 % of the specific activity of the medium glucose. This probably means that a large part of the protein carbon was derived from cellular carbon sources present at the moment of inoculation (or partly from CO_2). The figures for protein net synthesis are irregular owing to technical difficulties arising from the capsular substances.

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