PHOSPHORYLATION OF NUCLEOSIDES DURING GERMINATION OF BACTERIAL SPORES*

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As early as 1949, Hills (Hills 1950) had shown that certain compounds such as L-alanine, adenosine and tyrosine stimulate the germination of spores of a few species of the genus Bacillus. Later it was found that inosine also causes the spores to germinate at a very rapid rate. Although various theories have been proposed to explain the process of germination, concise data concerning the fate of the germinants is not available. In this communication we present evidence for the phosphorylation of inosine and the exchange of P^{32} into nucleotides during germination.

Two year old spore suspensions of B. cereus strain T were used in these experiments. The spores were suspended in a (0.05M) Tris-HCl buffer pH 7.4 and heat activated by placing in a bath of 65°C for 30 minutes. They were incubated with the respective germinants in this buffer in the presence of inorganic phosphates as well as labelled P^{32} as phosphoric acid. The reaction was stopped by the addition of cold 10% trichloracetic acid. The mixture was allowed to stand for some time and then centrifuged. The precipitate was discarded and the supernatant was analyzed for P^{32}-labelled nucleotides by adsorption on charcoal and subsequent hydrolysis with dil. hydrochloric acid (Crane and Lipmann 1953).

The details of incubation and the amount of exchanged P^{32} are given in Table 1.

In a second experiment, the spores were allowed to germinate in the presence of inosine for a period of 15 minutes and the exchanged nucleotides

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Table 1

The incubation mixture consisted of a total vol. of 5 ml. containing 0.1 ml. of spore suspension Tris-HCl buffer pH 7.4 (0.05M) potassium phosphate (10^{-3}M) P^{32} 10^4 cpm/ml. of incubation mix and the germinants in appropriate concentration.

No.	Germinant	Optical Density in Klett units at 0'	Optical Density in Klett units at 30'	Exchanged P^{32}-labelled nucleotides in 30 min. cpm/ml of incubation mix.
1	---	380	380	-----
2	alanine (0.8mg/ml)	375	380	-----
3	adenosine (20r/ml)	375	175	6.6×10^3
4	alanine (0.8mg/ml) + adenosine (20r/ml)	375	185	7.6×10^3
5	inosine	375	198	5.2×10^3

were trapped as before by adsorption on charcoal. The nucleotides were then eluted with 50% aqueous ethanol containing 3% concentrated ammonia. The eluate was evaporated to dryness under reduced pressure and the residue was taken in a small amount of water and separated on a Dowex-1 chloride column by a slight modification of the procedure described by W. E. Cohn (W. E. Cohn 1951.) Figure 1 gives the elution profile as well as the distribution of radioactivity in the fractions. The several fractions which showed a high u.v. absorption and radioactivity were concentrated and subjected to paper chromatography with a solvent isobutyric acid:acetic acid:water 100:1:50 (Turba 1951). The Rf's of the radioactive fractions were identical with those of inosinic acid (Rf0.36) and adenosine triphosphate (Rf 0.22). (Figure 2)

Discussion: The exchange of P^{32} with ATP and the formation of inosinic acid can be explained by the following over-all mechanisms.

$ATP \rightleftharpoons ADP + \sim P$ (1) $\qquad$ Inosine $+ \sim P \longrightarrow IMP$ (2)

Reaction (1) does not occur in the absence of the germinants as shown by the lact of exchange of P^{32} (Table 1).

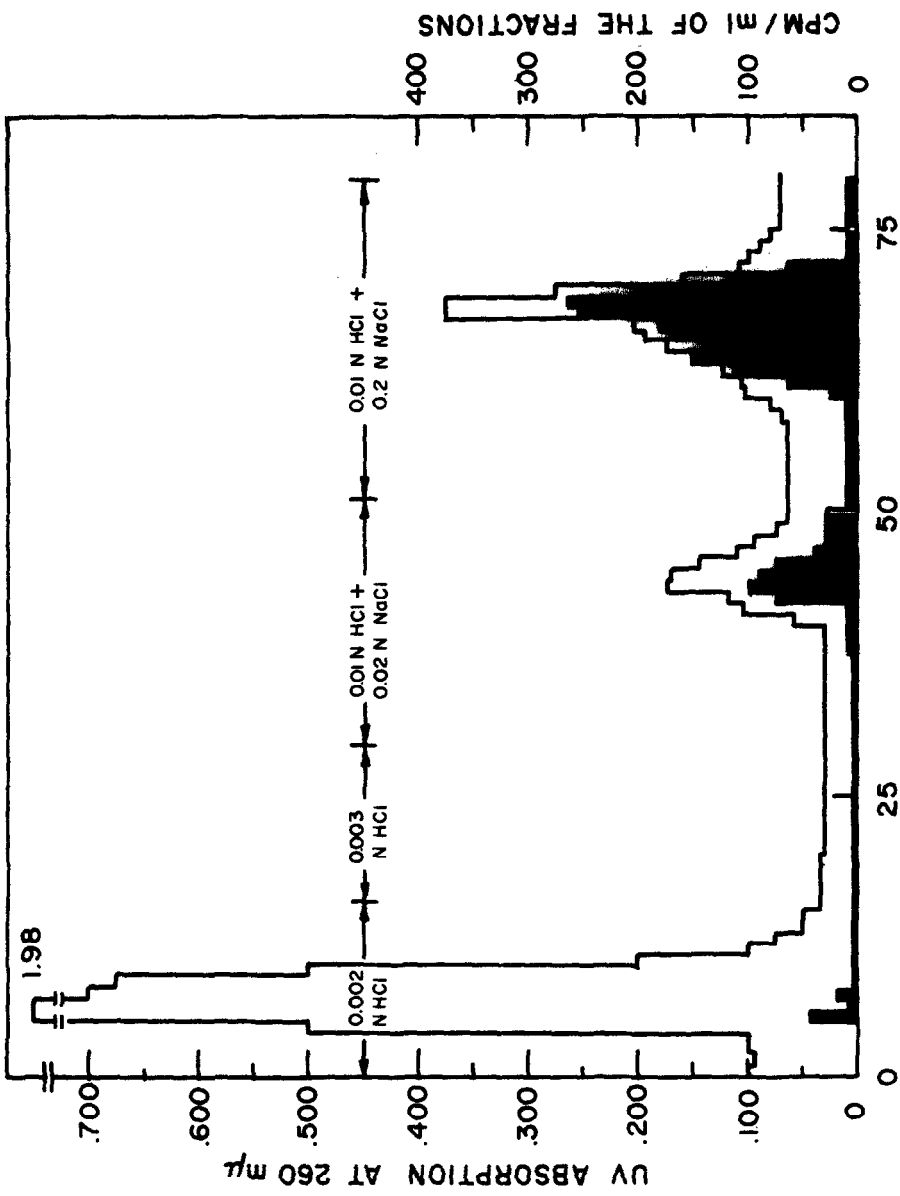

Figure 1

u.v. adsorption at 260 mμ.
Dowex-1-Cl, column 6 x 1.5cm.2
Eluents: 0.002N HCl 1-17 fractions: 0.003N HCl 18-36 fractions: 0.01N HCl and 0.02N NaCl 37-63 fractions: 0.01N HCl and 0.2N NaCl 64-80 fractions.
Flow rate: 1ml./min. each fraction 5 ml.

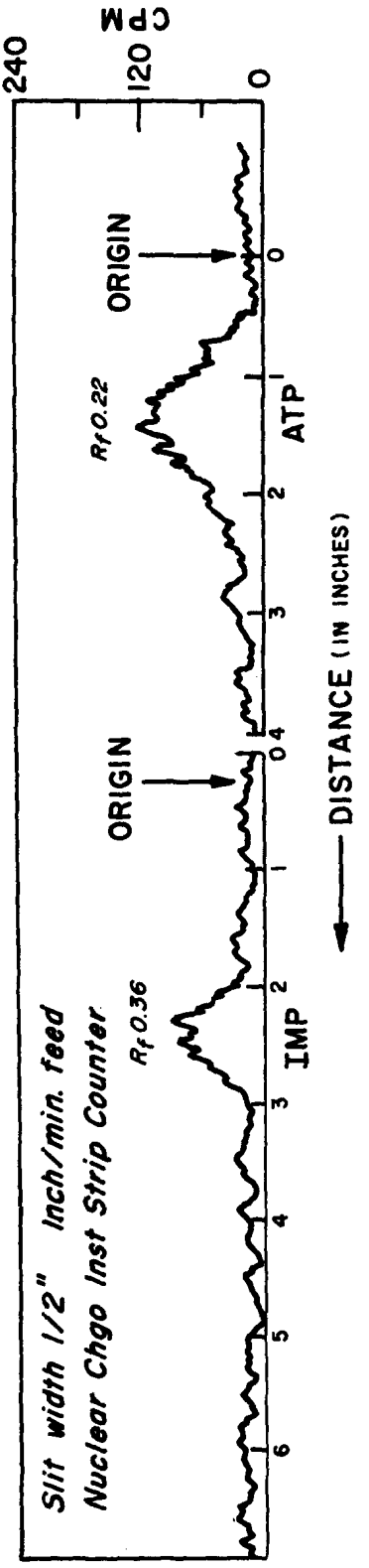

Figure 2
Recording of the radioactivity on paper chromatograms.

The fact that the exchange of P^{32} occurs not only in the presence of inosine but also with adenosine suggests that the reaction (1) may be common to both the methods of germination.

It is significant that the results show a direct phosphorylation of a nucleoside by an organism but its importance with regard to germination can only be revealed on further investigations.

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References

W. E. Cohn. J. Cellul. Comp. Physiol. Suppl. 1. 38, 21 (1951).

R. K. Crane and F. Lipmann. J. Biol. Chem. 201, 236 (1953).

G. M. Hills. J. Gen. Microbiol. 4, 38 (1950).

F. Turba and M. Turba. Naturwissenschaften 38, 188 (1951).