The Inhibitory Effect of Diaminopurine Riboside on the Growth of *Ophiostoma*

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The possible function of certain purines and purine ribosides as intermediates in the biosynthesis of nucleic acids has been a matter of discussion for several years. One way of gaining some insight into this problem is to study the response of purine-requiring mutants of microorganisms to various postulated intermediates. 2,6-Diaminopurine, free or in a ribosidic linkage, has been suggested as a possible precursor of nucleic-acid purines, and was therefore tested in experiments with various purine-less mutants as well as with wild type strains of the fungus *Ophiostoma multiannulatum*.

As was earlier shown [1], free diaminopurine is as capable as guanine in supporting the growth of the guanine-less mutants, and in contrast to what has been found in certain other organisms [2,3] it does not possess any inhibitory influence upon other *Ophiostoma* mutants or wild type strains.

Diaminopurine riboside, on the other hand, could not be utilized by any of the hypoxanthine-, adenine-, or guanine-less mutants, but proved to be a strong inhibitor of growth. In wild type strains a concentration of 30×10^{-6} *M* completely prevented growth, whereas 3×10^{-6} *M* still decreased the rate of growth considerably (Table 1). Diaminopurine riboside probably acts as an antimetabolite to adenosine, since this substance appeared more efficient than the other purine derivatives tested in counteracting the inhibition. A similar effect has been noted in recent studies on mammalian neoplastic tissues, where the toxicity of diaminopurine riboside can be blocked by adenosine (and adenine) [5,6].

As a general conclusion from the experiments with *Ophiostoma* it may be said that the results do not support the assumption of diaminopurine riboside being a normal intermediate in nucleic acid biosynthesis in this organism.

Table 1. Effect of 2,6-diaminopurine riboside (DAPR) on the growth of a wild type strain, No. 51, of Ophiostoma with and without a further addition of other purine derivatives. The fungus was cultivated in shake tubes (triplicates) as a conidial culture, and the growth was measured photometrically [4]. The figures in the table indicate the growth attained after two days in per cent of the control without any purines added.

DAPR 10^{-6} *M*	Further addition, each 50 μmoles/lit.					
	None	Diaminopurine	Adenine	Guanine	Adenosine	Guanosine
0	100	96	100	100	96	100
3	87	87	87	85	89	87
5	75	37	58	51	82	79
10	20	9.7	12	8.1	66	37
30	5.4	5.0	5.8	4.3	25	6.5
50	4.6	4.6	4.6	3.9	18	4.3

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The Content of Polyglucose of Glycogenic Nature * in *Escherichia coli B* during Growth in Media Deficient in Nitrogen and Carbon

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During an investigation of *Escherichia coli B* cultivated by the continuous technique at different growth rates, it was

* For the sake of brevity called "glycogen".

Table 1.

Exp.	Time after inoculation in min	Number of cells/ml $\times 10^8$	"Glycogen" % of dry weight	Mg "glycogen" per liter of culture	"Glycogen"/ /cell mg $\times 10^{-12}$
A	0	0.75	2.1	0.3	3.8
	30	0.80	8.8	1.7	20.8
L.F.: NH_4Cl	90	1.5	8.4	3.3	32.8
C.S.: lactate	140	2.7	6.1	4.8	20.6
	180	4.5	9.3	10.4	37.6
	220	4.6	12.2	13.6	50.6
NH_4Cl	260	4.9	15.3	18.2	63.1
⟶ added	270	5.3	13.8	17.2	58.5
	325	8.5	7.5	14.2	33.4
	475	—	0.4	3.5	—
B	0	1.0	2.1	0.3	2.5
L.F.: lactate	90	0.74	7.8	1.5	20.3
C.S.: lactate	360	7.0	4.1	5.4	7.7
	420	10.0	2.7	5.7	5.7
	480	15.0	2.8	7.0	4.7
	600	16.0	4.6	12.0	7.5
lactate	660	16.0	5.7	15.5	9.7
⟶ added	720	16.0	6.4	17.2	10.7
	780	22.0	6.9	26.8	12.2
	840	26.0	5.4	25.2	9.7
C§ L.F.: NH_4Cl	0	1.2	6.6	1.3	10.4
C.S.: glucose	60	1.2	13.4	3.2	26.0
NH_4Cl	150	1.7	11.0	5.6	33.0
⟶ added	300	2.8	21.0	21.2	75.7
	375	5.9	11.9	24.6	41.6
D	0	1.1	1.9	0.3	2.5
L.F.: NH_4Cl	140	2.1	5.4	3.7	17.7
C.S.: lactate	300	6.3	18.2	17.5	27.8
No addition of L.F.	540	5.7 §§	21.4	25.2	44.9

L.F.: limiting factor. C.S.: carbon source.
§ After 200 min. the number of cells remained constant at 2.8×10^8 per ml.
§§ Difficult to count in the Buerker chamber because of the scanty contrast.

found that the nature of the limiting factor had a profound influence on the "glycogen" content of the cells. In order to obtain additional information, it was regarded as essential that also cells obtained in batchgrowth were investigated, where the growth was limited by the nitrogen source (NH_4Cl) or by the carbon source (sodium lactate).

Experimental. In two experiments (A and B) the culture volume was 30 litres. In the other two (C and D) it was 5 litres. The cultivation and harvesting procedures are previously described [1]. The main substrate was Friedleins synthetic medium containing Na-lactate as the carbon source and NH_4Cl as the nitrogen source [2]. In one experiment (C) glucose was the carbon source [3]. In the media deficient in nitrogen (A, C, and D), the NH_4Cl was 0.75×10^{-3} *M*. The carbon deficient medium (B) was 1.67×10^{-2} *M* with regard to lactic acid. After the initial lag and multiplication periods, the cells stopped to divide because of the deficiency of the medium. After an additional time of 1.5 hours in the nitrogen deficient cultures and of 4 hours in the carbon deficient one, an amount of the limiting factor was added to make the cultures (A, C, and B) 0.02 *M* and 0.1 *M*, respectively.

Table 2.

Exp.	Time after inoculation in minutes	Cold TCA-N mg per liter of culture	+ hot TCA-N per cell mg × 10^{-12}	Cold TCA-N / Hot TCA-N ratio
B	360	5.8	8.3	1.5
L.F.: Na-lactate	480	9.9	6.6	1.6
	540	11.3	6.6	1.6
	600	10.3	6.4	1.4
	660	10.0	6.3	1.3
	720	9.6	6.1	1.5
	721	sodium lactate added		
	780	13.1	5.9	0.7
	840	15.5	6.0	0.4

The "glycogen" was isolated and the glucose obtained from the "glycogen" as described in a paper to follow [4]. The trichloroacetic acid (TCA) extraction in experiment B was performed according to Schneider [5].

Results. The results of the "glycogen" determinations are presented in Table 1. A very rapid increase of "glycogen" per mg of dry cells, and per cell was found during the first 60–90 minutes (the lag phase). Then the "glycogen" decreased until the cell division rate began to decrease, indicating the beginning of the starvation phase. From this moment on a rapid increase in "glycogen" content was found in the nitrogen deficient cultures. In the carbon deficient culture there was also an increase during the same phase, but its rate was slower. After the addition of the limiting factors the cells began to multiply again. During this second multiplication phase the "glycogen" content immediately decreased in the nitrogen deficient cultures. In the carbon deficient one there was a short lag phase during which the "glycogen" content of the cells increased slightly, followed by a decrease.

In order to obtain a rough estimate of the relationship between the low and high molecular nucleic acid fractions, the cells were extracted with TCA according to Schneider [5]. The cold TCA fraction contains mainly low molecular and the hot TCA fraction high molecular nucleic acid derivatives.

The ratio between the nitrogen in the cold and the hot TCA extracts was higher than 1.3 during the whole carbon deficient period in experiment B, where Na-lactate was the limiting factor. After the addition of lactate the ratio rapidly decreased (Table 2).

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On the Correspondence between Thermocells and Isothermal Cells

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A theoretical relationship between the thermopotential difference of two pure thermocells and the corresponding isothermal cell has been derived recently by Holtan [1,2].

Consider for instance the thermocell

$$\underset{T}{Pt(H_2);\ HCl_{aq};}\ \underset{T+\Delta T}{(H_2)Pt} \qquad (1)$$

For the differential thermopotential difference of this cell we write $(\Delta\varphi/\Delta T)_1$.

Consider then the thermocell

$$\underset{T}{Ag;\ AgCl,\ HCl_{aq},}\ \underset{T+\Delta T}{AgCl;\ Ag} \qquad (2)$$

For the differential potential difference of this cell we write $(\Delta\varphi/\Delta T)_2$.

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