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Short Communications

Synthesis of Polynucleotides in Slices from Regenerating Liver

PETER REICHARD and SUNE BERGSTRÖM

Biochemical Department, Karolinska Institutet, Stockholm, Sweden, and Department of Physiological Chemistry, University of Lund, Lund, Sweden

During the course of an investigation on the extent to which orotic acid is a natural intermediate for the synthesis of pyrimidines it became desirable to inquire into the possibilities of using an in vitro system capable of synthesizing the bases of polynucleotides. Greenberg [1] has shown that a homogenate prepared from pigeon liver can synthesize hypoxanthine from $C^{14}O_2$ or $HC^{14}OOH$, and Schulman *et al.*[2] have shown that the same system can synthesize hypoxanthine from C^{14}-labeled-4-amino-5-imidazole-carboxamide. Hypoxanthine however, is not a natural constituent of polynucleotides, nor can it be used as a precursor for polynucleotide purines by the rat [3]. After the start of the present investigation Weed et al [4] reported the occurence of C^{14} in the pyrimidine-mononucleotides from ribonucleic acid (PNA) after incubation of liver slices with C^{14}-orotic acid. These authors, however, did not investigate the distribution of the isotope between the pyrimidines and ribose.

The present communication reports studies on the synthesis in slices of regenerating liver of PNA and DNA (desoxyribonucleic acid) purines and pyrimidines with N^{15}-glycine [5] or N^{15}-orotic acid [6] as precursors. In each experiment 8 rats were subjected to partial hepatectomy. The animals were killed 24 hours after the operation; slices were prepared from their livers (ca 10 g) and suspended in 100 ml of solution. Two different media were used for this incubation of the slices: one medium which was synthesizing ATP [7], and a Krebs-Henseleit medium [8] where $CaCl_2$ had been omitted. Different amounts of either N^{15}-glycine or N^{15}-orotic acid were added to the medium in each experiment. The incubations were carried out for varying periods between 2 and 8 hours. At the end of each incubation the suspension was cooled in ice water, 10 ml of 30 % trichloro acetic acid were added and the slices were filtered off. After homogenization in a Waring Blendor with 100 ml ice cold 3 % trichloroacetic acid the slices were filtered again and dried with alcohol and ether. PNA and DNA were extracted and separated from the dry material [9]; purines and pyrimidines were prepared from the polynucleotides[10] and analyzed for N^{15}. The results are summarized in Table 1.

The differences in values for the two media are not thought to be significant. The N^{15} incorporation into the purines in the experiments with orotic acid is low enough to be probably explained by degradation of orotic acid. Specific contribution of glycine N^{15} to nucleic acid pyrimidines might also be questioned. The values, however, do indicate a considerable synthesis of both PNA and DNA pyrimidines from orotic acid and a synthesis of polynucleotide purines from glycine. In considering the relatively low N^{15} incorporation into the purines one should keep

Table 1. Incubation of slices from regenerating liver with N^{15}-glycine (excess N^{15} = 32 atom per cent) and N^{15}-orotic acid (excess N^{15} = 16.2 atom per cent). AO = ATP medium + 5 mg orotic acid; KO1 = Krebs-Henseleit medium + 5 mg orotic acid; KO2 = Krebs-Henseleit medium + 25 mg orotic acid; KG = Krebs-Henseleit medium + 50 mg glycine.

	Time of incubation	Excess N^{15}							
		PNA				DNA			
		Adenine	Guanine	Cytidine	Uridine	Adenine	Guanine	Thymine	Cytosine
AO	4 h			0.038	0.098			0.035	0.021
AO	8 h	0.008	0.006	0.102	0.281	0.007	0.003	0.096	0.073
KO1	4 h	0.017	0.009	0.057	0.140				
KO1	8 h	0.026	0.019	0.156	0.390	0.005	0.002	0.099	0.068
KO2	4 h	0.000	0.001	0.093	0.209				
KO2	8 h	0.015	0.011	0.227	0.504	0.006	0.009	0.160	0.139
KG	2 h	0.041	0.013						
KG	4 h	0.066	0.030	0.007	0.011				
KG	8 h	0.105	0.067	0.022	0.031	0.029	0.025	0.016	0.014

in mind that probably most of the isotope is located in only one nitrogen atom [5] out of five.

Corresponding experiments with homogenates from regenerating liver in both types of media did not show a significant incorporation of N^{15} into the bases; apparently this system does not synthesize polynucleotides from these precursors.

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On Carbon Balance and Carbon Dioxide Fixation in Thermophilic Cellulose Fermentation

L. ENEBO

Division of Microbiology, Royal Institute of Technology, Stockholm, Sweden

In experiments with six different pure cultures of thermophilic bacteria on the carbon balance in fermentation of cellulose McBee [1] was able to obtain a satisfactory recovery of the carbon from the fermented cellulose in only one case. Similarly Imsenecki [2] in his experiments with a possibly pure culture * obtained a recovery of only c. 75 % of the carbon added. It should be noticed, however, that both these authors neglected succinic acid which appeared only as a trace among the products.

It seems probable that small changes in the general condition of the fermentation

* In spite of the doubtful isolation method it seems with regard to more recent results of McBee [1, 3] and the author [4, 5] that the culture of Imsenecki really was pure.