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Purine and Pyrimidine Turnover of Ribo Nucleic Acid from Fractionated Rat Liver Cytoplasm

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In a recent publication Jeener [1] investigated the turnover of ribonucleic acid (PNA) in different parts of animal cells with the aid of isotopic phosphorus. For this purpose he applied the principles of the techniques of differential centrifugation as described by Hogeboom, Schneider and Pallade [2] for the separation of mitochondria, microsomes and "cell sap" from different organs. His results indicated that in all the organs investigated, PNA-phosphorus from various locations in one and the same cell contained different amounts of isotope, the results from the different fractions sometimes varying by as much as a factor of 40.

These results seemed to indicate the presence of at least metabolically different PNA's in one and the same cell. One might even suspect that the PNA in various parts of the cell might differ chemically. Such differences might be expected to show up on analysis for purine and pyrimidine bases.

The present investigation was carried out in order to investigate further these problems. Two different approaches seemed possible for this purpose. The first and more direct would have been to isolate mitochondria, microsomes and "cell sap" from some organ according to Hogeboom and co-workers' technique, isolate the PNA's from each fraction and analyze for purines and pyrimidines with the aid of existing methods [3]. This approach was not adopted, as it seemed that it would be difficult to avoid degradation of PNA during the preparation of the cell fractions. The second possibility was to investigate the turnover of the purines and pyrimidines with the aid of the stable isotope N^{15}. This approach was chosen and the isotope was administered in the form of glycine-N^{15} in order to permit correlation with previous experiments [4,5] which have been carried out in this laboratory with this substances. The most suitable organ for the present investigation seemed to be rat liver

because of its high content of PNA and the relative ease with which the different cytoplasmic fractions might be prepared. In order to get a higher and more significant labelling of the purines and pyrimidines without using too much of the isotope the synthesis of PNA was speeded up by partial hepatectomizing the rats before the administration of the isotope. After the rats had been killed the livers were rapidly removed and the cytoplasmatic cell fractions isolated according to the principles outlined by Hogeboom *et al*[2]. After preparation of PNA from the different fractions purines and pyrimidine nucleosides were isolated from each sample of PNA and analyzed for N^{15} in the mass spectrometer. The results are summarized in Table 1.

Table 1. Purines and pyrimidine nucleosides from PNA from rat liver cell fractions. The value $\frac{E_{max}}{\gamma N/ml}$ *is a test for purity with respect to foreign nitrogen. For standard values see Reichard*[7, 8].

	Excess N^{15}	$\frac{E_{max}}{\gamma N/ml}$
Adenine		
Mitochondria	0.224	0.177
Microsomes	0.224	0.183
"Cell sap"	0.234	0.177
Guanine		
Mitochondria	0.486	0.162
Microsomes	0.504	0.172
"Cell sap"	0.490	0.165
Cytidine		
Mitochondria	0.153	0.277
Microsomes	0.161	0.282
"Cell sap"	0.161	0.281
Uridine		
Mitochondria	lost	
Microsomes	0.176	0.332
"Cell sap"	0.185	0.312

As can be seen from the table the isotope contents of one and the same base from mitochondria, microsomes and cell sap are not significantly different from each other and thus under the present conditions do not indicate that a fractionation of cytoplasmatic PNA has occured.

EXPERIMENTAL

Administration of isotope. The isotope was administered as glycine-N^{15} (atom per cent excess = 32). 10 rats weighing about 200 g each were subjected to partial hepatectomy as previously described [4]. 16 hours after the operation each rat received a dose of 50 mg of glycine / 100 g of body weight by subcutanous injection. The same amount of isotope was given to each rat 2, 4, 6 and 8 hours after the first injection. Two hours after the last injection the rats were killed, the livers rapidly removed and after cutting into small pieces immersed in 200 ml ice cold 0.88 *M* solution of sucrose.

Isolation of cell fractions. The procedure was the same as that of Hogeboom *et al.*[2] only somewhat simplified. All operations described below were carried out in the cold. The pieces of liver in sucrose were homogenized in a Swedish mixer of the Waring blendor type ("hushållsassistent") at half speed until no macroscopic pieces could be distinguished. This usually took about 5—7 minutes. The resulting suspension was filtered through a double layer of muslin. Unbroken cells and cell nuclei were spun off by three more centrifugations at 600 g for 10 minutes in an »Ecco» centrifuge. The supernatant from the last centrifugation was poured off and centrifuged in a Korda angle centrifuge at 16.000 g for 1 hour. The sediment was considered to represent the mitochondria. The microsomes were obtained from the supernatant, which microscopically did not contain any mitochondria, by centrifugation at 60.000 g for 1 hour in an ultra centrifuge. The PNA in the supernatant from this centrifugation was precipitated by making the solution 70 % with respect to alcohol. None of the fractions were washed as the amount of material was rather limited and slight contamination of one fraction with another was not thought to be likely to effect the results seriously. Each fraction was dried with alcohol and ether.

Preparation of purines and pyrimidine ribosides. PNA's samples were prepared from each fraction according to the method of Hammarsten [9]. Purines and pyrimidine ribosides from each sample were then worked up according to methods worked out at this laboratory [6–8].

DISCUSSION

The present results sharply contrast with those obtained by Jeener using P^{32}. Before discussing this difference one must keep in mind that the experimental conditions in the present investigation were not the same as those used by Jeener. In the present work regenerating liver from the rat was the system investigated, whereas Jeener worked on mouse embryo, chicken embryo and pigeon crop gland. On the other hand the differences of turnover rate with P^{32} were obtained from such different organs that one might well expect them to be valid for all those organs from which the different cytoplasmatic fractions (mitochondria, microsomes and cell sap) can be isolated.

Another difference in the experimental conditions is the time chosen for the administration of the isotope. Because of the much smaller dilution which can be allowed for the stable nitrogen isotope as compared to radioactive phosphorus, the isotope administration was carried out over a period of 10 hours the total amount of isotope being divided into five doses. Jeener only

gave a single injection of isotope and killed the animals 2 hours later. These differences seem, however, to be far from enough to explain such fundamentally different results.

Neither are the slight differences in the methods of preparation of the cytoplasmatic fractions of any more considerable importance. As only one centrifugation has been applied it is not to be expected that complete separation of the fractions has been achieved. It seems especially likely that the isotope value of the mitochondrial purines and pyrimidines may have been considerably effected by the inclusion of microsomes in this fraction. This applies both to the present investigation and Jeeners original work. As the results, however, are more of qualitative than of quantitative significance this objection does not affect the main conclusion.

The main difference between Jeeners experiments and the present investigation, was the difference of the isotopic precursor used. The results obtained in this way as far as can be seen at present, seem to indicate that though phosphorus has a different turnover in different PNA fractions from cell cytoplasm, this is not true for the nitrogen of the purines and pyrimidines. This explanation can not of course be fully accepted until an investigation of fractionated PNA turnover from regenerating liver has been carried out with the aid of P^{32}.

The present results naturally do not indicate that there can not exist several PNA's within one and the same cell, each containing nitrogen with various different turnover rates. A significant difference between the turnover rates of the purines from cytoplasmic PNA and nuclear PNA has also been shown for the first time in an investigation from this laboratory on regenerating liver [4, 10]. The present author has also previously found some indirect evidence, that cytoplasmatic purines and pyrimidines may exist in different metabolic states [5]. The correct conclusions from the present experiment may therefore not necessarily be that there only exists one type of PNA in rat liver cytoplasm with respect to purine and pyrimidine turnover, but that the method of differential centrifugation may be too coarse for it to be possible to obtain a fractionation of metabolically different PNA's.

SUMMARY

After administration of glycine- N^{15}, PNA samples from mitochondria, microsomes and cell sap from regenerating rat liver were isolated. The isotope content from the corresponding purines and pyrimidines did not show any significant differences. The results were thus not in agreement with those obtained by Jeener [1] with P^{32}.

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