OCCURRENCE OF LABILE PHOSPHATE IN RAT LIVER NUCLEI

by

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Various functions have so far been ascribed to cell nuclei by genetical and cell physiological studies. Requirement of energy-rich phosphate bonds for cell nuclei is evidenced by the following facts: (1) the turnover of pentosenucleic acid (PNA) in the cell nucleus is more rapid than in the cytoplasm[1,2,3], and that of nuclear phosphoprotein is also quite extensive[4]; (2) an enzyme catalyzing the synthesis of diphosphopyridine nucleotide (DPN) from adenosine triphosphate (ATP) and nicotinamide mononucleotide is only found in nuclei and the formed DPN is probably transferred to the cytoplasm[5]; (3) the activity of the glycolytic enzyme system has been demonstrated in nuclei[6,7]. However, the oxidative enzyme system has not been found within nuclei[8,9]. This leads to the expectation that nuclei must contain ATP, even if the synthesis of this compound does not take place therein.

In 1950, NAORA AND SIBATANI[10] proposed a histochemical method to demonstrate the acid-soluble phosphates, including ATP, which were found in the nucleus as well as in the cytoplasm of rat liver, intestine and striated muscle. However, there remains much uncertainty about the validity of the technique employed.

Therefore a biochemical analysis has been made on the distribution of labile phosphate among nuclear and cytoplasmic fractions of rat liver separated with non-aqueous media. Present investigation has now confirmed the earlier observations that ATP occurs not only in the cytoplasm but also in the nucleus.

MATERIAL AND METHODS

Fresh rat livers (body weight 200–220 gram) were sectioned into small pieces and placed in a dry ice-ether mixture (−70° C) for five minutes. Then, the frozen tissue was dried in a freeze-drying apparatus[11] for ten hours at −20° C.

The principle of the isolation of nuclei from the dried tissue was essentially the same as that proposed by BEHRENS[12] AND ALLFREY *et al.*[13]. The medium used for the isolation of nuclei was carbon tetrachloride and petroleum ether.

The homogenate with petroleum ether was centrifuged at 3000 r.p.m. for 15 minutes. A three-layered sediment was obtained. The upper layer, characterized by its white color and fine granules, was carefully picked with a spatula. The same procedure was repeated on this fraction and, the cytoplasmic fraction was obtained. The nuclear fraction was purified from the middle layer of the sediment.

Although ALLFREY *et al.*[13] reported that nuclei of the normal rat liver were difficult to isolate without pretreatment of the animals, we could obtain a small amount of almost pure nuclei with a specific gravity of 1.265 to 1.273. But it seems that these nuclei represent a certain special type rather than the average in the nuclear population of rat liver.

When the cytoplasmic fraction was suspended at sp. gr. 1.102 and centrifuged, a turbid super-

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natant was obtained. The sediment was resuspended at sp. gr. 1.101, and the same procedure was repeated several times with increasing sp. gr. up to 1.153. These supernatants contained mitochondria of various sizes depending upon the specific gravity employed, so that these several supernatants were combined and used as the mitochondrial fraction.

The specific gravity of the mixtures to be used for the flotation or the sedimentation of nuclei can not be determined rigidly. It may be suggested that the composition of nuclei depends upon the nutritional conditions of animals, so that the specific gravity of the nuclei may be different from one animal to another.

The purity of the isolated nuclei was tested by staining with methyl green-pyronin or hematoxylin-eosin.

Acid-soluble phosphates were extracted from the whole homogenate and the different fractions with 7% trichloracetic acid for one hour at 0–5° C. Then the centrifuged extracts were neutralized with 7 *N* KOH. Inorganic phosphate and labile phosphate (*A*7-P) were determined by ALLEN's method[14]. SCHNEIDER's procedure[15] was employed for the determination of DNA and PNA (desoxyribose and ribose nucleic acid).

RESULTS

The fractionated morphological units can be seen in Figs. 1–3. As shown in Fig. 2, the isolated nuclei were almost free from cytoplasmic contamination. However, a few small nuclei, which may be of non-parenchymal origin, were found in the cytoplasmic fraction. It has been assumed that the numerous formed bodies found in the cytoplasmic fraction (Fig. 1) are cytoplasmic debris originating from parenchymal cells. The mito-

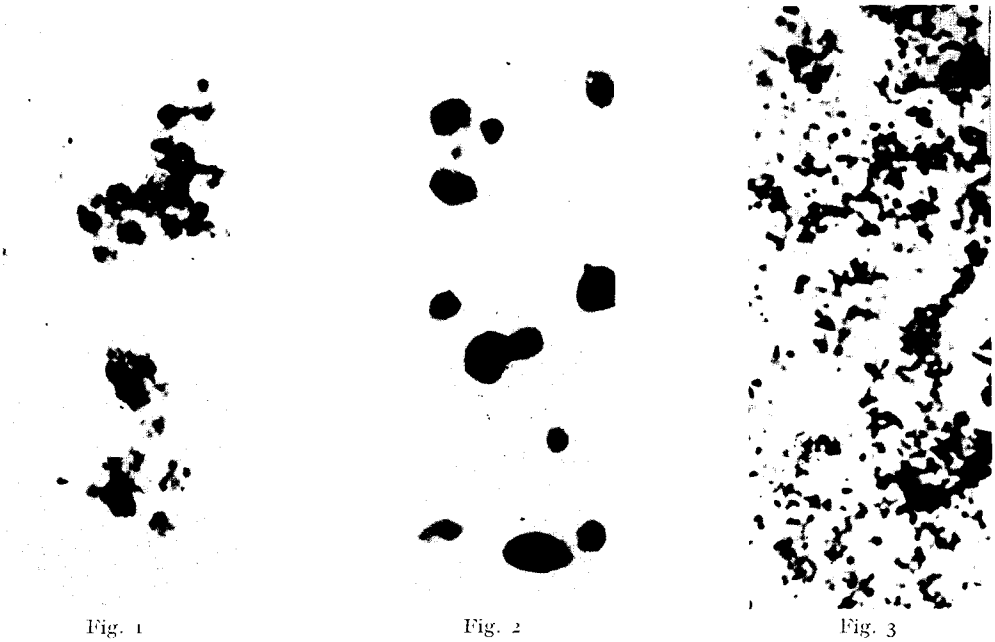

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Crude cytoplasmic preparation, showing cytoplasmic debris and a few small nuclei stained with methyl green-pyronin and photographed with Fuji E filter (monochromatic green filter, 5300 Å max.). × 1000.

Fig. 2. Intact and fragmented nuclei stained with methyl green-pyronin and photographed with Schott RG-1 filter. × 1000.

Fig. 3. Individual and aggregated mitochondria separated in non-aqueous media; stained by ALTMANN-KULL's procedure and photographed with Fuji E filter. × 2400.

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chondrial fraction is characterized by small and fine granules. The size of these granules could not be measured, because it was below the limit of the resolving power of the microscope objective. However, the size of these granules was neither too small nor too large to be mitochondria. Moreover, these granules stained by the Altmann-Kull's procedure. The purity of this fraction was much higher than for the others. Fig. 3 shows these granules stained by Altmann-Kull's procedure.

TABLE I

LABILE P AND PNA–P OF THE VARIOUS FRACTIONS OF RAT LIVER SEPARATED WITH NON-AQUEOUS MEDIA, EXPRESSED IN RELATIVE VALUES TO DNA–P OR PNA–P AS A REFERENCE STANDARD

	Homogenate	*Nuclear fraction*	*Cytoplasmic fraction*	*Mitochondrial fraction*
$\Delta 7$–P/DNA–P	0.885	0.396		
$\Delta 7$–P/PNA–P	0.130	1.19	0.009	0.284
PNA–P/DNA–P	6.79	0.333		

The results of the chemical analysis of the various fractions are summarized in Table I. Labile phosphate (or $\Delta 7$-P), which is assumed here to represent ATP, is found in all fractions. As shown elsewhere[16,17], the DNA content of the average liver nuclei is constant at a given growth stage of the rat. It is thus assumed that $\Delta 7$-P/DNA-P in the homogenate and in the nuclear fraction can be taken as the relative measure of the labile phosphate content per cell and per nucleus respectively. The labile phosphate/DNA-P of the nuclear fraction is 0.396, while that of the whole homogenate is 0.885. It should be noted that the nucleus contains 45% ATP per cell, if we assume that the isolated nuclei represent the modal population of nuclei in the total homogenate with respect to the chemical composition. The mitochondrial fraction contains a high quantity of ATP, although the ATP content of the total mitochondria of a single cell could not be determined.

The ratio of PNA-P/DNA-P of the homogenate, or the relative amount of PNA per cell, is 6.79, while PNA-P/DNA-P of the isolated nuclei, or the relative amount of PNA per nucleus, is 0.333. The cytoplasmic fraction contained a small amount of DNA. This does not mean that the cytoplasm contains DNA, since the cytoplasmic fraction was not free from nuclear contamination. The ratio of ATP to PNA in the cytoplasmic fraction is much smaller than in the mitochondrial fraction. Even if mitochondria are poorer in PNA than the remainder of the cytoplasm, the difference in these two ratios is too great to be caused by the difference in PNA content only. It may well be postulated that the major part of cytoplasmic ATP is confined to mitochondria.

DISCUSSION

The expression "labile phosphate content per cell" used in this paper has only a statistical meaning, for the liver tissue has various polyploid nuclei and non-parenchymal cells, such as Kupffer cells, etc. However, the isolated nuclei belong to a specialized type of liver cell nuclei, so that the term "labile phosphate content per nucleus" used here is less statistical than "labile phosphate content per cell". Thus, although it may not be pertinent to compare the labile phosphate content per cell with

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that per nucleus directly, nearly a half of the labile phosphate content of the cell is distributed in the nucleus if we *roughly* estimate the intracellular distribution of labile phosphate.

The significance of the presence of ATP within the cell nucleus might be better understood if one recalls the following facts.

Nuclei do not contain the enzymes which are needed for respiration and some other catabolic reactions[8]. However, CASPERSSON[18] pointed out that there is evidence of protein synthesis in the nucleus and that the function of the nucleus must be related to protein synthesis in the cytoplasm.

It should also be noted that a number of glycolytic enzymes have been found in isolated nuclei[6,7]. However, because of the lack of the succinic dehydrogenase system, it is assumed that the Krebs cycle is lacking within the nucleus[8,9]. Consequently, if the glycolytic enzyme system does not supply sufficient energy to the nucleus, the nucleus is "parasitic" on the cytoplasm for its energy supply; otherwise, some unknown energy-yielding system should be at work in the nucleus. The assumption that energy-rich compounds are transferred from the cytoplasm to the nucleus is rather consistent with the fact that naked nuclei cease to show normal metabolic activities sooner than enucleated cells[19].

Do "the action and the maintenance of the genes" require energy? Studies on this problem seem to be lacking. However, the contribution of energy-rich phosphate for "gene action" is worth studying.

It may be pertinent to remember that the mechanism of the contraction of nuclear elements, *i.e.* the chromosomes[20] and the spindle fibers[21], involves ATP, as is the case for muscular contraction. Furthermore, although the structure of the living interphase nuclei is homogeneous, the heterogeneous distribution of the desoxyribonucleohistone, which is observed in nuclei treated with fixatives and other reagents which are expected to interfere their functional state[22], may be correlated to the action of ATP in nuclei[23].

It will then readily be understood that the fact that nuclei contain ATP is of great cell-physiological significance.

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SUMMARY

Cell nuclei and mitochondria of the rat liver were isolated with non-aqueous media and the labile phosphate (may be ATP) content of the fractions was compared with that of the total homogenate or of the crude cytoplasmic fraction. The labile phosphate was concentrated in nuclei and also in mitochondria. The cell-physiological significance of the presence in the cell nucleus of ATP is discussed.

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RÉSUMÉ

Les noyaux cellulaires et les mitochondries du foie du Rat ont été isolés dans des milieux non-aqueux. La quantité de phosphate labile (peut-être de l'ATP) de chaque fraction a été comparée à celle de l'homogénat total et à celle de la fraction cytoplasmique totale. Le phosphate labile est surtout concentré dans les noyaux et les mitochondries. La signification biologique de la présence de l'ATP dans les noyaux cellulaires a été discutée.

ZUSAMMENFASSUNG

Die Zellkerne und Mitochondrien der Rattenleber wurden mit nichtwässerigen Medien isoliert, und die Menge des in einzelnen Fraktionen vorhandenen labilen Phosphats (wahrscheinlich von ATP) wurde mit derjenigen des gesamten Homogenisats sowie der groben Cytoplasmafraktion verglichen. Das labile Phosphat war in den Zellkernen und Mitochondrien konzentriert. Die zellphysiologische Bedeutung des Vorkommens von ATP im Zellkern wurde erörtert.

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Note added in proof

It has recently been shown by the authors and SUGIMURA that ATP in nuclei has more rapid turnover than that in the other fractions by ^{32}P tracer technique.