SHORT COMMUNICATIONS

BBA 43241

Comparison of inosine and glucose as a substrate for energy metabolism in isolated rat-thymus nuclei

Cell nuclei isolated from calf and rat thymus glands contain cytochromes[1,2] and show an oxidative phosphorylation[3,4] comparable with the mitochondrial process. The substrate supply for energy metabolism is not fully understood as yet. This problem is under investigation in this laboratory. It has been reported by BETEL AND KLOUWEN[5] that, in contrast to many other substrates, added inosine could stimulate ATP synthesis in isolated rat-thymus nuclei. In their opinion[6,7] the ribose part of inosine was metabolized *via* the hexose monophosphate pathway through the Embden–Meyerhof pathway and the citric acid cycle. The fact that glucose did not stimulate ATP synthesis was attributed[7] to an inability of glucose to be metabolized because of a lack of ATP which should block the first two enzymes of glycolysis. This phenomenon is found in erythrocytes[8,9] which have been stored for some time and where ribose from added inosine can bypass the initial part of glycolysis *via* the hexose monophosphate pathway. In thymus nuclei, however, this inability has never been found. Because of the limited experimental support for the hypothesis that inosine is superior to other substrates as a substrate for energy metabolism, the role of glucose was considered and compared with the effect of inosine.

TABLE I

EFFECT OF IODOACETIC ACID AND 2-DEOXYGLUCOSE ON GLYCOLYSIS AFTER ADDITION OF GLUCOSE OR INOSINE

Rat-thymus nuclei were isolated according to the method of KLOUWEN AND BETEL[10]. The nuclei were incubated in 15-ml Warburg flasks for 60 min at 25° with air as the gas phase. The incubation medium consisted of 0.25 M mannitol, 15 mM NaCl, 3 mM $CaCl_2$ and 50 mM Tris–HCl (pH 7.4). Each vessel contained 2.5 ml of nuclear suspension (corresponding with about 0.5 mg DNA-P from one thymus gland). Lactate was estimated by an enzymatic method[11]; DNA after DISCHE[12]. The results are expressed as means ± S.D. The number of the experiments is given in parentheses.

Substrate	*Inhibitors*	*Glycolysis (μmoles lactate per mg DNA-P)*
None	None	0.2 ± 0.1 (16)
Glucose (5 mM)	None	1.8 ± 0.2 (12)
	2-Deoxyglucose (20 mM)	0.3 ± 0.1 (4)
	Iodoacetic acid (0.05 mM)	0.2 ± 0.1 (10)
Inosine (5 mM)	None	1.1 ± 0.3 (10)
	2-Deoxyglucose (20 mM)	0.8 ± 0.2 (4)
	Iodoacetic acid (0.05 mM)	0.1 ± 0.1 (4)

TABLE II

THE INFLUENCE OF INOSINE ON GLUCOSE METABOLISM

Incubations were performed for 1 h at 37° in 60-ml Warburg-type flasks covered with rubber caps. A glass tube in the center well served as a receptacle for hyamine to absorb CO_2. The absorbent was injected from a tuberculin syringe through the rubber cap at the end of the incubation. Hereafter 0.2 ml of 3 M H_2SO_4 was injected in the main compartment to release CO_2. The incubation medium had the same composition as is mentioned in the legend to Table I. The added glucose had a specific activity of about 160000 disint./min per μmole glucose. Radioactivity was measured using liquid scintillation. Glucose and lactate were assayed enzymatically[11]. For the determination of the specific radioactivity of lactate, this compound was isolated with a Sephadex G-10 column[14].

Additions	*Glucose uptake*	*Lactate production*	$^{14}CO_2$ *total activity* (%)	[^{14}C]*Lactate specific activity* (%)
	(*μmoles/mg DNA-P per h*)			
[6-^{14}C]Glucose (2 mM)	2.5 ± 0.3 (4)	3.3 ± 0.2 (4)	(100)	(100)
[6-^{14}C]Glucose + inosine (10 mM)	1.4 ± 0.2 (4)	4.2 ± 0.3 (4)	64 ± 18 (4)	60 ± 2 (4)

TABLE III

EFFECT OF RIBOSE AND RIBOSE DERIVATIVES ON NUCLEAR GLYCOLYSIS

Nuclei were incubated under the same circumstances as described in the legend to Table I. All substrates were added to a final concentration of 5 mM.

Substrate	*Glycolysis* (*μmoles lactate per mg DNA-P*)	*Substrate*	*Glycolysis* (*μmoles lactate per mg DNA-P*)
None	0.2 ± 0.1 (16)	Deoxyinosine	0.3 ± 0.2 (4)
Ribose	0.2 ± 0.1 (4)	Deoxyadenosine	0.4 ± 0.2 (4)
Ribose-1-*P*	0.5 ± 0.2 (2)	Thymidine	0.3 ± 0.1 (4)
Ribose-5-*P*	0.4 ± 0.1 (4)	Cytidine	0.4 ± 0.1 (4)
Adenosine	1.3 ± 0.2 (5)	Uridine	0.3 ± 0.1 (4)
Guanosine	1.1 ± 0.2 (5)		

In our experiments, neither of the two substrates could stimulate respiration, while both stimulated lactate production. As can be seen in Table I, this stimulation was prevented when iodoacetic acid was added. With 2-deoxyglucose, however, lactate production with inosine as substrate was hardly influenced. In Table II, experiments with [6-^{14}C]glucose are shown. Only 1 % of the ^{14}C label of glucose taken up was recovered in $^{14}CO_2$ (not shown here). Thus, the citric acid cycle does not seem to be important for the metabolism of glucose[13]. Inosine depressed glucose utilization, total activity of $^{14}CO_2$ and the specific radioactivity of [^{14}C]lactate, while total lactate production was slightly stimulated. From the results in Tables I and II it is concluded that inosine and glucose are both suitable in providing substrates for the Embden–Meyerhof pathway and, to a minor degree, also for the citric acid cycle. To investigate whether the lactate production from inosine could also be demonstrated by other ribose derivatives, the compounds mentioned in Table III were tested. The data in Table III suggest that free ribose was not metabolized, which is in agreement with other reports concerning normal[15] and tumor cells[16]. The minimal increase in lactate

production when ribose 1-phosphate and ribose 5-phosphate were added may be due to permeation difficulties through the nuclear membrane[17]. As shown in Table III, only the purine ribonucleosides gave a considerable stimulation of glycolysis. To compare the effect of inosine and glucose on ATP synthesis it was tried to obtain nuclei with a low ATP synthesis in the control experiments because it was in these nuclei that former investigators[5] found the best stimulation effect of inosine. By shaking vigorously at 37°, instead of gentle shaking at 25° (which is the normal standard condition), a low ATP synthesis was found. In none of these experiments was a clear stimulating effect of added substrate observed. As can be seen in Fig. 1, the roughly treated nuclei lost a great deal of their acid-soluble ultraviolet ($A_{260\ m\mu}$)-absorbing material which may explain the observed low ATP synthesis. Incubations done at 5° to study substrate influence on the rate of ATP synthesis also had no positive result.

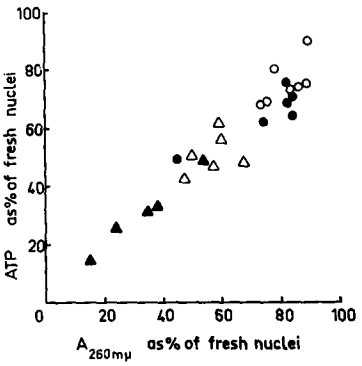

Fig. 1. Correlation between acid-soluble 260-mμ-absorbing substances inside the nuclei and ATP content. Fresh nuclei were kept under nitrogen for 15 min at 30° without shaking. This causes a decrease in ATP content to about 18% of the ATP level in fresh nuclei. Subsequent aerobic incubation during 30 min under different conditions gave variable ATP levels as can be seen in the figure. ATP was estimated enzymatically[11]. The different aerobic incubation conditions were as follows: vigorous shaking at 37° (▲) or at 25° (△) and gentle shaking at 37° (●) or at 25° (○).

The experiments reported in this paper show that inosine is not superior to glucose in providing substrate for the Embden–Meyerhof pathway and the citric acid cycle. Both compounds enhance lactate production, but clear stimulation of oxidative phosphorylation could not be detected. The latter is probably caused by the existence of high amounts of endogenous substrate in the isolated nuclei. The nature of the endogenous substrate is being investigated at present.

Laboratory of Physiological Chemistry,
University of Groningen,*
Groningen (The Netherlands)

A. W. T. KONINGS

1 S. YAMAGATA, K. UEDA AND R. SATO, *J. Biochem.*, 60 (1966) 160.
2 I. BETEL, *Biochim. Biophys. Acta*, 143 (1967) 62.
3 B. S. MCEWEN, V. G. ALLFREY AND A. E. MIRSKY, *J. Biol. Chem.*, 238 (1963) 758.
4 I. BETEL AND H. M. KLOUWEN, *Biochim. Biophys. Acta*, 131 (1967) 453.
5 I. BETEL AND H. M. KLOUWEN, *Biochim. Biophys. Acta*, 76 (1963) 327.

* Postal address: Bloemsingel 1, Groningen, The Netherlands.

6 I. BETEL AND H. M. KLOUWEN, *2nd Federation European Biochem. Socs. Meeting, Vienna, 1965*, Abstr. No. 161.
7 I. BETEL, *Oxidatieve Fosforylering in Kernen Geisoleerd uit Rattethymus*, Ph.D. Thesis, Rototype, Amsterdam, 1966.
8 G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 466.
9 T. SAITO AND S. MINAKAMI, *J. Biochem.*, 61 (1967) 211.
10 H. M. KLOUWEN AND I. BETEL, *Intern. J. Radiation Biol.*, 6 (1963) 441.
11 H. U. BERGMEYER, *Methods of Enzymatic Analysis*, Academic Press, New York, 1963, p. 117, 266 and 539.
12 Z. DISCHE, *The Nucleic Acids*, Vol. 1, Academic Press, New York, 1955, p. 287.
13 J. KATZ AND H. G. WOOD, *J. Biol. Chem.*, 238 (1963) 517.
14 M. V. RILEY, *Anal. Biochem.*, 22 (1968) 341.
15 E. KUN, P. TALALAY AND M. G. WILLIAMS-ASHMAN, *Cancer Res.*, 11 (1951) 855.
16 H. HARRINGTON, *J. Biol. Chem.*, 233 (1958) 1190.
17 B. S. MCEWEN, V. G. ALLFREY AND A. E. MIRSKY, *J. Biol. Chem.*, 238 (1963) 2579.

Received April 28th, 1969