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SUBSTRATE SUPPLY FOR NUCLEAR OXIDATIVE PHOSPHORYLATION

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SUMMARY

1. Cell nuclei isolated from rat thymus were examined for the identity of the endogenous substrate by which nuclear oxidative phosphorylation is driven. Under conditions of maximum inhibition of glyceraldehydephosphate dehydrogenase, respiration and ATP synthesis were not affected indicating that the energy source is not of a carbohydrate nature.

2. To measure oxidation of nuclear lipids a method was developed for preparing nuclei which are labelled with ^{14}C in several lipid components and which, in metabolic terms, are comparable with freshly isolated nuclei.

3. The amount of endogenous lipid oxidized during aerobic incubation, as calculated from the $^{14}CO_2$ production of ^{14}C-labelled lipids, is close to the theoretical amount of lipid which needs to be oxidized to account for one hour respiration on a lipid source.

4. Nuclear lipid oxidation is affected by oligomycin, antimycin A, 2,4-dinitrophenol and iodoacetate to a degree comparable with the influence of these compounds on nuclear respiration.

5. It is concluded that endogenous fatty acid oxidation is the principal energy-donating process for oxidative phosphorylation in isolated cell nuclei.

INTRODUCTION

Whereas opinions concerning the mechanism of energy production in cell nuclei isolated from liver are conflicting[1–6], general agreement[7–13] has been reached on the existence of nuclear oxidative phosphorylation in isolated thymus nuclei. The electron transport system in these nuclei reveals great similarity with the mitochondrial respiratory chain[12,14,15].

Nuclear oxidative phosphorylation *in vitro* can not be stimulated by the addition of oxidizable substrates[11] and is completely dependent on an endogenous source. It has been suggested that this substrate is of a carbohydrate nature[16,17].

In a preliminary report from this laboratory it has been shown, however, that inhibition of nuclear glycolysis by a low concentration of iodoacetate ($5 \cdot 10^{-2}$ mM) was not accompanied by an effect on respiration and ATP synthesis[18].

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In this paper further evidence is given on the independency of nuclear oxidative phosphorylation on carbohydrate breakdown via glycolysis and it will also be shown that endogenous fatty acid oxidation is the principal energy-donating process.

MATERIALS AND METHODS

Isolation of rat thymus nuclei

Female albino rats of a Wistar strain, weighing 100–130 g, were anaesthetized with ether and decapitated. Fractionation of thymus tissue and examination of the purity (by microscopy and by the use of succinate–cytochrome *c* reductase distribution) was performed as described before[19]. The nuclei were isolated in a medium that contained 0.25 M mannitol and 3.0 mM $CaCl_2$.

Incubation conditions

The incubations were performed in a medium containing 0.25 M mannitol, 15 mM NaCl, 3 mM $CaCl_2$ and 50 mM Tris–HCl (pH 7.4). Three types of incubation flasks were used. (a) 15 ml Warburg flasks for experiments in which oxygen consumption was determined. (b) 60 ml Warburgtype flasks sealed with rubber caps for most of the experiments in which $^{14}CO_2$ production was measured. (c) 50 ml centrifuge tubes in which ATP content was measured. Data on temperature, gas phase, incubation time and additions to the incubation medium are given in the legends to the tables and figures.

Labelling of nuclear lipids by preincubation of thymus tissue

Thymus fragments of about 8 mm^3 were incubated in a solution which was obtained after mixing 1 vol. 0.50 M mannitol and 1 vol. of a complex medium consisting of three solutions: (a), (b) and (c) which were mixed in a ratio of 14:15:1. The composition and the preparation of these solutions will be described below for an experiment in which 12 thymus glands were preincubated in 4 ml medium.

Solution (a) contained: 357 mM mannitol; 21 mM NaCl; 8.93 mM ATP; 0.179 mM CoA; 8.93% albumin; 8.93 mM glucose; 0.36 mM sodium-α-glycerophosphate and 71 mM Tris–HCl (pH 7.4).

Solution (b) was obtained by evaporating under nitrogen a solution of 10 μC uniformly ^{14}C-labelled palmitic acid or 10μC [1-^{14}C]palmitic acid in benzene and subsequently adding 0.6 ml 0.001 M NaOH. The specific activity of both radioactive solutions was 55 mC/mmole. The solution was heated (60°) just prior to use.

Solution (c) contained 60 mM $CaCl_2$.

For an experiment with 12 thymus glands 0.5 ml of the warm solution (b) was added slowly with the aid of a preheated pipet (about 60°) to 1.4 ml of solution (a) under thorough mixing. Hereafter 0.1 ml of solution (c) was added. The medium was completed by adding 2 ml 0.50 M mannitol.

The preincubation was performed for 30 min at 25° under gentle shaking with air as gas phase. Hereafter the thymus fragments were thoroughly washed with 0.50 M mannitol, minced with scissors and homogenized as in the normal procedure[19]. The isolation medium was supplemented with 0.5% albumin (Poviet, Amsterdam) and 0.1 mM sodium palmitate.

Analytical methods

Collection of $^{14}CO_2$. Experiments in which $^{14}CO_2$ was collected were usually performed in 60 ml Warburg-type flasks covered with rubber caps. A glass tube in the center well of the flask served as a receptacle for 1 M hyamine hydroxide in methanol, an absorbant for the carbon dioxide. The absorbant was injected from a tuberculin syringe through the rubber cap at the end of the incubation period. Hereafter 0.2 ml of 3 M H_2SO_4 was injected into the main compartment to release CO_2 from the medium during the next hour of shaking. In some experiments where oxygen consumption was measured simultaneously, $^{14}CO_2$ was collected in the center well of the 15 ml Warburg flasks. A piece of accordion-folded filter paper (Whatman 1) with 2 cm sides was inserted in the center well to which 0.1 ml 10% KOH had already been added. One of the side arms of the flask contained 0.3 ml 30% perchloric acid which was added to the main compartment at the end of the incubaton. After shaking for an additional hour to complete CO_2 absorption, the paper was taken out and the center well was cleaned twice with 0.4 ml hyamine solution. The piece of paper together with the hyamine solution were assayed for radioactivity as described below.

Estimation of ATP. ATP measurements were performed in nuclear suspensions of freshly isolated nuclei, in suspensions which were kept under nitrogen for 15 min at 30° and in suspensions which were subsequently incubated for 30 min at 25° with air as the gas phase. The suspensions were centrifuged for 7 minutes at 700 × *g* in the cold and the sediments were subsequently treated with perchloric acid to a final concentration of 3%. ATP was estimated enzymatically[20] in the neutralized acid soluble extracts of the sediments. ATP synthesis = ATP content after aerobic incubation *minus* ATP content before aerobic incubation[10].

Estimation of lactate. After the incubation and subsequent additional shaking period (to collect CO_2 completely) the suspension was treated with perchloric acid to a final concentration of 3%, allowed to stand for 15 min in ice and centrifuged for 15 min. at 1800 × *g*. The sediment was washed with 3% perchloric acid. The supernatant and washings were collected and neutralized by addition at 5 M K_2CO_3. Lactate was estimated enzymatically[20] in this solution.

Estimation of ^{14}C distribution in several classes of compounds. After thorough washing of the preincubated thymus fragments on a filter flask, the nuclei were isolated from these fragments as described above. The nuclear suspension was centrifuged for 7 min at 900 × *g*. The supernatant obtained was concentrated by lyophilization and tested for ^{14}C.

The fraction of nucleosides and nucleotides was controlled by extracting the sediment with cold 5% trichloroacetic acid. The amount of ^{14}C-label was estimated in the extracts obtained. The residue of the extraction was treated with 96% ethanol for 15 min at 50–60° and centrifuged. The sediment was treated with a mixture of ethanol and ether (1:1; v/v) for 15 min at 40°. The supernatants obtained were gathered and counted for ^{14}C. This fraction was considered to be the lipid fraction. The residue was treated for 30 min at 70° with 5% trichloroacetic acid to control the presence of label in the nucleic acid fraction. The remaining residue was considered to be the protein fraction.

Estimation of lipids. To inititate lipid extraction of the nuclear suspensions (concentration approx. 1 mg DNA P per ml) two volumes of methanol and one volume of chloroform were added under thorough mixing in a centrifuge tube. The extraction

was completed according to the procedure of BLIGH AND DYER[21]. Nonlipid contaminants were removed by Sephadex column chromatography[22]. Total lipids were separated into neutral lipids and phospholipids by silicic acid column chromatography[23]. Quantitative determinations of total, neutral and phospholipids were performed by evaporating aliquots of the extracts under nitrogen and weighing the lipid on a Cahn "Gram" electrobalance. Neutral lipids were separated by a two-step thin-layer chromatographic method[24] and determined by oxidation in acid dichromate as described by AMENTA[25]. Phospholipids were separated by thin-layer chromatography according to the method of SKIPSKI[26] and determined by the method described by BROEKHUYSE[27].

Measurement of radioactivity

All radioactive measurements were performed by liquid scintillation counting (Nuclear Chicago type 725). Counting efficiency was determined by the channel ratio method[28]. Hyamine hydroxide solutions (1 ml) were mixed with 15 ml of toluene supplemented with 0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP (1,4-bis-(5-phenyloxazolyl-2)-benzene). Aqueous solutions were counted in the toluene–Triton-X-100 system of PATTERSON AND GREENE[29]. Radioactivity in proteins was determined after treatment with digestin (Merck, Germany) for two hours at 60° and counted as described above for the hyamine hydroxide solution. Lipid extracts were evaporated under nitrogen in counting vials and (after addition of a few drops of ethanol) counted in the toluene–PPO–POPOP medium. Lipids from the thin-layer plates were directly counted in a gel of 3.5% Cab-O-Sil (Packard, U.S.A.) in toluene–PPO–POPOP. $^{14}CO_2$ on filterpaper was determined by adding the piece of paper (together with the center well washings) to a counting vial and mixing it with 0.4 ml dibutylphosphate, 1 ml ethyleneglycol monethylether and 13.6 ml of the toluene–PPO–POPOP medium[30].

RESULTS

The effect of iodoacetate on carbohydrate catabolism and nuclear oxidative phosphorylation

Iodoacetate is frequently used as an inhibitor of glycolysis, because of its ability to block glyceraldehydephosphate dehydrogenase (EC 1.2.1.12). The inhibitory effect of this compound on nuclear respiration and ATP synthesis has been used by several investigators as an argument for the dependency of oxidative phosphorylation on the glycolytic pathway as a route for substrate supply[16,17,31]. To verify this conclusion, the effect of increasing concentrations of iodoacetate on the production of lactate and $^{14}CO_2$ from [6-^{14}C]glucose was tested and compared with the effect on oxygen consumption and ATP synthesis. The results of these experiments are illustrated in Fig. 1. The production of lactate and $^{14}CO_2$ is affected (each in an opposite direction) by concentrations of iodoacetate above $1 \cdot 10^{-3}$ mM. Respiration and ATP synthesis are not influenced by iodoacetate concentrations up to $5 \cdot 10^{-2}$ mM. The additional presence of 20 mM 2-deoxyglucose did not influence oxygen consumption and ATP synthesis. This indicates that the oxygen consumption in the presence of iodoacetate is not due to oxidation of NADPH which is generated *via* the hexose monophosphate pathway because 2-deoxyglucose is known to inhibit glucose-6-phosphate conversions in several tissues[32,33]. The latter will also hold for thymus

nuclei because $^{14}CO_2$ production from [1-^{14}C]glucose is completely inhibited by addition of 2-deoxyglucose (unpublished observations).

The stimulation of $^{14}CO_2$ production from [6-^{14}C]glucose by iodoacetate as shown in Fig. 1 may be caused by an increased decarboxylation *via* the hexose monophosphate pathway of those hexose monophosphates which have been recombined from triose phosphates in such a way that 6-C of added glucose has been transferred to the 1-C position. A mechanism like this has been described before by other workers in a study on Ascites tumor cells[34–36]. Experiments are in progress to verify this hypothesis.

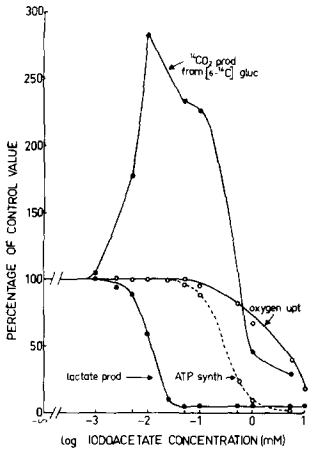

Fig. 1. The effect of iodoacetate on glycolysis and nuclear oxidative phosphorylation. The incubations were performed at 37° during one h in the medium described in the text plus 10 mM glucose. Oxygen consumption and ATP synthesis were also measured in the presence of 20 mM 2-deoxyglucose which resulted in the same curves. Without iodoacetate there was a lactate production of 6.2 μmoles/h per mg DNA P; ATP synthesis, Δ ATP = 0.30 μmoles mg DNA P; oxygen uptake, 6.8 μmoles/h per mg DNA P; $^{14}CO_2$ production, 4350 disint./min per mg DNA P; the specific activity of glucose was approx. 160000 disint./min per μmole glucose; glucose consumption 3.6 μmoles/h per mg DNA P.

Possible role of lipids

In a series of 5 experiments the respiratory quotient of isolated rat thymus nuclei was determined as 0.80 $\pm$ 0.05. The result supports the conclusion that carbohydrate is not preferably oxidized in these nuclei and led us to investigate the role of nuclear lipids as oxidizable substrate. In an aerlier paper data on lipid content and lipid composition of rat thymus nuclei have been reported[37]. It has been shown that these nuclei contain about 2.2 mg lipids per DNA P. To account for one hour's respiration (6.7 μmoles O_2 per mg DNA P) on a lipid source, about 5% of the lipids present has to be oxidized (75 μg palmitate).

Labelling of nuclear lipids

To be able to measure endogenous lipid oxidation a method was developed which yields nuclei labelled with ^{14}C solely in the lipid fraction. These nuclei could be isolated from thymus fragments which were preincubated with [^{14}C]palmitate. This fatty acid was chosen for the labelling method because it was found to be present

in rat thymus nuclei in considerable amounts[38]. During the development of the labelling procedure it was observed that the time elapsing between the killing of the animals and the start of the preincubation should not exceed 15 min. The time of preincubation has to be limited to about 30 min. If these conditions are not fulfilled, the nuclei obtained no longer demonstrate a clear stimulation of respiration by 2,4-dinitrophenol nor an inhibition of respiration by oligomycin. This led us to conclude that coupling of oxidation to phosphorylation has been lost in these nuclei. The thymus fragments which are preincubated must not be cut into pieces smaller than about 8 mm^3, or else the pieces tend to form a gel during preincubation or during the subsequent washing procedure. The procedure outlined under MATERIALS AND METHODS yields nuclear suspensions of high quality, which are completely comparable with freshly isolated nuclei.

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN NUCLEAR LIPIDS

After preincubation of thymus fragments with uniformly ^{14}C-labelled palmitate, the nuclei were isolated and lipids were extracted, separated and assayed as described in the text. For the measurement of radioactive distribution neutral lipids (4429 disint./min) and phospholipids (2995 disint./min) were applied to the thin-layer plate. For the determination of weight distribution 842 μg of neutral lipid and 673 μg of phospholipids were used. The specific activity of total lipids in this experiment was 20.4 disint./min per μg lipid; of neutral lipids 26.3 disint./min per μg lipid; and of phospholipids 17.8 disint./min per μg lipid.

Components	*Neutral lipids*		*Components*	*Phospholipids*	
	% ^{14}C	% *Weight**		% ^{14}C	% *Weight***
Total neutral lipids	(100)	(100)	Total phospholipids	(100)	(100)
Cholesterol esters	7	22	Phosphatidylethanolamine	10	27
Triglycerides	38	31	Phosphatidylinositol + Phosphatidylserine	4	8
Diglycerides	25	8			
Cholesterol	1	32	Phosphatidylcholine	84	53
Free fatty acids	18	3	Sphingomyelin	1	2
Monoglycerides	4	2	Lysophosphatidylcholine	1	1
			Solvent front	4	9

* Weight as % of total neutral lipid weight.
** P as % of total phospholipid P.

Distribution of radioactivity

The fractions of nucleosides, nucleotides, nucleic acids and proteins were found to be free of radioactivity. The ^{14}C-label was solely found in the lipid fraction. The specific activity of the nuclear lipids varied between 20 and 70 disint./min per μg lipid. The amount of lipids extracted from these nuclei was found to be 2.4 $\pm$ 0.4 mg per mg DNA P in 8 experiments. About 70% of these lipids were phospholipids.

It can be seen from Table I that most of the radioactivity of the neutral lipid fraction is found in the triglycerides and diglycerides. Most of the ^{14}C-label in the phospholipids is recovered in phosphatidylcholine. The total amount of lipids and the distribution of weight within the two groups are comparable with the data obtained from freshly isolated nuclei[37].

TABLE II

OXIDATION OF ENDOGENOUS NUCLEAR LIPIDS

The nuclei were isolated after preincubation of rat thymus fragments with uniformly ^{14}C-labelled palmitate. One part of the nuclei was used for the determination of specific activity in the total lipids and the other part was incubated in triplicate. The incubation was performed in 50 ml Warburg-type flasks during one h at 37° with air as the gas phase. The $^{14}CO_2$ evolved was absorbed in a solution of 1 M hyaminhydroxide in methanol.

Experiment	*Before incubation*	*After incubation*	*"Oxidized lipid"*
	Specific activity of total lipids (disint./min per μg)	*$^{14}CO_2$ produced (disint./min per mg DNA P per h)*	*(μg/mg DNA P per h)*
1	33.9	3 569	105
2	33.0	3 578	108
3	20.4	2 132	105
4	57.1	5 073	89
5	67.0	7 660	114
6	42.1	4 251	101
7	25.3	2 664	105
8	32.8	3 647	111
9	51.4	5 595	109
10	30.0	3 172	106
			mean 105 ± 7

Oxidation of endogenous lipids

Cell nuclei obtained after the labelling method described above were divided into two parts. One part of the suspension was used for direct lipid extraction and subsequent determination of specific activity. The other part of the suspension was incubated in triplicate for one h under aerobic conditions and tested for the production of $^{14}CO_2$. The results of these experiments are given in Table II. It can be seen from this table that although the figures of specific activity and the figures of $^{14}CO_2$ evolved are rather different when the 10 experiments are compared, a fairly constant figure is obtained by dividing the figures of the third column by those of the second column. The value obtained is 105 ± 7 and may be considered as the approximate amount of lipid, expressed as μg per mg DNA P, which is oxidized during one hour of aerobic incubation at 37°. It has been mentioned before that about 75 μg palmitate needs to be oxidized to account for one h respiration on a lipid source. Thus the theoretical amount of lipid is close to the amount of lipid which is calculated from the $^{14}CO_2$ evolved during incubation of nuclei labelled in the lipid fraction.

Further studies are in progress to settle the question as to which of the lipid classes preferentially provides fatty acids for oxidation. Some preliminary experiments indicate a decrease of triglycerides and phosphatidylcholine in the nuclei after aerobic incubation, with a simultaneous increase of free fatty acids and lysophosphatidylcholine.

Relation of respiration and lipid oxidation

Fig. 1 shows that inhibition of glycolysis in isolated thymus nuclei does not necessarily affect respiration and ATP synthesis. Expt. 1 of Table III shows that lipid oxidation and oxygen consumption are both affected to the same extend by iodoacetate. Similar correlations were found after addition of oligomycin and anti-

TABLE III

THE INFLUENCE OF SEVERAL COMPOUNDS ON RESPIRATION AND LIPID OXIDATION

The incubations were performed in 15-ml Warburg flasks during 1 h at 37° Oxygen consumption and $^{14}CO_2$ production in the control of the first experiment amount to 3.8 μmoles O_2/flask and 2503 disint./min per flask, respectively; in the second experiment, 2.3 μmoles O_2/flask and 1715 disint./min per flask. The concentration of the suspensions was approx. 0.5 mg DNA P/flask. Concentration of the additions: oligomycin, 5 μg per mg DNA P; antimycin A, $1 \cdot 10^{-6}$ M; 2,4-dinitrophenol, $5 \cdot 10^{-5}$ M.

Expt. 1			*Expt. 2*		
Additions	*Respiration* (%)	*Lipid oxidation* (%)	*Additions*	*Respiration* (%)	*Lipid oxidation* (%)
None	(100)	(100)	None	(100)	(100)
0.01 mM Iodoacetate	100	102	Oligomycin	38	39
0.05 mM Iodoacetate	103	96	Antimycin A	3	2
0.10 mM Iodoacetate	97	99	2,4-Dinitrophenol	97	94
1.00 mM Iodoacetate	47	55	2,4-Dinitrophenol + glucose	181	99

mycin A as is demonstrated in the second experiment of the same table. In this experiment it is also shown that after addition of 2,4-dinitrophenol, an uncoupler of nuclear oxidative phosphorylation[10,19], no net effect on respiration and lipid oxidation was observed. Addition of glucose to the uncoupled nuclei resulted in a clear stimulation of respiration, while no net effect on lipid oxidation was found. To obtain more insight in the latter phenomenon time course experiments were performed (see Fig. 2). In incubations without added glucose, 2,4-dinitrophenol stimulates both respiration and lipid oxidation during the first half hour followed by an inhibitory effect during the second half hour. While glucose addition in the absence of 2,4-dinitrophenol affected neither respiration nor lipid oxidation, in the presence of 2,4-dinitrophenol a two fold increase of respiration during the whole incubation period could be observed. Lipid oxidation in the uncoupled nuclei is only slightly enhanced by the addition of glucose. Some experiments were performed in order to investigate the effect of oligomycin on lipid oxidation in uncoupled nuclei. The de-

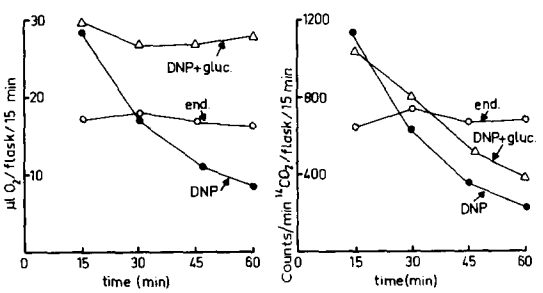

Fig. 2. Time course experiment of the influence of 2,4-dinitrophenol on respiration and lipid oxidation. The incubations were performed in 15 ml Warburg flasks for 1 h at 37° as described in the text. The additions in these experiments were $2 \cdot 10^{-2}$ M glucose (gluc.) and $5 \cdot 10^{-5}$ M 2,4-dinitrophenol (DNP). The concentration of the suspension was about 0.5 mg DNA P/flask. In the absence of 2,4-dinitrophenol addition of glucose does not influence the endogenous rate of respiration and $^{14}CO_2$ production (end.).

pressed lipid oxidation as shown in Fig. 2 during the second half hour could, however, not be prevented by oligomycin.

DISCUSSION

The existence of nuclear oxidative phosphorylation in thymus tissue is generally accepted at the moment[10,11,13,39]. Isolated thymus nuclei contain the cytochromes *a*, a_3, *b*, *c* and c_1 (see ref. 12). Most investigations on energy metabolism in isolated thymus nuclei have been performed without the addition of substrate. The effect of a number of substrates on nuclear ATP synthesis was studied by BETEL AND KLOUWEN[40] some years ago. According to their results inosine was preferentially utilized as a substrate for nuclear ATP synthesis. This, however, could not be confirmed in this laboratory[11]. The discrepancy cannot be due to a loss of those nuclear enzymes which are involved in the hexose monophosphate pathway or in glycolysis because in a series of studies published before[11,19,38], it was shown that purine nucleosides, including inosine, could be metabolized towards lactate very rapidly. Because inosine does stimulate ATP synthesis in whole thymus cells (unpublished observations), the presence of thymocytes in the nuclear preparations of BETEL AND KLOUWEN[40], might explain the different results. Glucose appeared to be even a better substrate for glycolysis than the purine nucleosides. However, only about 1% of the [6-^{14}C]-glucose utilized could be recovered as $^{14}CO_2$, which means that no more than 5% of the total oxygen consumption may be caused by glucose oxidation. With these data in mind, it is conceivable that a 3-fold stimulation of $^{14}CO_2$ production from [6-^{14}C]glucose by the addition of iodoacetate (Fig. 1) does not result in a measurable increase in oxygen consumption.

From experiments with inhibitors of glycolysis MCEWEN *et al.*[16] concluded that respiration and ATP synthesis in thymus nuclei were dependent on a substrate supply *via* the glycolytic route. From the data in their paper it is notable that addition of 1 mM iodoacetate resulted in a reduction of ATP content of 89% while the inhibition of respiration was only 32%. From this phenomenon it may not simply be concluded that glycolytic substrate-level phosphorylation is an important process in these nuclei, because addition of oligomycin results in an almost complete inhibition of ATP synthesis as was found by BETEL AND KLOUWEN[10] and in this laboratory (unpublished observations). Fig. 1 of this report strongly suggests that the effect of iodoacetate on nuclear oxidative phosphorylation is not specifically caused by an inhibition of glyceraldehydephosphate dehydrogenase. An alternative explanation might be that iodoacetate increases the permeability of the nuclear membrane, because it was observed that after addition of iodoacetate at concentrations above $5 \cdot 10^{-2}$ mM a considerable loss of at 260 nm absorbing material occurred. The decrease in ATP at high concentrations of iodoacetate may be caused by the proposed leakage of the nuclear membrane. The effect of iodoacetate at those higher concentrations on nuclear respiration may then most easily be understood by assuming an inhibited lipid oxidation, caused by a lack of cofactors necessary for fatty acid oxidation. The parallel inhibition of respiration and lipid oxidation caused by several compounds (Table III) is in line with this assumption.

Previously[19] it was found that addition of [6-^{14}C]glucose to uncoupled nuclei resulted in an enhanced respiration while glucose consumption and $^{14}CO_2$ production

were also increased. It was concluded from these studies that glucose could serve as a substrate for nuclear oxidation in uncoupled nuclei only. Fig. 2 is in accordance with these former results. It can be seen in this figure that addition of 2,4-dinitrophenol to the nuclei inhibits both fatty acid oxidation and respiration after an initial stimulatory period. Addition of glucose strongly stimulates respiration in these uncoupled nuclei while lipid oxidation is little affected. It was observed during these experiments that the respiratory quotient in uncoupled nuclei was enhanced by about 15% after addition of glucose[38]. Further study is needed to explain why fatty acids are preferentially oxidized in coupled nuclei while this no longer holds after addition of 2,4-dinitrophenol.

The following data militate against the suggested[16,17,40] important role of carbohydrate as source for nuclear energy supply: (a) Only 5% of the observed respiration can be covered by glucose oxidation (see refs. 11 and 19). (b) Nuclear oxidative phosphorylation is not influenced by addition of 0.05 mM iodoacetate, while glycolysis, under these conditions, is inhibited in such a way that insufficient intermediates are left to account for nuclear respiration. (c) The amount of endogenous lactate in the isolated nuclei which may be used as a substrate is too low to account for total nuclear respiration (approx. 35%, see ref. 18). (d) The respiratory quotient of isolated thymus nuclei is low (0.80).

The quantitative contribution of endogenous lipids to the substrate supply for nuclear energy will probably be overestimated by the labelling method described in this paper. The amount of lipid which is probably oxidized (105 μg/mg DNA P per h) is calculated from the specific activity of the total lipid fraction. However, the specific activity of those particular fractions that provide the labelled fatty acids may be expected to be higher than the specific activity of the total fraction.

Although elucidation of the regulatory mechanism of nuclear energy supply needs further study, it is obvious from this report that fatty acids are the principal substrate for nuclear oxidative phosphorylation.

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