practice. This indicates, after making due allowance for possible species differences, that tubular function may be significantly affected in man during clinical anesthesia. The present data also raise the possibility that many of the reports of renal function during anesthesia in man require re-evaluation when such studies depend upon the rate of clearance from blood of compounds known to be transported by the tubular organic acid transport system. In view of the effect of thiopental noted in the present study, this applies particularly to the decrease in renal plasma flow reported in man after thiopental administration[2]. The only critical test made in man of measuring renal vein PAH levels to make certain clearance per se is not affected by anesthetics under clinical conditions is that by DEUTSCH et al.[3]. They found that halothane did not affect PAH clearance, which agrees with the present in vitro results and with the observation by MILLER et al.[4], that halothane affects clearance rates only in proportion to levels of arterial hypotension produced by the anesthetic.

Table I. Time to appearance of first detectable intraluminal dye

	n	Control min[a]	Experimental min[a]
Buffer	9	7.1 ± 1.27	6.8 ± 1.64
Buffer + 20 mg/100 ml thiopental Na	7	8.4 ± 1.99	22.6 ± 3.51
Buffer + 10 mg/100 ml thiopental Na	6	6.5 ± 0.84	9.5 ± 1.38
80% Nitrous oxide	12	9.1 ± 1.56	13.7 ± 3.50
1.2 % Methoxyflurane in O_2	8	5.9 ± 1.46	8.4 ± 2.32
2.0% Halothane in O_2	6	6.3 ± 1.75	7.0 ± 1.79

[a] Mean ± standard deviation.

Table II. Time to maximal concentration of intraluminal dye

	n	Control min[a]	Experimental min[a]
Buffer	9	27.9 ± 4.36	32.6 ± 3.80
Buffer + 20 mg/100 ml thiopental Na	7	35.3 ± 3.16	59.1 ± 5.48
Buffer + 10 mg/100 ml thiopental Na	6	39.2 ± 1.25	59.8 ± 8.87
80% Nitrous oxide	12	37.1 ± 3.46	43.7 ± 8.45
1.2% Methoxyflurane in O_2	8	37.2 ± 2.03	54.9 ± 5.63
2.0% Halothane in O_2	6	39.2 ± 2.68	44.1 ± 6.40

[a] Mean ± standard deviation.

The present investigation of the effects of anesthetics upon organic acid transport systems in proximal convoluted tubules requires supplementary in vitro studies on other tubular reabsorption mechanisms before the effects of anesthesia on renal function can be fully defined in the absence of hormonal and renal vascular changes associated with clinical anesthesia[5].

Zusammenfassung. Die Fähigkeit zur Anreicherung von rotem Chlorphenol wird durch Stickoxydul, Methoxyfluran und Thiopental bei isolierten Fischharnkanälchen herabgesetzt, während Halothan ohne Wirkung blieb. Es wird gefolgert, dass allgemeine Betäubungsmittel den Transport organischer Säuren in benachbarten Nierenkanälchen beeinflussen.

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The Influence of Iodoacetate on the Mechanism of Nuclear Glucose Oxidation

Cell nuclei isolated from thymus tissue demonstrate a clear oxygen consumption and an oxygen dependent ATP synthesis[1–4]. It is also known that these organelles contain the cytochromes a_1, a_3, b, c and c_1 within the inner nuclear membrane[5] In these aspects thymus nuclei resemble mitochondria. In contrast to mitochondrial oxidative phosphorylation the nuclear process cannot be stimulated by the addition of external substrate[4]. Very recently[6] evidence has been obtained from this laboratory that endogenous lipids are the principal source of energy for isolated thymus nuclei. In earlier studies of McEWEN et al.[7] it was stated that nuclear oxidative phosphorylation was dependent on glycolysis. It could be shown[8], however, that this conclusion was obtained from experiments in which a dose of iodoacetic acid (IAA) was used too high to give a specific inhibition of glyceraldehyde-phosphate dehydrogenase (1.2.1.12) alone. From these studies it appeared that 0.05 m*M* IAA already completely blocked glycolysis while oxygen consumption was not yet influenced.

This communication reports on a study of IAA on glucose oxidation. The effect of this compound on $^{14}CO_2$ production from (6-^{14}C) glucose and (2-^{14}C) glucose was investigated.

The nuclei were prepared from rat thymus in 0.25 *M* mannitol and 3.0 m*M* $CaCl_2$ as described before[9]. Details concerning the incubation procedure are given in the legends of the tables. Termination of the incubation was preformed by the addition of 3 *M* H_2SO_4 and after a shaking period of 1 h (to release $^{14}CO_2$ from the medium) perchloric acid was added to a final concentration of 3%. After centrifugation of the suspension for 15 min at 1800 *g* the sediment was washed in 3% perchloric acid. The supernatant and the washings were collected, neutralized by the addition of 5 *M* K_2CO_3 and used for the enzymatic determination of lactate[10].

From Table I it can be seen that addition of increasing amounts of iodoacetate to thymus nuclei in the presence of (6-^{14}C) glucose results in a stimulation of $^{14}CO_2$ production while the lactate production is inhibited. In order to understand this unexpected result, some experiments with (2-^{14}C) glucose were performed. In general it may be assumed that production of $^{14}CO_2$ via the hexose monophosphate pathway is obtained from this compound after the hexosephosphates have been recycled one time[11].

In Table II it is shown that addition of IAA to (2-^{14}C) glucose inhibits the $^{14}CO_2$ production considerably while this product from (6-^{14}C) glucose is stimulated. This is most easily understood by assuming that IAA addition influences a mechanism by which the order of the positions of the C atoms in the hexosephosphates which are oxidized via the hexosemonophosphate pathway is changed. While addition of methylene blue alone has only a moderate stimulatory effect especially on the $^{14}CO_2$ production from (6-^{14}C) glucose, a high stimulation is found in the presence of IAA. It is known that methylene blue can accelerate the oxidations via the hexosemonophosphate pathway by regenerating NADP$^+$[12]. From these data it is concluded that the stimulation of $^{14}CO_2$ production form (6-^{14}C) glucose by IAA may be caused by an increased decarboxylation via the hexosemonophosphate pathway of those hexosemonophosphates which have been recombined from triosephosphates in such a way that the C-6 of added glucose has been transferred to the C-1 position. The recombination of triosephosphates may be the result of an accumulation of these products caused by the inhibition of glyceraldehydephosphate dehydrogenase.

McEWEN et al.[7] studied the effect of IAA on oxygen consumption, ATP synthesis and on the production of $^{14}CO_2$ of (6-^{14}C) glucose. They found that 0.1 m*M* IAA and 1 m*M* IAA inhibited both oxygen consumption and ATP synthesis. The effect of IAA on the $^{14}CO_2$ production was only reported for a concentration of 1 m*M*. No figure was given for the effect of 0.1 m*M* IAA. In the study reported in the present paper this concentration increased $^{14}CO_2$ production up to 200%.

In ascites tumor cells the formation of fructose-1,6 diphosphate from triosephosphates in the presence of IAA has been demonstrated and also the further conversion to hexosemonophosphates[13–15].

The quantitative significance of these reactions in thymus nuclei have still to be investigated.

Table I. Effect of iodoacetate (IAA) on the production of $^{14}CO_2$ and lactate from (6-^{14}C) glucose

Addition of IAA m*M*	$^{14}CO_2$ production cpm/flask	%	Lactate production µmoles/mg DNAP	%
None	1124	(100)	6.2	(100)
0.001	1176	105	6.3	102
0.005	1912	170	5.3	86
0.010	3019	269	3.6	58
0.050	2755	245	0.3	5
0.100	2374	211	0.3	5
0.500	1033	92	0.4	6
1.000	481	43	0.3	5

The incubation was performed in 60 ml Warburg-type flasks covered with rubber caps. A glass tube in the center well of the flask served as a receptable for 1 ml 1 *M* hyamine-hydroxide in methanol, an absorbent for the carbon dioxide. The absorbent was injected from a tuberculin syringe through the rubber cap at the end of the incubation period. Thereafter 0.2 ml of 3 *M* H_2SO_4 was injected to the main compartment to release CO_2 from the medium during the next hour of shaking. The hyamine-hydroxide solutions were mixed with 15 ml of toluene supplemented with 0.4% PPO and 0.01% POPOP and assayed for radioactivity by liquid scintillation counting. The counting efficiency in all samples of this experiment was the same (about 70%). The incubation medium contained 0.25 *M* mannitol, 15 m*M* NaCl, 3 m*M* $CaCl_2$, 50 m*M* *Tris* HCl (pH 7.4) and 10 m*M* glucose (specific activity about 160,000 dpm per µmole).

Table II. Effect of methylene blue (MB) on the mode of glucose oxidation in the presence of iodoacetate

Addition	$^{14}CO_2$ from (6-^{14}C) glucose cpm/flask	%	$^{14}CO_2$ from (2-^{14}C) glucose cpm/flask	%
None	1037	(100)	2627	(100)
0.05 m*M* IAA	2280	220	740	28
0.01 m*M* MB	1415	136	4136	157
IAA + MB	6793	655	1313	50

The incubation was performed as mentioned in the legend of Table I.

Résumé. Les expériences présentées montrent que dans les noyaux isolés du thymus de rat l'inhibition de glycéraldehyde-phosphate déhydrogénase stimule la production de $^{14}CO_2$ de (6-^{14}C) glucose. Un méchanisme capable d'expliquer cet effet est proposé et discuté.

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