L-asparaginase from *Erwinia aroideae* contains no carbohydrate. The N-terminal amino acid is L-alanin, as determined by dansylation of the pure enzyme followed by hydrolysis and chromatography[7]. 3.3% glutaminase activity was found at pH 8.5 with the original enzyme, and 2.5% with the acylated enzyme. The dependence of the activity upon pH is shown in Figure 3. L-asparaginase from *Erwinia aroideae* loses its enzyme activity rather quickly in plain buffer, between pH 4 and pH 9, when exposed to temperatures above 30 °C; however at pH 10 it is rather stable under the same conditions (Figure 4). Data from L-asparaginases from *Erwinia aroideae* and from *E. coli* have been compiled for comparison in Table II.

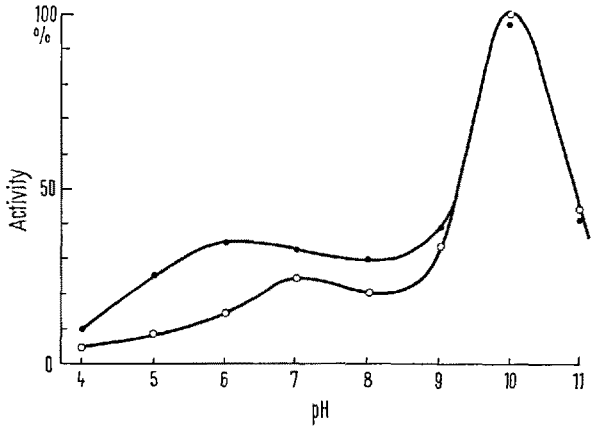

Fig. 4. Heat stability of L-asparaginase from *E. aroideae*. Residual activity was measured after exposure to 37 °C for 1 h at pH 4–11. ●—●, L-asparaginase; ○—○, acyl L-asparaginase.

The clinical application of L-asparaginase from *Erwinia* will show whether this enzyme is an agent as suitable as L-asparaginase from *Escherichia coli* for the treatment of certain types of leukaemia[15].

Zusammenfassung. L-Asparaginase wurde aus *Erwinia aroideae* gewonnen und durch Fraktionierung wie Fällungsschritte und Chromatographie um das 500fache angereichert. Das Enzym zeigt keine immunologische Kreuzreaktion mit L-Asparaginase aus *Escherichia coli*. Durch Acylierung lässt sich das basische Protein L-Asparaginase aus *E. aroideae* in ein saures Protein unter weitgehendem Erhalt der Enzymaktivität überführen.

J. STAERK, O. ZWISLER
and H.-RONNEBERGER

Behringwerke AG, D-355 Marburg/Lahn (Germany), 15. September 1970.

[15] Acknowledgments. We are grateful to Dr. T. KRANZ for running the ultracentrifugal analysis and to Dr. P. STEIN for the suggestion of the method for acylating L-asparaginase from *Erwinia aroideae*. The skilled technical assistance of M. SALLERMANN and K. WIEGAND is greatly appreciated.

Effect of Anesthetics on the Organic Acid Transport System of Renal Tubules

Although the effects of anesthesia on renal function are often measured by determining the rate at which compounds are secreted by the organic acid transport system of proximal convoluted tubules, data are not available on the effects of anesthetics on this transport system. Using the method of FORSTER and TAGGART[1] we evaluated in vitro the response of this system to clinically effective concentrations of several anesthetics.

Adult goldfish (*Carassius auratus*) were decapitated and both kidneys rapidly removed and placed in chilled fish Ringer's solution (NaCl 100.0, KCl 2.5, $CaCl_2$ 1.5, $MgCl_2$ 1.0, NaH_2PO_4 0.5, and $NaHCO_3$ 10.0 mM/l) where they were teased apart into individual tubules which were then placed in split Petri dishes containing $10^{-5}M$ chlorphenol red in 24 °C fish Ringer's. One side of the dish served for control studies (without anesthetic) while tubules from the same kipney were simultaneously exposed to anesthetic on the other side of the divider. Control observations confirmed that functional viability persisted for over 6 h. Nitrous oxide (80% in oxygen) was compared to nitrogen (80% in oxygen) while halothane (2.0%) and methoxyflurane (1.2%) in oxygen were compared to paired controls exposed to 100% oxygen. Thiopental studies were performed during exposure to room air. Because of the high pH of thiopental solutions, $0.01M$ secondary-tertiary sodium phosphate buffer, pH 7.4, was added to the fish Ringer's solution and comparisons were made between effects of buffer alone and buffer plus thiopental. Anesthetics were insufflated through the media for 1 h before the tubules were added. The time required for the first discernable appearance of dye intraluminally was determined visually using low power (×50–400) light microscopy, together with the time required before attainment of maximal concentration. Means and standard deviations were calculated for each series and its paired controls. The two-sample t-test was used to determine the statistical significance of the difference between two means. Significance was assumed if resulting p values were 0.05 or less.

As shown in Tables I and II halothane had no effect on the rate at which dye was transported by renal tubules. Methoxyflurane did not affect the time required for appearance of detectable amounts of intraluminal dye but did prolong the time for maximal concentrations to be achieved. Nitrous oxide increased the time to first appearance of dye but had no effect on the time required for peak dye concentrations to be reached. Both concentrations of thiopental significantly prolonged the time of first dye appearance, as well as the time of peak concentration.

The fact that nitrous oxide and methoxyflurane had different effects on the 2 measurements (time of dye appearance and time for attainment of peak concentration) suggests that different anesthetics may act in different ways. The fact that halothane had no effect implies that inhibition of this tubular transport system is not a characteristic of all anesthetics.

The concentrations of anesthetics used in the present study correspond to concentrations used in clinical

[1] R. P. FORSTER and J. V. TAGGART, J. Cell comp. Physiol. *36*, 251 (1950).

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.

D. F. DEDRICK and N. M. GREENE

Division of Anesthesiology,
Yale University School of Medicine,
New Haven (Connecticut 06504, USA), 11 August 1970.

[2] D. V. HABIF, E. M. PAPPER, H. F. FITZPATRICK, P. LOWRANCE, C. McC. SMYTHE and S. E. BRADLEY, Surgery *30*, 241 (1950).
[3] S. DEUTSCH, M. GOLDBERG, G. W. STEPHEN and W. H. WU, Anesthesiology *27*, 793 (1966).
[4] J. R. MILLER, V. K. STOELTING and R. K. RHAMY, Anesth. Analg. *45*, 41 (1966).
[5] This work was supported by the Harold C. Strong Anesthesia Research Fund.

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].