REDOX STATE OF THE LIVER DURING ANAESTHESIA AS STUDIED BY PARENTERAL GALACTOSE LOADS IN CHILDREN

BY M. Kekomäki, T. Suutarinen and M. A. K. Mattila

SUMMARY

The elimination rate of a parenteral dose of galactose, an indirect indication of the redox state of the liver hepatocytes, was determined in 46 children during anaesthesia and surgery. The anaesthetics used were thiopentone sodium, propanidid, halothane, diethyl ether, and nitrous oxide, administered in seven different combinations. The results suggest no direct involvement by any of the types of anaesthesia used in the liver redox system. Nevertheless, the normal oxidative metabolism may be affected indirectly by thiopentone-halothane combinations, unless an adequate alveolar ventilation is provided by appropriate measures.

A decreased oxygen supply to tissues with aerobic metabolism is manifested biochemically through an impaired oxidation of reduced co-enzymes via the mitochondrial electron transfer chain, which, in turn, leads to an increased ratio of NADH± to NAD in cells, or, in other words, to an increased redox potential of the tissue and to the accumulation of acid radicals in the cytosol (Henderson, 1969). In experimental investigation, the tissue contents of either co-enzymes proper or those of the appropriate “redox pairs”, reflecting the redox states of separate cellular compartments (Krebs, 1967), can now be assayed by the methods of enzymatic analysis (Hohorst, Kreutz and Bücher, 1959).

The diagnosis and prevention of tissue hypoxia constitutes one of the principles of safe practice in anaesthesia. However, the demonstration of inadequate oxygenation of a particular organ is difficult. Practically, there are only two ways to substantiate an insufficient respiration of a given organ: one of these is to measure the concentrations of some metabolites, which are in equilibrium with each other through an oxidation-reduction reaction (e.g., that of lactate and pyruvate), in the venous blood of this organ; the other is to quantitate one or more of the energy-consuming chemical reactions, specific for this organ (McDowall, 1969).

Our interest was directed to the oxidative metabolism of the liver during routine anaesthesia and surgery in children. As the criterion of the adequacy of liver respiration, we employed the determination of the rate of galactose elimination from the blood. This test is known to apply to this purpose for the following reasons. (1) After a standard parenteral loading, the elimination of galactose is an exclusive function of the liver in man (Tygstrup and Winkler, 1954), which thus fulfils the requirement of the organ specificity of the test. (2) Galactose is metabolised via phosphorylated intermediates to uridyldiphosphoglucone (UDP-glucose; fig. 1); the phosphorylation requires ATP and, therefore, necessitates a sufficient rate of ATP formation by the hepatocytes; furthermore, because of the high activity of galactokinase in human liver, capable of phosphorylating approximately 60 mg galactose/min/1.5 kg of liver tissue (Anderson, Kalckar and Isselbacher, 1957), the amount of high-energy phosphate bonds hydrolyzed per unit time in galactose phosphorylation is many times as great as that required for the elimination of, e.g., a test dose of sulphobromophthalein. (3) Since the (uridyldiphosphate-)galactose-4-epimerase (reaction No. 4 in fig. 1) is inhibited by the presence of reduced nicotinamide nucleotides (Isselbacher and Krane, 1961), the rate of galactose assimilation...
is strikingly diminished by an elevation in the content of NADH in liver cells. Thus the rate of galactose assimilation is a measure of the redox state of the liver tissue (Robinson, Kalckar and Troedsson, 1963; Forsander, 1966). Since such shifts from the physiological redox state of the liver as caused by the oxidation of ethanol are capable of diminishing the rate of galactose elimination appreciably (Tygstrup and Winkler, 1958), we regarded the last-mentioned feature of galactose metabolism as the most important one in the demonstration of possible liver hypoxia during anaesthesia. Lastly, the only observations reported so far on the rate of galactose elimination in experimental animals during general anaesthesia suggested considerable changes in liver metabolism after thiopentone administration (Salaspuro and Salaspuro, 1968). We determined the rate of galactose assimilation by children during seven different forms of general anaesthesia. Special attention was directed to combinations of anaesthesia with halothane, the other anaesthetics used being diethyl ether, nitrous oxide, thiopentone sodium and propanidid.

**MATERIALS AND METHODS**

**Test Subjects and Controls.**

Forty-nine children between 3 and 14 years old were included in the study group. None of these had a history of liver disease. This was the first general anaesthesia in all patients except for one subject of group A. The children were subjected to the following routine procedures of elective paediatric surgery: circumcision, herniorrhaphy, orchidopexy, elective appendicectomy, some operations of plastic surgery, and some procedures of paediatric urology. The original material included two patients operated on for aortic coarctation. Both of these children (group A) and only they displayed a clearly diminishing fractional rate of galactose elimination during the observation period. This may have been due to a significant interference with liver perfusion by the tying-off of the collaterals. These patients were excluded from the analysis of results; a third patient was omitted from the series in group F because of an evident error in injecting the test dose of galactose. The final study group thus consisted of 46 children.

---

**Fig. 1**
Pathway(s) of galactose metabolism in human liver.

1. Galactokinase
2. GAL-1-P uridylyltransferase
3. UDP-GAL pyrophosphorylase
4. UDP-GAL-4-epimerase
5. UDP-GLU pyrophosphorylase
The control group included a total of 76 non-anaesthetized children of age from 4 to 12 years, all without clinical signs of liver disease. These children were in part studied in our department, and in part included in a recent study from our neighbour hospital (Relander, 1968). Since the rate of blood galactose elimination is a function of the age of the individual (Relander, 1968), and since the mean age of separate study subgroups varied, the control material was treated as follows: the mean age of one study subgroup being \( N \), all controls of age from \((N-2)\) years to \((N+2)\) years were taken as its representative controls.

**Methods.**

Premedication.

Our standard system of premedication consisted of pethidine hydrochloride (Pethidin, Orion Oy, Helsinki, Finland) and of atropine sulphate (Atropin; Orion Oy), 1 mg/kg and 10–15 \( \mu \)g/kg, respectively, both given intramuscularly approximately 45 minutes before induction. Before the start of surgery, another similar dose of pethidine hydrochloride was administered intravenously to each patient. The mean duration of fasting prior to anaesthesia was 12 hours.

**Types of anaesthesia employed (fig. 2).**

**Group A.** Anaesthesia was induced with 4–6 mg/kg body weight of thiopentone sodium (Intraval-Natrium; Pharma-Rhodia A/S, Birkeroed, Denmark), and muscle relaxation obtained with approximately 0.5 mg/kg body weight of tubocurarine chloride (Tubocurarin; Orion Oy). During anaesthesia nitrous oxide: oxygen mixture (2:1 vol/vol) was given by positive pressure ventilation, either manually or with an Engström respirator. The depth of anaesthesia was adjusted individually and according to the needs of the surgery with intravenous thiopentone sodium and/or with pethidine hydrochloride.
Group B. Halothane (Fluothane; ICI) was used as the main anaesthetic with spontaneous ventilation. Halothane concentrations, adjusted with a Fluotec Mark III apparatus, needed for surgical anaesthesia ranged from 1.5 to 3.0 per cent (v/v); nitrous oxide-oxygen mixture was as for group A and all gases were administered with a non-rebreathing system.

Group C. Thiopentone was used for induction as in group A. Ventilation was spontaneous, and the depth of anaesthesia was adjusted by adding appropriate concentrations (1–2.5 per cent v/v) of halothane to a similar mixture of nitrous oxide and oxygen as in group A.

Group D. Induction with thiopentone was followed by spontaneous inhalation of halothane (1–2.5 per cent v/v) in 100 per cent oxygen.

Group E. Thiopentone was used for induction as in group A. Approximately 0.5 per cent (v/v) of halothane was added to the standard mixture (group A) of nitrous oxide and oxygen. Ventilation was of the intermittent positive pressure type, and muscle relaxation was obtained with 0.25 mg/kg body weight of alcuronium chloride (Alloferin; F. Hoffmann-La Roche & Co. AG, Basle, Switzerland).

Group F. Propanidid 50 mg/kg (Epontol; Farbenfabriken Bayer AG, Leverkusen, Germany) was used intravenously for induction. Ventilation and regulation of the anaesthesia was as in group C.

Group G. Anaesthesia was induced with thiopentone as in group A. Diethyl ether (Aether ad narcosin; H. Lundbeck & Co. A/S, Kopenhagen, Denmark) was mixed with the standard (2:1) nitrous oxide:oxygen mixture in an ether vaporizer.

Complications.
Two patients in group B displayed a fall of blood pressure below 80 mm Hg for less than 5 minutes. No other complications were recorded by the standard observation system.

Galactose loads.
During the first 15 minutes of surgical anaesthesia, 0.45 g/kg body weight of d(+)-galactose as a 30 per cent solution in water (Galaktos; Ab Kabi, Stockholm, Sweden) was injected rapidly to a peripheral vein. Venous blood was drawn prior to the load (= zero reference) and 10, 15, 20, 30 and 40 min after it. Appropriate samples were immediately precipitated for the determination of blood galactose (Hjelm, 1966) and glucose (Hjelm and deVerdier, 1963) concentrations. From the galactose concentrations obtained, the half-time of galactose elimination (Gal-T$_{1/2}$) was determined graphically.

Statistical calculations.
For the estimation of the significance of differences in Gal-T$_{1/2}$ values between the anaesthetized and control subjects, the Student t-test was used.

RESULTS
Elimination rate of galactose during different types of anaesthesia.
Patients in groups A and E excluded, the mean Gal-T$_{1/2}$ values were slightly higher in the anaesthetized children than in the controls (fig. 2). A consistently decreased rate of galactose elimination was found in one of the subgroups only (group C), i.e. in the children given thiopentone for induction and halothane-nitrous oxide for maintenance. This increase in the mean value was significantly different from the mean normal values. The same basic combination, thiopentone-halothane, appeared to affect the Gal-T$_{1/2}$ less if the content of oxygen was increased in the inhaled gas mixture (group D), or if the ventilation was controlled (group E). In none of these subgroups was the change statistically significant. Such findings suggest the possibility that the lengthened galactose elimination time in group C may result from a decreased alveolar ventilation. Neither propanidid-halothane nor thiopentone-diethyl ether combinations gave statistically abnormal Gal-T$_{1/2}$ values. Summarizing, our results thus suggest a remarkable stability of the galactose elimination rate in the types of anaesthesia employed, with no definite sign of direct interference with liver metabolism.

One patient (group E) displayed a grossly abnormal Gal-T$_{1/2}$ value during anaesthesia. When the test was repeated postoperatively, a similar value (22.5 min) was found, while other measures of liver function did not differ from the normal. Heterozygosity for a galactosaemic trait is the most plausible explanation for this observation.
Observations on blood glucose concentration.

The mean zero value for blood glucose did not differ from the normal fasting values measured for children in our hospital. After loading with galactose, increasing blood levels of glucose were the general finding (fig. 3), as they are in unanaesthetized controls. The greatest average increment (50 ± 4 mg/100 ml blood, mean ± SEM) during the first 20 min of loading was observed in children anaesthetized with the propanidid-halothane combination. Our results are thus in agreement with the outcome of the investigation of Clarke (1970) that a hyperglycaemic response is higher and more common in patients anaesthetized with propanidid than in those given thiopentone for induction.

Discussion

The liver participates in the metabolism of both volatile (Van Dyke and Chenoweth, 1965) and non-volatile (Shideman et al., 1949; Mark et al., 1965; Rahn, Dayton and Frederickson, 1969) anaesthetics in many ways. On the other hand, certain anaesthetic agents affect the metabolism of the liver, either directly at the molecular level, or indirectly, by altering the oxygen transport to the liver, or in both ways. Therefore details of the mutual relationships between the liver and the agents used in general anaesthesia have increased in significance.

Our aim was to examine the redox state of the liver, a close consequence of the oxidative metabolism of the liver (Henderson, 1969), during some types of routine anaesthesia in children. We interpreted the blood galactose half-time after a single rapid intravenous injection as an indication of the state of liver respiration. For the reasons mentioned in the introduction, the rate of galactose elimination under standardized conditions is a sensitive indirect measurement of the redox state of the hepatocyte (Isselbacher and Krane, 1961; Robinson, Kalckar and Troedsson, 1963; Forsander, 1966).

In this study, special attention was paid to combinations of anaesthetics in which halothane was included, since it has been suspected (cf., e.g., Pichlmayr and Pichlmayr, 1964) but not substantiated (e.g., Virtue et al., 1958; Little and Barbour, 1958; Bunker, 1968) that halothane is hepatotoxic, and because of its increasing clinical use.

As compared to the ethanol-induced reduction of galactose assimilation (Tygstrup and Winkler, 1958), the deviations from normal were small with any of the types of anaesthesia employed in this study. Our findings thus suggest stability of the redox state of the liver cell during anaesthesia or, which is a prerequisite for this, non-hypoxia of the liver cell during anaesthesia (Henderson, 1969). The only type of combination anaesthesia giving Gal-T1/2 values higher than normal was the thiopentone-induced halothane-nitrous oxide anaesthesia. However, patients undergoing similar treatment but with higher alveolar oxygen tensions or controlled ventilation showed less deviation from normal. This indicates that liver hypoxia, with or without concomitant hyper-
capnia (Ahlgren et al., 1966), resulting both in part from an insufficient ventilation, may have diminished the rate of galactose assimilation in the children in group C. Furthermore, essentially normal rates of galactose breakdown were seen in patients anaesthetized with a combination of propanidid, nitrous oxide and halothane, or with thiopentone induction followed by diethyl ether and nitrous oxide. Thus, a synergistic depressant action of thiopentone and halothane on respiration is possible.

A series of pharmacological observations, made both at the organismal and molecular levels, indicate that halothane, when administered without proper precautions, may, but does not unavoidably, interfere with the oxidative metabolism of the liver. Since its introduction to clinical anaesthesia, halothane has been shown to diminish cardiac output. Gattiker and associates (1966) recorded, however, a reduction of splanchnic blood flow, which exceeded the average reduction in cardiac output. This specific influence of halothane, brought about by regional splanchnic vasoconstriction (Price et al., 1966), evidently increases the risk of liver hypoxia. Nonetheless, the following facts are not consistent with a significant hepatic hypoperfusion and consequent hypoxia during halothane anaesthesia: an oxygen saturation of at least 40 per cent in the hepatic vein was found during halothane anaesthesia, when the inhaled gas mixture contained 40 per cent (v/v) of oxygen (Gattiker et al., 1966). Furthermore, neither Lowenstein, Clark and Villareal (1964) nor Price and associates (1966) observed any excess lactate production or oxygen debt during or after halothane anaesthesia, which also suggests an uninhibited oxidative metabolism. According to the experimental investigations of Hoech, Matteo and Fink (1966), a 2 per cent concentration of halothane diminished the consumption of oxygen by liver slices, but did not affect the rate of glycolysis. High (3 per cent) levels of halothane in the exchange gas mixture reversibly preclude the oxygen uptake by isolated perfused bovine livers (Middleton et al., 1966). In rat liver mitochondrial preparations, halothane is known to uncouple oxidative phosphorylation at three sites (Snodgrass and Piras, 1966); in vivo, however, levels of halothane capable of inducing general anaesthesia leave the efficiency of oxidative phosphorylation (the P/O quotient) of the liver tissue unchanged (Snodgrass and Piras, 1966). According to Kunz (1966), the content of reduced nicotinamide co-enzymes in rat liver mitochondria decreases rather than increases, when halothane is administered intermittently.

Although halothane is thus potentially capable of affecting the oxidative metabolism of the liver cell both by diminishing liver perfusion and by its direct action on the hepatocyte, the undesirable consequences of these phenomena can largely be prevented by providing an adequate alveolar ventilation during anaesthesia and by an accurate administration of halothane. This statement excludes any possible idiosyncratic reactions to halothane, which lack proper explanations in terms of molecular pharmacology, and to which certain disastrous results of halothane anaesthesia have been attributed (Bunker, 1968).

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to the nursing staff of the operating department of Children's Hospital.

A preliminary report of this study was given at the IX Congress of the Scandinavian Society of Anaesthesiologists in Bergen, June 1969.

This study was supported by a grant from the Finnish Medical Foundation (Duodecim), Helsinki. Laboratory facilities were kindly provided by the Foundation for Pediatric Research in Finland, Helsinki.

REFERENCES


ETAT REDOX DU FOIE DURANT L'ANESTHESIE, ETUDIER PAR CHARGE PARENTERALE DE GALACTOSE CHEZ DES ENFANTS

Le taux d'élimination d'une dose parentérale de Galactose, indirectement indicateur de l'état redox des hépatocytes du foie, a été déterminé chez quarante-six enfants durant l'anesthésie et l'opération. Les anesthésiques utilisés étaient le thiopentone sodique, le propanidid, l'halothane, le diéthyléther et le protoxyde d'azote, administrés en sept combinaisons différentes. Les résultats suggèrent qu'aucun des types d'anesthésie n'intervient directement dans le système redox du foie. Mais le métabolisme normal d'oxydation peut être influencé indirectement par l'association thiopentone-halothane, sous condition qu'une ventilation alvéolaire adéquate n'est pas garantie par des mesures appropriées.

ZUSAMMENFASSUNG