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# Effects of Halothane and Other Anesthetic Agents on the Concentrations of Rat Liver Metabolites *in Vivo*

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SUMMARY

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The technique of freeze-clamping has been used to study metabolic changes occurring in the livers of rats *in vivo* after acute or chronic exposure to halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), an anesthetic agent in common clinical use. For comparison similar experiments were carried out with methoxyflurane, trichlorethylene, or diethyl ether. In fed animals, exposure to halothane (1.5%, v/v) for 60 min caused glycogenolysis in the liver, with a significant decrease in tissue glycogen and increases in tissue and blood glucose concentrations. These changes were accompanied by a 50% decrease in the concentrations of 2-oxoglutarate and glutamate. The oxidation-reduction states of the NAD couple in both cytoplasm and mitochondria were unaffected by halothane. One hour after withdrawal of halothane the decrease in glycogen persisted, whereas glucose returned to normal within 30 min. On repeated exposure of animals to halothane (six successive periods of anesthesia for 60 min at 48-hr intervals) the glycogenolytic response was less marked, because glycogen stores were not replenished between periods of anesthesia. At the end of the sixth anesthesia period the levels of 2-oxoglutarate and glutamate were 25% and 40% lower, respectively, than in the unanesthetized controls. Depletion of glycogen persisted for more than 3 days, but less than 4 weeks, after repeated exposure to halothane. None of the liver metabolite changes brought about by halothane in fed rats were found in rats fasted for 24 hr or in livers from rats adapted to a high-fat diet. Glycogenolysis was a metabolic response common to all four anesthetic agents tested. Only halothane brought about decreases in 2-oxoglutarate and glutamate concentrations: a single exposure (30 min) to trichlorethylene (1%, v/v, in $O_2$) caused a 60% increase in 2-oxoglutarate concentration over the unanesthetized control value.

## INTRODUCTION

Previous experiments on the isolated, perfused liver (1, 2) have shown striking metabolic effects of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), an anesthetic agent

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in wide clinical use. The main effects of halothane were to stimulate glycolysis in livers from fed rats, and to inhibit gluconeogenesis and urea synthesis in livers from animals fasted for 48 hr. The effects could be largely attributed to the inhibition by halothane of $O_2$ uptake, and evidence was presented to suggest that fatty acids may have a protective effect on the observed metabolic changes, because oleate oxidation was relatively unaffected by halothane.

Although the study of the effects of halothane on the isolated, perfused liver gave information which could not readily have been obtained *in vivo*, such experiments exclude the physiological responses associated with anesthesia, such as changes in cardiovascular, respiratory, and endocrine function. These changes, as well as the anesthetic agent or its breakdown products, could contribute to liver dysfunction during or after anesthesia. The study of the biochemical effects of halothane has therefore been extended to the rat *in vivo*. Since halothane in the isolated organ had effects on carbohydrate metabolism and energy production, the liver content of a variety of metabolites associated with these processes has been determined in livers freeze-clamped after single and repeated exposure to halothane in normal fed, 24-hr-fasted, and fat-fed animals. Similar, though less extensive, investigations, using methoxyflurane, trichlorethylene, and diethyl ether, were carried out in order to test whether the halothane-induced metabolic changes were simply a general response associated with the state of anesthesia, irrespective of the anesthetic agent. The results show that metabolic changes peculiar to halothane occur in the liver, and that a physiological state in which fatty acids provide the main fuel has a protective effect.

## MATERIALS AND METHODS

*Rats.* Male Wistar rats (180–220 g) were obtained from Scientific Products Farm, Manston Research Center, Margate, Kent, and were kept on Oxoid pasteurized breeding diet for rats and mice.

*Anesthesia.* The volatile anesthetic agents were administered in $O_2$ at the following concentrations: halothane, 1.5% (v/v); trichlorethylene, 1% (v/v); diethyl ether, 6% (v/v); methoxyflurane, 1.0% (v/v). The anesthetic vaporizers were as previously described (1). The vaporizers used for diethyl ether (E.M.O. Inhaler or Abingdon Vaporizer) were obtained from Longworth Scientific Instruments, Ltd., Abingdon, Berks. The anesthetics were administered through a tube into the exposure chamber, a plastic box of approximately 2 cu. ft. The gas flow was high (6 liters/min). Care was taken to avoid obstruction of the airways of the animals during both anesthesia and recovery. All anesthesia was begun between 9 and 10 a.m.; after exposure to the anesthetic, $O_2$ continued to be flushed through the exposure chamber until the animals regained consciousness and were able to crawl unsteadily round the chamber. This period became progressively shorter on repeated halothane anesthesia.

*Preparation of liver extracts.* Freeze-clamping (3) of livers was carried out after single or repeated doses of anesthesia, and at different stages of recovery of the animals from the anesthetic. The rats were killed by dislocation of the neck, immediately followed (mean time interval, 7 sec) by freeze-clamping of the liver using aluminum clamps as described by Wollenberger *et al.* (3) and liquid $N_2$. Both awake and anesthetized animals were treated in precisely the same way. At least three control (awake) animals were included with each group of experimental animals in the freeze-clamping experiments. Liver extracts were prepared as described by Williamson *et al.* (4).

*Tail vein blood samples.* In some experiments involving repeated anesthesia, samples of blood (approximately 0.2 ml) were taken from the tail tips of both control and experimental groups into heparinized tubes. A known volume (0.1 ml) was pipetted into 1 ml of 2% (v/v) $HClO_4$. The blood samples were taken from the experimental group after approximately 45 min of anesthesia. Halothane caused vasodilation, which facilitated the collection of blood samples. For the

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control groups, however, the tail was warmed in water at approximately 50° to increase blood flow.

*Determination of metabolites.* For the determination of glycogen as glucose, a small volume (1 ml) of the liver homogenate in $HClO_4$, prepared after freeze-clamping, was stored at 4° overnight to extract the glycogen. A measured volume of the supernatant extract was neutralized with KOH and treated with Diazyme (crude amylo-α-1,4-α-1,6-glucosidase from *Aspergillus*) as described by Krebs *et al.* (5). At the end of the incubation the mixture was again neutralized before the determination of glucose by the method of Slein (6). The free glucose content of the liver was subtracted from the total obtained after Diazyme treatment.

Other metabolites were determined spectrophotometrically, as described by Biebuyck *et al.* (1), by enzymatic methods involving the oxidation or reduction of nicotinamide adenine nucleotides.

*Reagents.* Casein for the special high-fat (low-carbohydrate) diet was obtained from B.D.H. Chemicals, Poole, Dorset. Diazyme was obtained from Miles Chemical Corporation (this crude preparation has been superseded by a purer preparation of α-1,4-glucan 4-glucanohydrolase from *Bacillus subtilis*). Florisil was obtained from Koch-Light Laboratories, Ltd., Colnbrook, Bucks. Other reagents were from the sources described previously (1). Diethyl ether (anesthetic ether, B.P.) was obtained from MacFarlan Smith, Ltd., Edinburgh.

*High-fat (low-carbohydrate) diet.* For some experiments rats were transferred for 5 days prior to anesthesia to a diet consisting of 70% margarine, 25% light white casein, and 5% McCollum's salt mixture to which vitamins and choline chloride were added.

## RESULTS

*Effects of halothane on liver content of some metabolites in vivo.* Livers of fed rats were freeze-clamped after 60 min of exposure to 1.5% (v/v) halothane in $O_2$. Other animals were allowed to recover from the anesthetic, and their livers were freeze-clamped when the animals had regained consciousness and were able to move around the exposure chamber (approximately 30 min). The livers of a further group were freeze-clamped after complete recovery from the anesthetic (after 60 min). The changes in metabolite content in the experimental groups compared to the controls are shown in Fig. 1 and Table 1. After 60 min of exposure to halothane, the glucose concentration was significantly increased (from 6.59 to 10.3 μmoles/g, wet weight), with a 40% decrease in glycogen. The levels of both 2-oxoglutarate and glutamate were 50% lower than in the controls, although there was no change in the oxidation-reduction state of the mitochondrial

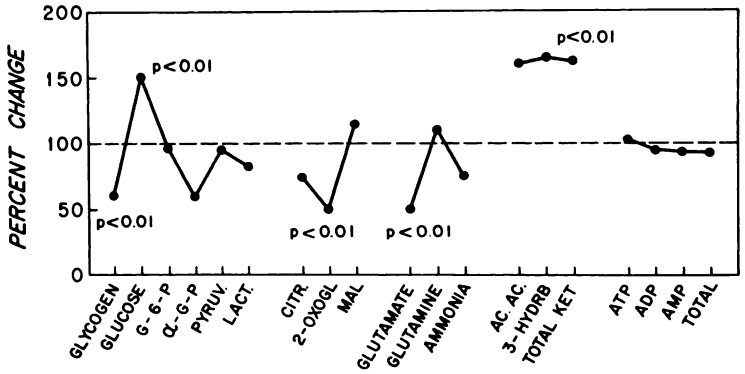


FIG. 1. *Tissue content of metabolites in livers of fed rats exposed to halothane (1.5%, v/v) for a single period of 60 min*

The livers were freeze-clamped as described under MATERIALS AND METHODS. The results are expressed as percentage change of the control (unanesthetized) animal values (see Tables 1 and 2). The *p* values are derived from the data in Tables 1 and 2, not from the percentage changes. G-6-P, glucose 6-phosphate; α-G-P, α-glycerophosphate; MAL, malate; AC. AC., acetoacetate; KET, ketones.

NAD couple as calculated from the 3-hydroxybutyrate and glutamate dehydrogenase systems (Table 2). The oxidation-reduction state of the cytoplasmic NAD couple, as calculated from the lactate dehydrogenase system, was also unaffected by halothane, although the actual concentrations of lactate and pyruvate were lower in the anesthetized animals. This lack of effect on glycolysis is in marked contrast to the findings in the isolated, perfused livers of fed rats, where halothane caused a 16-fold increase in the rate of lactate production from glycogen stores, and shifted the oxidation-reduction state of the NAD couples of cytoplasm and mitochondria in favor of reduction (1). There was slight increase in the concentrations of ketone bodies and of glutamine, both of which continued to increase throughout the 60-min recovery period (Fig. 2). By the end of the 30-min recovery glucose concentrations had returned to the control values, although glycogen remained low during the entire period studied (Fig. 3). After the decrease in 2-oxoglutarate concentration during halothane treatment, there was an overshoot during recovery to higher concentrations than in the controls (0.29 vs. 0.21 μmole/g, wet weight) (Fig. 3). There was no similar

TABLE 1

*Liver metabolite content in awake, fed rats*

Livers were freeze-clamped as described under MATERIALS AND METHODS. Values are means ± standard errors, with the number of observations in parentheses.

| Metabolite | Controls (awake animals) |
|---|---|
| | *μmoles/g, wet wt* |
| Glycogen | 336 ± 22.5 (18) |
| Glucose | 6.59 ± 0.19 (21) |
| Glucose-6-P | 0.23 ± 0.01 (14) |
| α-Glycerophosphate | 0.15 ± 0.01 (14) |
| ATP | 2.52 ± 0.04 (21) |
| ADP | 1.36 ± 0.05 (21) |
| AMP | 0.27 ± 0.18 (21) |
| Total nucleotides | 4.16 ± 0.07 (21) |
| Glutamine | 4.70 ± 0.25 (14) |

TABLE 2

*Effect of halothane on oxidation-reduction state of livers of fed rats in vivo*

Well-fed male rats were exposed to halothane (1.5%, v/v, in $O_2$) for 60 min as described under MATERIALS AND METHODS. Livers were freeze-clamped at this time. The oxidation-reduction state of the cytoplasm ([free $NAD^+$]$_c$/[free NADH]$_c$) was calculated from the lactate dehydrogenase system. The oxidation-reduction state of the mitochondria was calculated from the glutamate dehydrogenase ([free $NAD^+$]$_{m1}$/[free NADH]$_{m1}$) and 3-hydroxybutyrate dehydrogenase ([free $NAD^+$]$_{m2}$/[free NADH]$_{m2}$) systems. Values are means ± standard errors, with the number of observations in parentheses.

| Metabolite | Controls (no halothane) | After 60 min of halothane |
|---|---|---|
| | *μmoles/g of tissue* | *μmoles/g of tissue* |
| Pyruvate | 0.10 ± 0.01 (21) | 0.09 ± 0.01 (8) |
| Lactate | 0.76 ± 0.07 (21) | 0.68 ± 0.10 (8) |
| [Lactate]/[pyruvate] | 8.2 ± 0.6 (21) | 8.0 ± 0.6 (8) |
| [Free $NAD^+$]$_c$/[free NADH]$_c$ | 1220 ± 81 (21) | 1290 ± 112 (8) |
| 2-Oxoglutarate | 0.21 ± 0.02 (21) | 0.11 ± 0.03[a] (8) |
| Ammonia | 0.84 ± 0.04 (18) | 0.64 ± 0.04 (5) |
| Glutamate | 3.19 ± 0.09 (18) | 1.61 ± 0.17[b] (5) |
| [Free $NAD^+$]$_{m1}$/[free NADH]$_{m1}$ | 15.3 ± 1.4 (18) | 14.2 ± 2.5 (5) |
| 3-Hydroxybutyrate | 0.09 ± 0.01 (20) | 0.13 ± 0.03 (8) |
| Acetoacetate | 0.13 ± 0.01 (20) | 0.21 ± 0.03 (8) |
| Total ketone bodies | 0.22 ± 0.01 (20) | 0.34 ± 0.04[b] (8) |
| [Free $NAD^+$]$_{m2}$/[free NADH]$_{m2}$ | 37.3 ± 4.5 (20) | 44.1 ± 8.3 (8) |

[a] $p < 0.05$ compared with controls.

[b] $p < 0.01$ compared with controls.

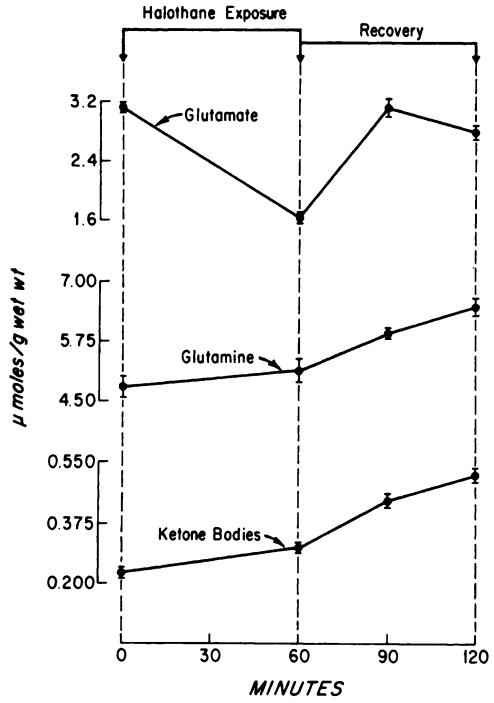


FIG. 2. *Time course of alterations in liver tissue concentrations of glutamate, glutamine, and ketone bodies in fed rats exposed to a single 60-min period of halothane (1.5%, v/v)*

The zero-time results represent the values obtained in unanesthetized control animals. The 60-min period represents the results during halothane anesthesia, and the 90- and 120-min periods represent the periods of recovery following withdrawal of the anesthetic at 60 min. The horizontal bars represent standard errors of the mean.

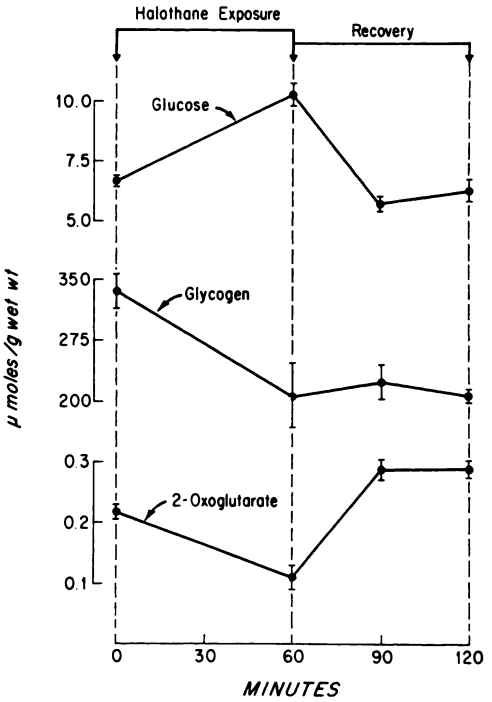


FIG. 3. *Time course of alterations in liver tissue concentrations of glucose, glycogen, and 2-oxoglutarate in fed rats exposed to a single 60-min period of halothane (1.5%, v/v)*

The zero-time results represent the values obtained in unanesthetized control animals. The 60-min period represents the results during halothane anesthesia, and the 90- and 120-min periods represent the periods of recovery following withdrawal of the anesthetic at 60 min. The horizontal bars represent standard errors of the mean.

overshoot for glutamate. The concentrations of all metabolites, other than glutamine and ketone bodies, did not change significantly between 30 and 60 min of recovery.

*Effect of repeated exposure to halothane on rat liver metabolites.* In view of the possible clinical association between repeated halothane anesthesia and hepatoxicity (7), the biochemical response was investigated in rats exposed repeatedly to halothane. The experiments were designed to study whether the metabolic changes found following acute anesthesia became less readily reversed after multiple halothane exposures.

Animals were exposed to 1.5% (v/v) halothane for 60 min on six successive occasions at intervals of 48 hr. On each occasion blood samples for glucose determination were taken from the tail as described in MATERIALS AND METHODS. After the first anesthetic period halothane caused a large increase in blood glucose concentration, but the response became progressively less marked during subsequent treatments (Table 3). The average weight gain of the halothane-treated rats was also lower during the first 6 days of the experiment (Table 3). The pattern of metabolite changes found in the liver after six separate exposures to the anesthetic was qualitatively the same as after a single 60-min period of anesthesia, although the decreases in concentration of lactate, pyruvate, citrate, and malate were greater. Although it appears that the decrease in liver glycogen

content was of the same order as after a single anesthetic exposure, it became clear from long-term recovery experiments (see following section) that liver glycogen stores were not replenished in the 48-hr intervals between doses of halothane (Fig. 4). This also explains why glucose increases in both tissue and blood were less responsive on repeated anesthesia. As after a single dose of halothane, the levels of both 2-oxoglutarate and glutamate were decreased, though to a lesser extent, without affecting the oxidation-reduction state of the mitochondrial NAD couple. Short-term recovery showed the same characteristics as after the single exposure to halothane. There was an overshoot of 2-oxoglutarate, and the concentrations of ketone bodies and glutamine continued to increase during the 60-min recovery period.

*Long-term metabolic changes produced by halothane.* Two groups of rats subjected to repeated halothane anesthesia were returned to normal living conditions to see whether any of the metabolic changes still present 60 min after withdrawal of the anesthetic would persist for days, or even weeks. These animals took part in the experiments of Fig. 4. They were given no further halothane

TABLE 3

*Effect of repeated halothane anesthesia on blood glucose concentrations of fed male rats*

Fed male rats were exposed to halothane (1.5%, v/v, in $O_2$) for 60 min at 48-hr intervals as described under MATERIALS AND METHODS. Glucose concentrations were determined in blood samples obtained from the tails of the experimental group after 45 min of anesthesia and from the nonanesthetized controls. At the time of anesthesia each animal was weighed. The data in the table refer to the net gain in body weight between anesthetics (i.e., per 48 hr), and are expressed as means ± standard errors for the number of observations shown in parentheses.

| Time | No anesthetic | | Halothane | |
|---|---|---|---|---|
| | Glucose | Weight gain | Glucose | Weight gain |
| *hr* | *μmoles/ml blood* | *g* | *μmoles/ml blood* | *g* |
| 0 | 5.46 ± 0.36 (8) | | 6.33 ± 0.21 (12) | |
| 48 | 5.19 ± 0.12 (9) | 13.5 ± 1.8 (12) | 8.32 ± 0.18 (12) | 7.2 ± 1.0 (20) |
| 96 | 5.35 ± 0.11 (8) | 10.9 ± 0.9 (12) | 7.62 ± 0.21 (12) | 5.8 ± 1.1 (19) |
| 144 | 4.92 ± 0.11 (9) | 9.7 ± 1.2 (9) | 5.48 ± 0.10 (12) | 8.0 ± 2.9 (19) |
| 192 | 5.16 ± 0.25 (8) | 11.4 ± 2.1 (12) | 5.81 ± 0.05 (12) | 11.3 ± 2.6 (19) |

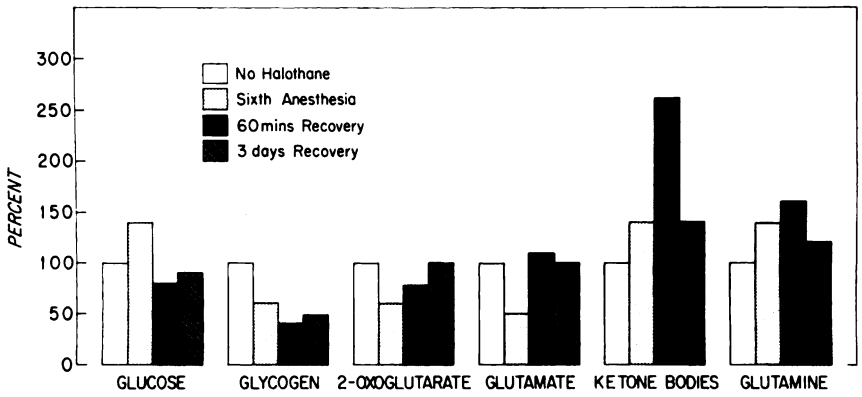


FIG. 4. *Percentage changes in liver metabolite concentrations in animals exposed to six 60-min periods of halothane (1.5%, v/v) on alternative days*

The changes represented are those present during the sixth anesthetic, at 60 min of recovery after the sixth anesthetic and 3 days following the sixth anesthetic. The livers were freeze-clamped as described under MATERIALS AND METHODS.

after the sixth exposure, and their livers were freeze-clamped 3 days or 4 weeks later.

After 3 days there was still a significantly decreased ($p < 0.05$) liver glycogen content [178 ± 14.6 (nine experiments) vs. 336 ± 22.5 (19 experiments) μmoles/g, wet weight, in the controls]. The concentration of glucose was somewhat lower [5.95 ± 0.22 (nine experiments) vs. 6.59 ± 0.19 (21 experiments) μmoles/g, wet weight] than in the controls. The raised glutamine and ketone body concentrations also persisted (5.55 ± 0.25 and 0.35 ± 0.08 μmoles/g, wet weight, respectively; for control values, see Tables 1 and 2). The malate concentration also remained lower (0.19 ± 0.02, six experiments) than in the controls. It should be emphasized that these metabolic changes persisted even though the rats had been living under the same conditions as the controls for 3 days since the previous anesthesia.

Four weeks after halothane anesthesia all metabolite concentrations measured were normal.

*Administration of halothane to 24-hr-fasted rats.* When rats fasted for 24 hr were exposed to halothane (1.5%, v/v) and their livers were freeze-clamped at 60 min, or at 30 or 60 min of recovery, the pattern of liver metabolites in the halothane-treated group was very similar to that of the 24-hr-fasted controls. With the exception of glycogen, the metabolites determined were those given in Fig. 1. There were no significant differences in any metabolite measured, so that the metabolic response of 24-hr-fasted rats exposed to halothane was very different from that of fed animals. Even the glycogenolytic response was very small [glucose increased from 3.06 ± 0.12 μmoles/g in the control group (three animals) to 3.69 ± 0.12 in the halothane-treated group (three animals)], because the liver glycogen was already depleted by deprivation of food.

*Effect of high-fat diet in preventing metabolic changes brought about by halothane.* The lack of effect of halothane on liver metabolites in fasted animals, and the previously reported (2) effect of oleate in preventing metabolic changes in isolated perfused livers, lent support to the argument that fatty acid oxidation is relatively unaffected by halothane. Animals were therefore fed a high-fat (low-carbohydrate) diet (see MATERIALS AND METHODS) for 5 days prior to halothane exposure. Halothane caused few changes in the metabolite pattern (Fig. 5). An increase (14%) in the liver glucose concentration occurred, with no change in the already low liver glycogen content (90.5 μmoles/g, wet weight). Halothane caused a change toward a more oxidized state of the low free $NAD^+$: free NADH concentration ratios found in fat-fed animals. The cytoplasm showed the most marked change in oxidation-reduction state, because the lactate concentration was

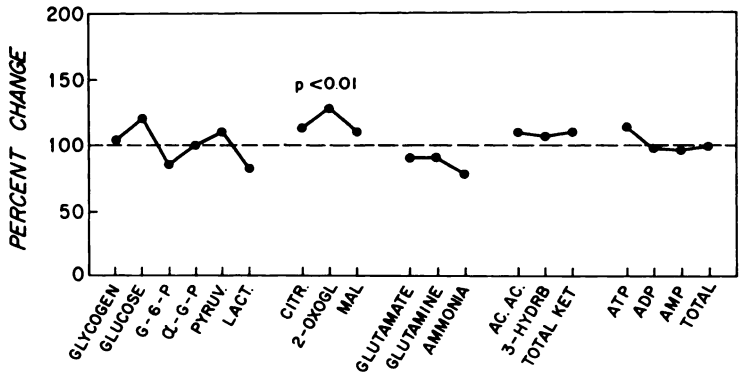


FIG. 5. *Tissue content of metabolites in livers of rats fed high-fat diet preceding exposure to halothane (1.5%, v/v) for a single period of 60 min*

The high-fat (low-carbohydrate) diet is described under MATERIALS AND METHODS. The livers were freeze-clamped as described in the text. The results are expressed as percentage change in the control (unanesthetized) values (see Tables 1 and 2). The $p$ values are derived from the data in Tables 1 and 2, not from the percentage changes. The abbreviations are defined in the legend to Fig. 1.

lower and that of pyruvate was higher than in the controls. Although the level of glutamate was slightly decreased by halothane, 2-oxoglutarate showed a significant increase, in marked contrast to the findings in the absence of exogenous fatty acid (1, 2). The ATP content was somewhat increased, with a corresponding decrease in ADP concentration.

*Effect of repeated methoxyflurane anesthesia on liver metabolite concentrations.* Rats were anesthetized for 60 min with methoxyflurane (1%, v/v, in $O_2$) at 48-hr intervals as described for similar experiments with halothane. Livers were freeze-clamped at 60 min during the sixth anesthetic exposure. Other animals were allowed to recover consciousness after the anesthetic. This took considerably longer than after exposure to halothane, so that livers were freeze-clamped only after 60 min of recovery. There was no glycogenolytic response after the sixth exposure to methoxyflurane (for details of the glycogenolytic response, as reflected in blood glucose concentrations during each preceding administration of anesthetic, see ref. 8). Liver lactate concentration was lower, and the total ketone body concentration more than 2-fold higher, than in the controls. No significant change in 2-oxoglutarate or L-glutamate levels occurred.

*Effects of short-term exposure to halothane, trichlorethylene, and diethyl ether on liver metabolites.* Livers of fed rats were freeze-clamped after 30 min of exposure to halothane (1.5%, v/v, in $O_2$), trichlorethylene (1.0%, v/v), or diethyl ether (6%, v/v, in $O_2$). The main differences between the three agents (Table 4) were a 5-fold decrease in $\alpha$-glycerophosphate content and an approximately 2-fold decrease in lactate during halothane anesthesia; a 2-fold increase in lactate during diethyl ether anesthesia; and an approximately 50% increase in total ketone bodies after halothane and trichlorethylene exposure, compared with a 25% decrease when diethyl ether was the anesthetic agent. The most marked difference

TABLE 4

*Effects of various anesthetic agents on liver metabolite concentrations*

Fed male Wistar rats were exposed to 30 min of anesthetic as described in the text. At the end of this time the livers were freeze-clamped as described previously. Results are means ± standard errors of the number of observations shown in parentheses.

| Metabolite | Controls (no halothane) | 30 min of halothane (1.5% in $O_2$) | 30 min of trichlorethylene (1% in $O_2$) | 30 min of diethyl ether (6% in $O_2$) |
|---|---|---|---|---|
| | *μmoles/g* | *μmoles/g* | *μmoles/g* | *μmoles/g* |
| Glucose | 6.59 ±0.19 (21) | 8.17 ±0.23[a] (3) | 9.69 ±1.4 (3) | 8.59 ±0.22[a] (3) |
| Glucose-6-P | 0.232±0.014 (14) | 0.157±0.014[b] (3) | 0.217±0.056 (3) | 0.347±0.069[b] (3) |
| $\alpha$-Glycerophosphate | 0.145±0.011 (14) | 0.030±0.002[a] (3) | 0.104±0.030 (3) | 0.213±0.070 (3) |
| Pyruvate | 0.102±0.011 (21) | 0.060±0.005 (3) | 0.120±0.030 (3) | 0.190±0.010 (3) |
| Lactate | 0.762±0.070 (21) | 0.44 ±0.07 (3) | 0.92 ±0.31 (3) | 1.71 ±0.19[a] (3) |
| [Lactate]/[pyruvate] | 8.2 ±0.6 (21) | 7.0 ±1.0 (3) | 7.0 ±1.0 (3) | 9.0 ±0.3 (3) |
| 2-Oxoglutarate | 0.214±0.019 (21) | 0.064±0.005[a] (3) | 0.333±0.090[b] (3) | 0.172±0.010 (3) |
| Malate | 0.294±0.022 (17) | 0.573±0.183[a] (3) | 0.754±0.240[a] (3) | 0.291±0.071 (3) |
| 3-Hydroxybutyrate | 0.088±0.011 (20) | 0.164±0.070 (3) | 0.122±0.054 (3) | 0.044±0.002 (3) |
| Acetoacetate | 0.129±0.007 (20) | 0.160±0.024 (3) | 0.184±0.040 (3) | 0.113±0.005 (3) |
| Sum of 3-hydroxybutyrate and acetoacetate | 0.217±0.014 (21) | 0.324±0.080[b] (3) | 0.301±0.101 (3) | 0.154±0.012[b] (3) |
| [3-Hydroxybutyrate]/[acetoacetate] | 0.69 ±0.09 (20) | 1.03 ±0.53 (3) | 0.57 ±0.23 (3) | 0.37 ±0.05 (3) |

[a] $p < 0.01$ compared with controls.

[b] $p < 0.05$ compared with controls.

in metabolic response occurred in the case of 2-oxoglutarate, which was decreased 70% by halothane, increased 60% by trichlorethylene, and unchanged by diethyl ether. All three anesthetic agents caused a glycogenolytic response which is described in detail elsewhere (8).

## DISCUSSION

*Differences in metabolic effects of halothane in vivo and in vitro.* The experiments reveal major differences between the isolated, perfused rat liver (1) and the situation *in vivo* in their metabolic response to halothane. (a) Halothane *in vivo* caused glycogenolysis with no concomitant increase in the steady-state lactate concentration, whereas in the perfused liver it caused a 16-fold increase in lactate production from liver glycogen. (b) The tissue content of ATP was decreased significantly by halothane when livers from 48-hr-fasted rats were perfused with lactate (a gluconeogenic precursor) or ammonium chloride plus ornithine (precursors of urea). The decrease in ATP content was associated with a shift of the cytoplasmic and mitochondrial NAD couples to a more reduced state. These changes in ATP and oxidation-reduction state did not occur when halothane was administered *in vivo*. (c) Halothane caused a 55% inhibition of $O_2$ uptake by the perfused liver of fed rats. The maintenance of ATP and the oxidation-reduction state of the NAD couples *in vivo* is an indication of unimpaired oxygen consumption, possibly by utilization of substrates by reactions which can to some extent bypass NADH dehydrogenase (i.e., $\beta$-oxidation of fatty acids to ketone bodies). These differences may be due in part to the responses of other tissues which can provide alternative fuels and hormonal regulation, thus modifying the response of the liver to halothane *in vivo*. Such mechanisms are absent in an isolated, perfused liver system. For instance, the difference with regard to lactate production on administration of halothane may be related to the fact that some glucose could be utilized by adipose tissue for fatty acid synthesis *in vivo*. The large increases in blood insulin and glucose that occur on halothane administration (8) would provide conditions favorable to fat synthesis.

*Common metabolic effects of halothane in vivo and in vitro.* The most striking effect of halothane was that in all experiments in which the livers contained reserves of glycogen, the anesthetic caused a large decrease in the liver content of 2-oxoglutarate. This again was most marked in the perfusion experiments, where the decrease was 5–8-fold (1). In all experiments *in vitro* the decrease in 2-oxoglutarate contents rather than an increase in glutamate or $NH_4^+$, was responsible for the reduced state of the mitochondrial NAD couple, and probably was related to the inhibition of NADH dehydrogenase by halothane (1, 2, 9–11). *In vivo* the decrease in 2-oxoglutarate concentration was only 50%, and there was a parallel decrease in glutamate, with no change in the mitochondrial oxidation-reduction state. The parallel change in the concentrations of only two reactants of this dehydrogenase system cannot be explained. While many of the factors that establish links between metabolites are well understood, the reasons why individual metabolites are maintained at a particular concentration in any given physiological state are not.

*Long-term effects of halothane.* The only long-term effect of halothane found was related to carbohydrate metabolism. Glycogen remained low after repeated exposures in normal fed rats, although the other metabolic changes found were apparently readily reversible. The erratic weight gain during the course of the experiment (Table 3) suggests that other, less readily reversible effects may also have occurred.

During anesthesia and the recovery period from single or repeated anesthetics, progressive rises (to above the concentrations in the controls) of ketone bodies and glutamine occurred. The content of 2-oxoglutarate also increased above the control value during the recovery period. The increases in ketone body concentration also occurred in the blood (8), and are probably related to the increased $\beta$-oxidation of fatty acids (requiring also increased lipolysis) during halothane anesthesia. However, increased rates of ketone body formation were not found in the liver perfusions when oleate was present in the medium (2), because, at this concentration of oleate, ketone body formation is already near maximal (12).

The overshoot of 2-oxoglutarate concentration after disturbance of the reactants of the glutamate dehydrogenase system (Table 1) was observed previously after injection of $NH_4Cl$ into rats *in vivo* (13), and is associated with the re-establishment of the physiological near equilibrium of this reaction. The increase in glutamine concentration is less easily explained. Although it is metabolically very closely related to glutamate, there is no evidence that the glutamine concentration is related either to the glutamate content or to the oxidation-reduction state of the liver cell.

*Physiological implications of metabolic effects of halothane.* These experiments show that distinct metabolic changes occur with halothane as compared to diethyl ether, trichlorethylene, and methoxyflurane. The finding that fasting or exogenous administration of fatty acids prevents some of the metabolic disturbances brought about by halothane is of special interest in relation to the view, long held, that a high liver glycogen content protects against damage during anesthesia (14–17).

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