The Effects of Feeding on the Development of Metabolic Acidosis in the Rat: Comparison Between Perfused Liver *in situ* and Whole Animal

Michiko SAKAI, Mikiko YAMAKAWA, Hideo HORIKAWA and Kunio ICHIYANAGI

Rat liver perfused *in situ* was charged with two concentrations of halothane. Lactate increased in a time- and dose-dependent manner in the liver of the fed rat, whereas its increase in the starved rat was much milder (1.7 → 9.2 mmol·l^{-1} *vs* 0.6 → 1.4 mmol·l^{-1} after a 3 hr charge with 6.0% halothane). Base excess decreased also more markedly in the fed rat. Glucose increased 2.3 times the control value in the fed rat, whereas it did not change significantly in the starved rat. Changes produced by enflurane were very similar to those produced by halothane. It was inferred that in the presence of halothane and enflurane, hepatic glycogen was transformed into glucose and then to lactate by the inhibition of NADH dehydrogenase. In the liver of the starved rat, glucose, hence lactate, did not increase because of the depletion of glycogen.

When halothane (1.9%) was given to the whole animal, changes in lactate, base excess and glucose in the arterial blood were very mild. Marked disparities in these parameters between the two experimental models were inferred to be due to: 1) possible insinuation of anaerobic metabolism in the perfusion experiments, 2) a well-kept balance between the suppression of cellular metabolic activity and inhibition of energy production by halothane in the whole animal, and 3) involvement of neural and humoral factors in the intact whole animal. (Key words: feeding, liver perfusion, glucose, metabolic acidosis, halothane)

(Sakai M, Yamakawa M, Horikawa H, et al.: The effects of feeding on the development of metabolic acidosis in the rat: Comparison between perfused liver in situ and whole animal. J Anesth 6: 480–489, 1992)

The liver, as one of the major storage sites in the body, contributes to the regulation of blood concentrations of nutritional substances and the maintenance of acid-base balance. We have noticed, in our previous rat liver perfusion studies, that the nature of the liver perfusate often differs between fed and starved rats, i.e., the perfusate of the liver from the fed rat tends to be more acidic. In this investigation we studied the effects of feeding on the glucose metabolism and acid-base balance of the rat. Comparison was made

Department of Anesthesia, Yamagata University School of Medicine, Yamagata, Japan

Address reprint requests to Dr. Sakai: Department of Anesthesia, Yamagata University School of Medicicne, Iida-Nishi 2-2-2, Yamagata, 990-23 Japan

Table 1. Experimental groups

	Inhalation Anesthetics	Body Weight (g)	Notation in Text
Fed	halothane 6.0%	230 ± 21	FH 6.0
	halothane 1.9%	231 ± 22	FH 1.9
	enflurane 6.9%	222 ± 12	FE 6.9
	enflurane 3.8%	251 ± 42	FE 3.8
	none	226 ± 23	FC
Starved	halothane 6.0%	202 ± 17	SH 6.0
	enflurane 6.9%	189 ± 19	SE 6.9
	none	219 ± 22	SC

F: fed, S: starved, C: control, H: halothane, E: Enflurane, n = 7 in each group

between two models, the liver perfused *in situ* and the whole animal, since it can easily be assumed that there might occur a large difference in these diverse models. Comparison was also made between two anesthetics, halothane and enflurane, to determine whether or not halogenated hydrocarbons share similar metabolic effects on the liver.

Study I: Liver Perfusion Study

Methods

1. Materials: Fifty-six Wistar rats, 168–308g, were divided into eight groups of seven animals each according to the mode of feeding and to the anesthetic used (table 1). The animals in the fed groups were allowed free access to animal chow (CRF-1, Charles River, Atsugi, Japan), and those in the starved groups were not given any food but tap water for 48 hours prior to the experiment.

2. Liver perfusion: An *in situ* liver perfusion model of Mortimore type was used[1]. The animal was anesthetized with an intraperitoneal injection of 50 $mg \cdot kg^{-1}$ of pentobarbital. Through a midline laparotomy an 18 gauge Teflon catheter was placed in the portal vein and was connected to the inflow side of the perfusion circuit (fig. 1). Through a midsternotomy a 16 gauge Teflon catheter was inserted into the inferior vena cava through the right atrium and was connected to the outflow side of the circuit. After the ligation of the hepatic artery and the inferior vena cava peripheral to the hepatic vein, perfusion of the liver was started. Perfusion was considered adequate when the liver showed a vivid "liver" color and was not swollen, and the P_{O_2} of the perfusate from the inflow side was higher than 200 mmHg and that from the outflow side was lower than 60 mmHg.

Perfusion circuit: Figure 1 shows the principle of the perfusion circuit. It was housed in a plastic chamber with the temperature set at 37 ± 0.5°C. With a rotary pump a flow rate of 10 $ml \cdot min^{-1}$ was maintained. The perfusate was equilibrated with 40% O_2, 5% CO_2 and 55% N_2 through a 4m Silastic tubing in an equilibration chamber and a series of bulbiform equilibration chambers.

Composition of the perfusate: Bovine red blood cells were suspended in a Krebs-Ringer bicarbonate solution to a hemoglobin concentration of 7 $g \cdot dl^{-1}$. Bovine serum albumin 2 $g \cdot dl^{-1}$ and glucose 100 $mg \cdot dl^{-1}$ (5.6 $mmol \cdot l^{-1}$) were added, and the pH of the solution was adjusted to 7.4 at P_{CO_2} 40 mmHg with sodium bicarbonate. The total amount of the perfusate used was

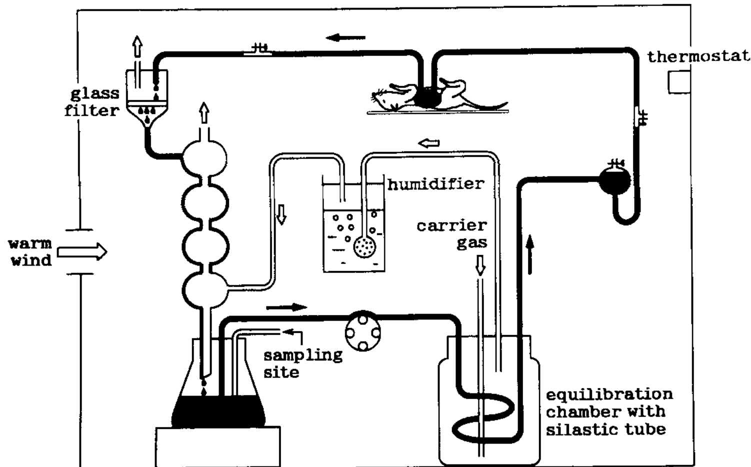

Fig. 1. Perfusion circuit.

120 ml.

3. Inhalation anesthetics: A Fluotec mkIII® (Cyprane, Keighley, England) and an Enfluwick type 200® (Murako Medical, Tokyo) were used for vaporization of halothane and enflurane, respectively. The carrier gas flow was set at 0.5 $l \cdot min^{-1}$, and the vaporizer dial was set at 2 or 5%. The actual concentrations determined by gas chromatography are shown in table 1.

4. Analyses of the perfusate: A perfusate sample was analyzed for pH and blood gases (ABL 30, Radiometer, Copenhagen, Denmark) 15 min after the initiation of perfusion, and metabolic acidosis, if present, was corrected with sodium bicarbonate. A second sample was analyzed 15 min after the first sample and the values obtained served as the control. Inhalation anesthetics were then added to the gas mixture (except in the FC and SC groups). One, two and three hr after the addition of the anesthetics, perfusate samples were analyzed for pH and blood gases, lactate, pyruvate, glucose and hemoglobin. Lactate-pyruvate ratio (L/P) was calculated from the lactate and pyruvate concentrations in $mmol \cdot l^{-1}$. Oxygen content was calculated from the oxygen carrying capacity of hemoglobin and the amount of dissolved oxygen. Oxygen consumption was calculated as the product of the flow rate (10 $ml \cdot min^{-1}$) and the difference in oxygen content between the inflow and outflow sides.

5. Statistical analyses: All the results are presented as the mean ± standard deviation. Analysis of variance with a repeated measure design and Bonferroni multiple comparison test were applied for statistical analyses. Differences were considerd significant when $P < 0.05$.

Study II: Whole Animal Study

Methods

1. Material: Twenty male Wistar rats, 290–400g, were divided into two groups of 10 animals each: a fed group and a starved group. The mode of feeding was the same as described in Study I.

2. Anesthesia: The animals were anesthetized with intraperitoneal pentobarbital, 50 $mg \cdot kg^{-1}$, and the trachea was intubated and the lungs ventilated with a pump ventilator. The animal was infused with Ringer solution via a cannula placed in the internal jugular vein at a rate of 10 $ml \cdot kg^{-1} \cdot hr^{-1}$. Pancuronium was given as needed. The

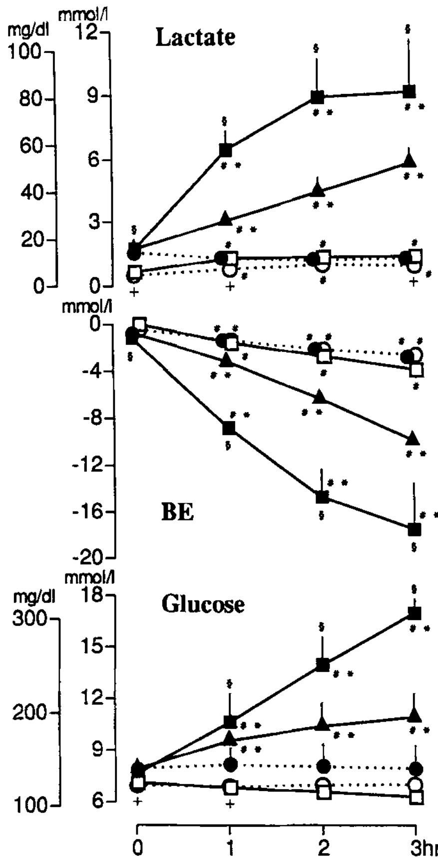

Fig. 2. Effects of halothane on lactate, BE and glucose.

■-■ FH6.0; ▲-▲ FH1.9; ●-● FC

□-□ SH6.0; ○-○ SC

* vs FC or SC ($P < 0.05$); # vs 0 hr ($P < 0.05$)

§ FH6.0 vs SH6.0 ($P < 0.05$); + FC vs SC ($P < 0.05$)

Values are mean ± SD.

femoral artery was cannulated for continuous blood pressure display and arterial blood sampling. After a stable condition, i.e., rectal temperature 37 ± 0.5°C, Pa_{CO_2} 35–45 mmHg and base excess (BE) ± 4.0 mmol·l^{-1}, had been obtained for 30 min, the animals were given 1.9% halothane in oxygen for three hr.

3. Analyses of arterial blood: Arterial blood samples were analyzed for pH and blood gases, total protein and hematocrit prior to and at the end of the inhalation of halothane. In order to reduce the sample size, lactate, pyruvate and glucose were measured only at the end of the halothane charge. Another group of 12 rats, 6 each in fed and starved groups, were anesthetized in the same fashion as in the experimental group except for the halothane charge. An arterial blood sample drawn when a stable condition was reached was analyzed for lactate, pyruvate and glucose and these values served as the control.

4. Statistical analyses: Student's paired and non-paired t tests were used for the statistical analyses. Differences were considered significant when $P < 0.05$.

Results

Study I

A. Effects of halothane on lactate, BE, glucose, L/P and oxygen consumption:

Lactate (fig. 2): In neither the FC group nor the SC group did the lactate change significantly throughout the experiment, but it was always higher in the FC group than in the SC group and this difference was significant at all times except at 2 hr of perfusion. The lactate increased in the FH groups in a time- and concentration-dependent manner, and was higher than that in the FC group at all the sampling points of the experiment. Increase in the lactate in the SH 6.0 group was very mild, and the difference between the FH groups and the SH group was significant at all the sampling times.

Base Excess (BE) (fig. 2): BE decreased in the FC and SC groups slightly, but there was no difference between the groups at any time. It decreased in the FH groups in a time- and concentration-dependent manner, reaching −17.5 mmol·l^{-1} after 3 hr of halothane charge in the FH 6.0 group. Values were significantly lower than

Table 2. Changes in lactate/pyruvate in the liver

	FC	FH 6.0	FE 6.9	SC	SH 6.0	SE 6.9
0 hr	9.1 ± 2.7	9.1 ± 2.7	11.4 ± 1.7	6.9 ± 1.5§	10.4 ± 2.4	9.7 ± 2.3
1 hr	7.3 ± 0.9#	21.7 ± 2.5*#	19.9 ± 4.9*#	4.0 ± 0.8#§	7.6 ± 0.5*§	6.9 ± 1.4*#§
2 hr	6.9 ± 0.8#	30.1 ± 6.6*#	28.1 ± 5.2*#	5.6 ± 0.7§	14.7 ± 3.3*#§	8.3 ± 1.4*§
3 hr	6.9 ± 0.8#	30.3 ± 3.7*#	29.9 ± 8.0*#	5.0 ± 0#§	22.9 ± 6.4*#§	7.9 ± 1.6*§

Mean ± SD * *vs* FC and *vs* SC ($P < 0.05$)
vs 0 hr ($P < 0.05$)
§ F groups *vs* S groups ($P < 0.05$)

Table 3. Changes in oxygen consumption ($ml \cdot min^{-1} \cdot 100g^{-1}$ wet weight of the liver) in the liver

	FC	FH 6.0	FE 6.9	SC	SH 6.0	SE 6.9
0 hr	3.7 ± 0.5	4.0 ± 1.0	3.8 ± 0.4	3.4 ± 0.8	3.9 ± 0.5	4.7 ± 0.7§
1 hr	3.9 ± 0.6	2.5 ± 0.6*#	3.3 ± 0.4#	3.3 ± 0.5	2.1 ± 0.4*#	3.0 ± 0.6#
2 hr	3.7 ± 0.4	2.5 ± 0.7*#	3.1 ± 0.6*#	3.2 ± 0.5	1.5 ± 0.5*#§	2.7 ± 0.7#
3 hr	3.6 ± 0.4	2.3 ± 0.6*#	3.0 ± 0.4*#	3.1 ± 0.6	1.7 ± 0.3*#§	2.9 ± 0.4#

Mean ± SD * *vs* FC and *vs* SC ($P < 0.05$)
vs 0 hr ($P < 0.05$)
§ F groups *vs* S groups ($P < 0.05$)

those in the FC group.

Glucose (fig. 2): In neither the FC group nor the SC group did the glucose change significantly during perfusion. It was about 1.1 $mmol \cdot l^{-1}$ higher all the time in the FC group. In the FH groups the glucose increased in a manner similar to lactate. Glucose increased linearly in the FH 6.0 group, reaching 16.9 $mmol \cdot l^{-1}$ (304 $mg \cdot dl^{-1}$) after 3 hr. Values were significantly higher than those in the FC group. In the SH 6.0 group it kept decreasing, though only slightly. The difference between the FH 6.0 group and the SH 6.0 group was significant at all times.

L/P (table 2): In the FC and SC groups L/P values slightly decreased from the control values and were smaller than 10. In the FH groups they increased significantly from the control values and were higher than those in the FC group at all times. In the SH 6.0 group it increased at 2 and 3 hr but this increase was significantly less than in the FH 6.0 group. A similar relationship was seen between the SE 6.9 and FE 6.9 groups.

Oxygen consumption (table 3): In the FC and SC groups the oxygen consumption was nearly stable throughout the experiment. It fell in the FH 6.0 group and values were significantly lower than the control value at all times during the perfusion. In the SH 6.0 group it fell markedly and was significantly smaller than in the FH 6.0 group at all times.

B. Effects of enflurane on lactate, BE, glucose, L/P and oxygen consumption:

In the FE groups the lactate and glucose increased and BE decreased in a time- and concentration-dependent fashion (fig. 3). Changes in L/P were similar to those in the halothane groups (table 2). The oxygen consumption decreased in the FE groups but these changes were less marked than those in the FH groups (table 3). The general picture was very similar to that of the halothane groups.

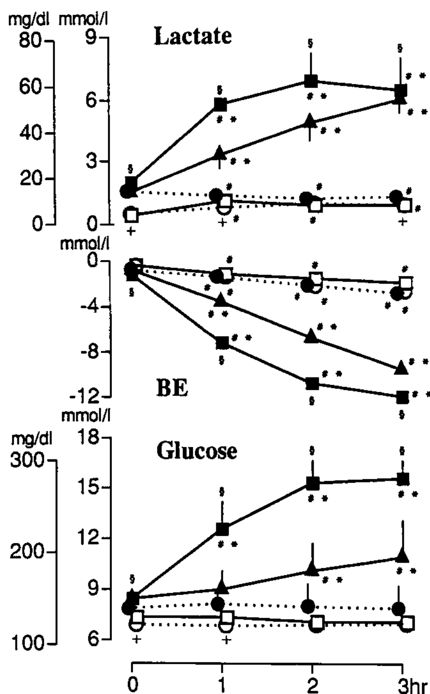

Fig. 3. Effects of enflurane on lactate, BE and glucose.

■-■ FE6.9; ▲-▲ FE3.8; ●-● FC

□-□ SE6.9; ○-○ SC

* *vs* FC or SC ($P < 0.05$); # *vs* 0 hr ($P < 0.05$)

§ FE6.9 *vs* SE6.9 ($P < 0.05$); + FC *vs* SC ($P < 0.05$)

Values are mean ± SD.

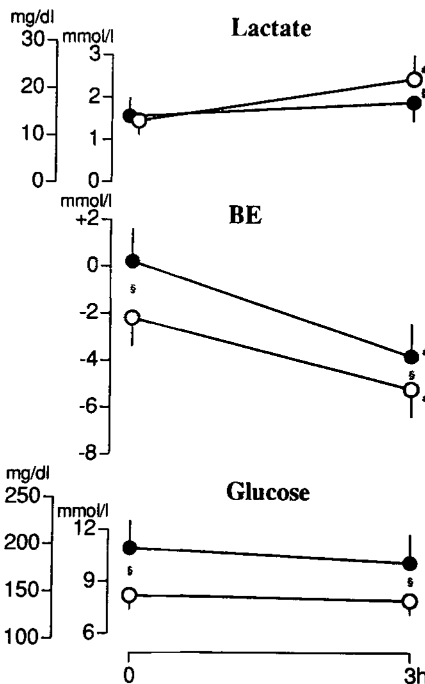

Fig. 4. Effects of halothane in whole animal.

●-● FH1.9; ○-○ SH1.9

vs 0 hr ($P < 0.05$); § *vs* groups ($P < 0.05$)

Values are mean ± SD.

Study II

In the starved group, after 48 hr starvation, the animals' body weight decreased from 358 ± 8g to 317 ± 6g, the latter being significantly less than 367 ± 5g in the fed group.

Mean arterial pressure (MAP): There was no difference in MAP between the two groups before the halothane charge. MAP was lowered in both groups by halothane, reaching 65 ± 12 mmHg in the fed group and 56 ± 10 mmHg in the starved group, but the difference between the groups was not significant.

Hematocrit (Ht): Ht changed from 45 ± 1% to 41 ± 1% in the fed animals and from 48 ± 1% to 43 ± 1% in the starved animals after the halothane charge. Intergroup difference was not significant at any time.

Lactate (fig. 4): Three hr after the halothane charge the lactate increased to 1.3 times the control value in the fed group and 1.9 times in the starved group, the latter being significantly higher.

BE (fig. 4): There was as significant difference in the control value of BE: 0.2 ± 1.5 mmol·l^{-1} in the fed group and −2.2 ± 1.3 mmol·l^{-1} in the starved group. There was, however, no difference between the two groups in the magnitude of the change after the 3 hr halothane charge (−3.9 ± 1.4 mmol·l^{-1} in the fed group *vs* −3.0 ± 1.2 mmol·l^{-1} in the starved group).

Glucose (fig. 4): No significant

change was produced in the glucose level in either group by halothane. It was, however, higher in the fed group both before and 3 hr after the halothane charge.

Discussion

Liver Perfusion Study

Woods et al.[2] reported that, in the liver of the fed rat perfused with 150 ml of perfusate, lactate increases rapidly in the early stages of perfusion reaching as high as 2 mmol$\cdot l^{-1}$, whereas glucose increases to 6 mmol$\cdot l^{-1}$ (108 mg$\cdot$dl^{-1}). These values of lactate and glucose were remarkably similar to those found in the blood plasma of their whole animals. In regard to the fact that the lactate stabilizes at certain levels, they claim that it is hard to explain this fact solely by the depletion of hepatic glycogen and the suppression of hepatic cellular function. Based on their findings that glycogen was in fact not depleted, though decreased, and ATP was maintained at the normal level, they suggested the possibility of certain feedback mechanisms playing a role in this phenomenon.

In our study, the lactate in the FC group did not change significantly (1.56 mmol$\cdot l^{-1}$ at 0 hr and 1.33 mmol$\cdot l^{-1}$ at 3 hr). This last value was very close to the one found in our whole animal study, indicating that the lactate stabilizes at a level commonly seen in the blood plasma of the whole animal, as has been reported by Woods et al.[2] The glucose level in the FC group was maintained at around 8 mmol$\cdot l^{-1}$, coinciding with the finding by Woods et al.[2] that the glucose also stabilizes at a certain level during perfusion. In their study the glucose was found to keep increasing up to 6 mmol$\cdot l^{-1}$ where it stabilized. This seems to be due to the lack of glucose in their perfusate, whereas our perfusate contained 5.6 mmol$\cdot l^{-1}$ of glucose.

Woods et al.[2] reported that in the fed rat, the lactate increased rapidly in the early stage of the perfusion and reached a plateau within 45 min. In the starved rat, since the rate of lactate production was so small, the lactate did not reach an equilibrium even after 135 min of perfusion. In our study the lactate in the FC group was 1.56 mmol$\cdot l^{-1}$ at 0 hr and remained unchanged, whereas in the SC group it increased from 0.54 to 0.92 mmol$\cdot l^{-1}$ during the 3 hr perfusion. Since 30 min had elapsed between the initiation of the perfusion and the first sampling (0 hr), the lactate must have reached a plateau (1.56 mmol$\cdot l^{-1}$) during this period of time in the fed group, whereas in the starved group lactate production continued even at 3 hr of perfusion. The whole picture fairly closely resembled that presented by Woods et al.

Biebuyck et al.[3] loaded the isolated rat liver with 2.5% halothane for 30 min and observed changes in the lactate in the hepatic tissue. The lactate increased markedly from 1.29 to 5.82 μmol$\cdot$g^{-1} wet weight in the fed rat, whereas it increased only mildly from 5.01 to 5.80 μmol$\cdot$g^{-1} in the starved rat. Their results are not directly comparable to ours because of the difference in the experimental design and mode of halothane charge (only one concentration of halothane and one analysis after a short exposure). Nevertheless, they were qualitatively fairly similar to ours.

In the present study the lactate and glucose increased in the perfusate of the liver of the fed rat in a time- and concentration-dependent manner after the liver was loaded with halothane (lactate 3.4 and 5.5 times and glucose 1.4 and 2.3 times the control values at 3 hr with 1.9% and 6.0% halothane, respectively) (fig. 2). Changes in the lactate and glucose of the perfusate reflect changes in the production and

the uptake of lactate and glucose by the hepatic tissue. In the whole organism, when the cellular metabolism tends to be anaerobic, e.g. in circulatory shock, the electron transfer system is suppressed and NADH accumulates. In the process of oxidation of this NADH, the lactate increases (NADH + pyruvate → NAD + lactate). Halothane, in an *in vitro* experiment using a mitochondrial suspension, has been found to inhibit in a concentration-dependent manner the state 3 mitochondrial respiration using glutamate as a substrate[4]. Biebuyck et al.[3] claim that the site of action of halothane is NADH dehydrogenase on the basis of their findings that NAD/NADH decreases in the cytosol as well as in the mitochondria of the rat liver when exposed to halothane. In our study, changes in the pyruvate were not marked compared with those in the lactate, and the L/P increased to 3 times the control value in the FH 6.0 group. The fact that the increase in the lactate and the decrease in the oxygen consumption are dependent on the concentration of halothane added to the perfusate also points to the possibility that the inhibition of NADH dehydrogenase was the major contributing mechanism to the increase in the lactate induced by halothane in the study of Biebuyck, et al., and in our study as well.

Halothane charge decreased BE of the perfusate in a time- and concentration-dependent fashion, and on the whole the picture was similar to the change in the lactate. The magnitude of the increase in the lactate corresponded to about a half that of the decrease in BE. The other half should be accounted for by increases in other acidic substances, which in the present study were not measured.

Enflurane has been known to inhibit the state 3 respiration of mitochondria *in vitro* in a manner similar to halothane[4]. In our *in situ* perfusion study a similar mechanism to that of halothane is most probably accountable for the metabolic effects of this anesthetic observed. The applicability of this assumption regarding the metabolic effects of enflurane to other inhalation anesthetics, fluorinated hydrocarbons in particular, must be further verified using other experimental modes.

Whole Animal Study

MAP was lowered moderately by loading the animal with 1.9% halothane (1.7 MAC). However, pH, blood gases and temperature remained in the normal range, and there were no differences in BE, lactate and L/P between the fed and starved groups, denying the presence of circulatory failure or accompanying anaerobic metabolism in either group. The control value of BE was lower in the starved group than in the fed group, suggesting that mild metabolic acidosis developed after starvation of 48 hr. BE decreased and lactate increased only very mildly in both groups after a 3 hr charge with 1.9% halothane. Intra- and intergroup differences were not significant. The direction of changes in the lactate and BE in the whole animal caused by halothane was the same as in the perfused liver, but the magnitude of the changes was much smaller.

In regard to the effects of halothane on the glucose metabolism in the whole organism there seems to be no agreement of opinions. Merin et al. (adults)[5], Kaniaris et al. (children)[6] and Oyama et al. (adults)[7] all reported that the blood glucose increased by inhalation of halothane, whereas Black et al.[8] claimed that halothane had little effect on the glucose metabolism in children. Yamazaki et al.[9] also reported little change in the lactate and pyruvate in children. In our study the glucose level was lower in the

starved group both before and after the halothane charge. However, the values were within normal range. No significant change occurred in the glucose level after the inhalation of halothane in either group.

If anaerobic metabolism insinuates during the inhalation of halothane, the tissue content of ATP may decrease. Nilsson et al.[10] found no change in the ATP content of the rat brain. Horikawa et al.[11] observed in the dog that halothane did not affect the rate of recovery of hepatic ATP content suppressed by hypoxia, denying the possibility that anaerobic metabolism plays an important role during halothane anesthesia. Hall et al.[12] claim that the lack of effect of halothane on the tissue ATP content in the whole animal can be attributed to a well-kept balance between the suppression of cellular metabolic activity and the inhibition of ATP production by halothane. In our whole animal study the lactate increased only very slightly, suggesting that suppression by halothane of aerobic cellular metabolism did not play any significant role.

Thus, the most conceivable reason for the difference in the observed metabolic phenomena between the perfused liver and the whole animal is that in the perfused liver anaerobic mechanism might have insinuated, although the oxygen delivering capability of the perfusion was sufficient, whereas in the whole animal there was no evidence of anaerobic metabolism attributable to the inhalation of halothane.

The difference in the metabolic reactions seen between the two experimental models might also be attributed partly to the involvement of neural and humoral mechanisms in the whole animal, such as the mobilization of catecholamines and insulin.

In conclusion, when the perfused rat liver was charged *in situ* with halothane the lactate and glucose increased and base excess decreased more markedly in the fed than in the starved rat. These changes brought about by halothane were time- and concentration-dependent. Similar changes were observed with enflurane. When the whole animal was made to inhale halothane, the glucose level and the degree of metabolic acidosis were not affected by whether the animal was fed or not.

The authors wish to thank Mr. Tadayoshi Karube for his assistance in statistical calculations and for preparing figures.

This study was supported in part by a Grant-in-Aid for Scientific Research (No. 61771105) from the Ministry of Education, Science and Culture, and presented in part at the 35th (1988, Kanazawa) and 36th (1989, Yamaguchi) Annual Meeting of the Japan Society of Anesthesiology.

(Received Sep. 5, 1991, accepted for publication Mar. 27, 1992)

References

1. Mortimore GE, Tietze F: Studies on the mechanism of capture and degradation of insulin-I^{131} by the cyclically perfused rat liver. Ann NY Acad Sci 82:329–337, 1959
2. Woods HF, Krebs HA: Lactate production in the perfused rat liver. Biochem J 125–139, 1971
3. Biebuyck JF, Lund P, Krebs HA: The effects of halothane (2-bromo-2-chloro-1,1,1-trifuluoroethane) on glycolysis and biosynthetic process of the isolated perfused rat liver. Biochem J 128:711–720, 1972
4. Cohen PJ: Effect of anesthetics on mitochondrial function. Anesthesiology 39:153–164, 1973
5. Merin RG, Samuelson PN, Schalch DS: Major inhalation anesthetics and carbohydrate metabolism. Anesth Analg 50:625–633, 1971
6. Kaniaris P, Lekakis D, Kykoniatis M, et al: Serum free fatty acid and

blood sugar levels in children under halothane, thiopentone and ketamine anesthesia. Canad Anaesth Soc J 22:509–518, 1975
7. Oyama T, Takazawa T: Effects of halothane anesthesia and surgery on human growth hormone and insulin levels in plasma. Br J Anaesth 43:573–580, 1971
8. Black GW, Mckane RV: Respiratory and metabolic changes during methoxyflurane anaesthesia. Br J Anaesth 37: 409–414, 1965
9. Yamazaki T, Naito H, Nakamura K, et al: Lactate, pyruvate, and excess lactate during ether and halothane anesthesia in infants and children. Anesthesiology 43:410–415, 1975
10. Nilsson L, Siesjo BK: The effect of anesthetics upon labile phosphates and upon extra- and intracellular lactate, pyruvate and bicarbonate concentrations in the rat brain. Acta Physiol Scand 80:235–248, 1970
11. Horikawa H, Ito Y, Naito E, et al: Halothane anesthesia and adenine nucleotide contents of canine liver and muscle. Acta Med Biol 27:43–47, 1979
12. Hall GK, Kirtland SJ, Baum H: The inhibition of mitochondrial respiration by inhalational anaesthetic agents. Br J Anaesth 45:1005–1009, 1975