Transplant Int (1990) 3: 128–132

TRANSPLANT
International

Postoperative recovery of mitochondrial function of the human liver graft procured and preserved with University of Wisconsin (UW) solution

N. Osaki[1], B. Ringe[1], H. Bunzendahl[1], Y. Taki[1], G. Gubernatis[1], M. Oellerich[2], E.-R. Kuse[1], M. Burdelski[3], S. Uemoto[4], M. Kimoto[4], Y. Yamaoka[4], K. Ozawa[4], and R. Pichlmayr[1]

[1] Clinic for Abdominal and Transplantation Surgery, [2] Clinic for Clinical Chemistry, and [3] Children's Clinic, Medizinische Hochschule Hannover, Konstanty-Gutschow-Strasse, D-3000 Hannover 61, Federal Republic of Germany
[4] The 2nd Department of Surgery, Faculty of Medicine, Kyoto University, 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto 606, Japan

Received December 6, 1989/Received after revision March 28, 1990/Accepted April 23, 1990

Abstract. Changes in arterial blood ketone body ratio (KBR) were investigated in 47 human liver transplantations. Of the 20 grafts preserved with University of Wisconsin (UW) solution, 10 had a cold preservation period of less than 10 h (UWS group) and 10 of more than 10 h (UWL group). In 27 other cases, grafts were preserved with EuroCollins (EC) solution for less than 10 h (EC group). In the EC group, KBR increased over 0.7 within 6 h after reperfusion of the graft in 17 cases (63%) and within 24 h in 7 cases (26%). In the 3 other cases, KBR failed to recover, and these patients underwent retransplantation. In the UW group, KBR recovered within 6 h in 13 cases (65%) and within 24 h in 7 cases (35%). There were no significant differences between the UWS and UWL groups. It is shown that the mitochondrial function of liver grafts preserved with UW solution can be well maintained even after extended preservation periods of more than 10 h.

Key words: Liver preservation, UW solution – Mitochondrial function, in liver preservation – Preservation, liver, UW solution – Ketone body ratio, liver

In the past 10 years, liver transplantation has emerged as an important therapy in the treatment of end-stage liver diseases [2, 15, 18], but it is still restricted by the shortage of donor grafts and by the limited preservation time of the grafts. UW solution, developed by the University of Wisconsin group, has been reported to be valuable for extending the period of organ preservation up to 24–48 h in canine and rabbit livers [5, 6]. Positive results have also been reported in clinical liver transplantation using a graft harvested and preserved with UW solution after extended preservation of more than 10 h [7]. If and when the effectiveness of this new solution is fully established, it will facilitate the transport of a harvested organ over longer distances, allowing liver transplantation to be performed under optimal conditions as an elective operation.

To evaluate the effects of the preservation solutions, it is necessary to clarify the postoperative factors affecting graft viability. According to Tanaka et al. [21], liver viability basically depends on the energy-producing capacity of the liver mitochondria, the functional state of which can be evaluated through the changes in arterial blood ketone body ratio (KBR; acetoacetate/3-hydroxybutyrate) reflecting the mitochondrial Redox state or NAD^+/NADH. That the functional viability of liver grafts depends on the mitochondrial function is supported by a number of experimental [9, 19] and clinical [20] studies. It has also been reported that a decrease in KBR after surgery is an early indicator of hepatic failure [12–14, 25].

In this study, in order to evaluate the effects of preservation solutions on the postoperative recovery of mitochondrial function of the graft, the early postoperative courses of patients who had received liver allografts harvested and preserved with UW solution were investigated in relation to the changes in KBR after recirculation of the graft, and the results were compared with those of patients receiving grafts with EuroCollins (EC) solution.

Materials and methods

Patients

From January 1988 to January 1989, a total of 77 adult liver transplantations were performed at the Klinik für Abdominal- und Transplantationschirurgie, Medizinische Hochschule Hannover. We studied 47 of these procedures in 43 patients (4 were retransplanta-

Table 1. Indications for liver transplantation

	EC group	UWS group	UWL group
Liver cirrhosis	8	3	4
Chronic hepatitis	4	1	2
Primary biliary cirrhosis	4	1	1
Budd-Chiari syndrome	2	0	0
Sclerosing cholangitis	0	1	0
Hepatocellular carcinoma	4	2	1
Other malignancies	1	1	1
Retransplantation	3	0	1
Other	1	1	0
Total	27	10	10

Offprint requests to: B. Ringe

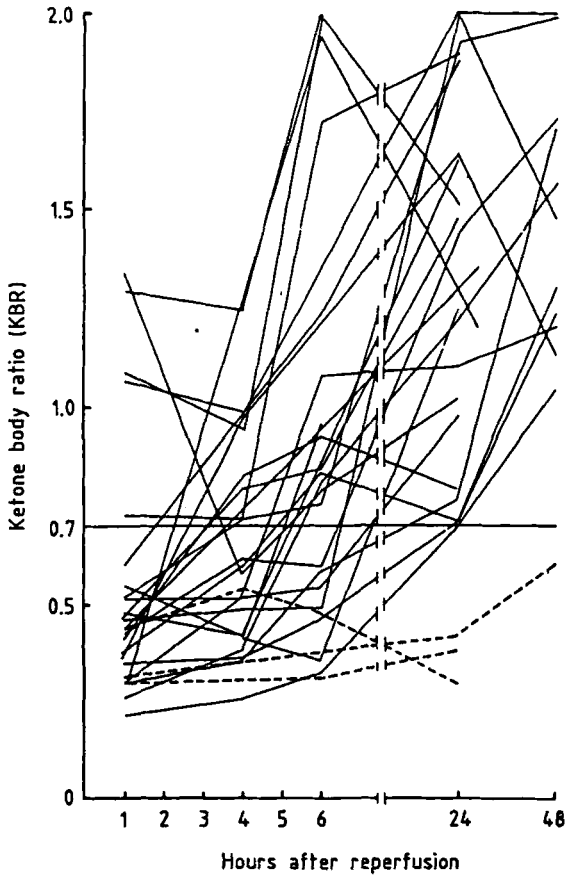

Fig. 1. Changes in ketone body ratio (KBR) after recirculation in the EC group ($n = 27$). Changes in cases of initial nonfunctioning graft are shown as *dotted lines*

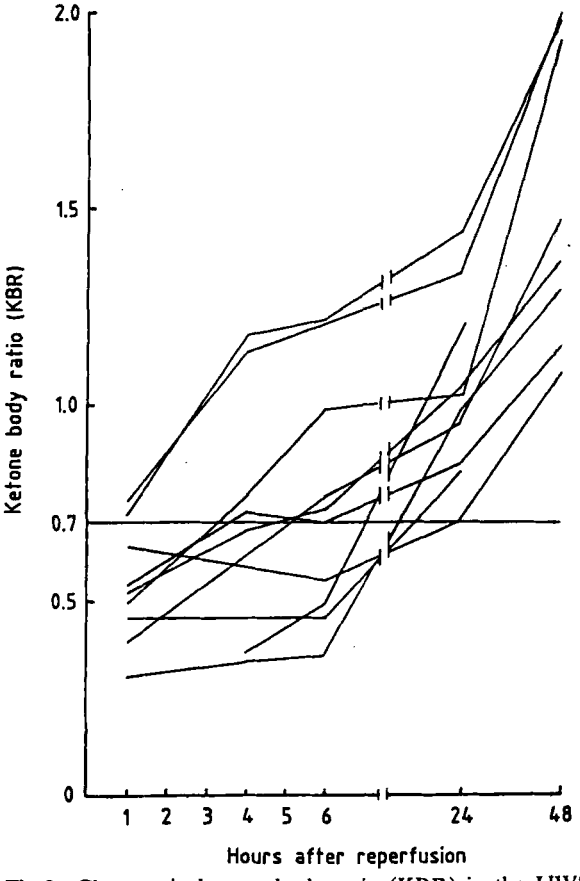

Fig. 2. Changes in ketone body ratio (KBR) in the UWS group ($n = 10$)

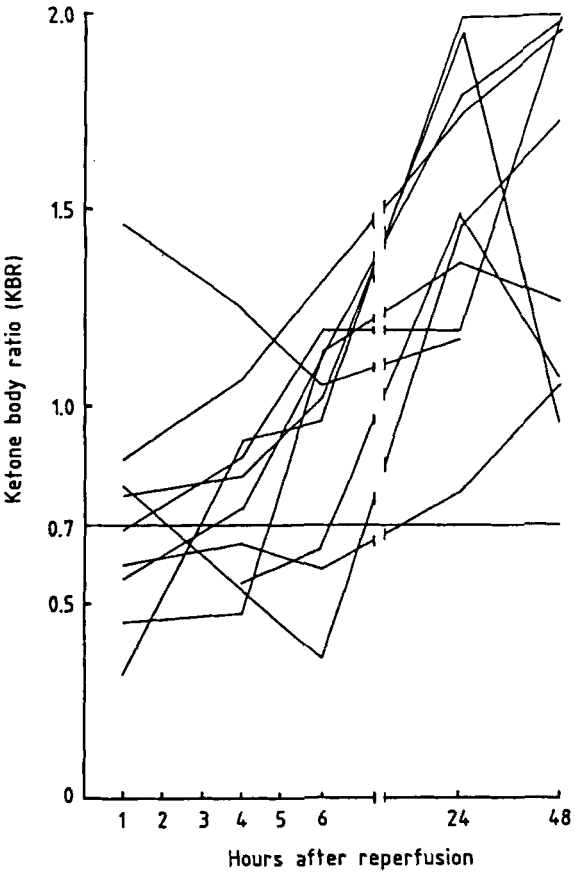

Fig. 3. Changes in ketone body ratio (KBR) in the UWL group ($n = 10$)

tions). Twenty of the liver grafts were harvested and preserved with UW solution (UW group) and 27 with EuroCollins solution (EC group). In the EC group, the average cold ischemia time was 5 h 59 min (range 2 h 46 min–9 h 55 min). The UW group was further divided into two groups: one of 10 patients with a cold ischemia time of less than 10 h (UWS group) and another of 10 patients with a cold ischemia time of more than 10 h (UWL group). The average preservation time was 7 h 24 min (range 5 h 36 min–9 h 9 min) in the UWS group and 17 h 33 min (range 10 h 19 min–24 h 18 min) in the UWL group. The primary diseases in each group are listed in Table 1.

Organ procurement and preservation

Donor hepatectomy was performed using techniques described elsewhere [10]. Following in vivo flushing of the aorta and portal vein with 2000 ml of ice-cold preservation solution (either EC or UW), the grafts were stored at 4 °C until use.

Recipient operation

Recipient hepatectomy and hepatic replacement were performed using techniques previously reported [16]. Postoperative immunosuppression consisted of cyclosporin A, low-dose steroids, and azathioprine [17].

Sampling protocol

Arterial blood sampling was performed in the anhepatic period at 1, 4, 6, 24, and 48 h after reperfusion. Ketone bodies (acetoacetate and 3-hydroxybutyrate) were measured enzymatically using a KETO-

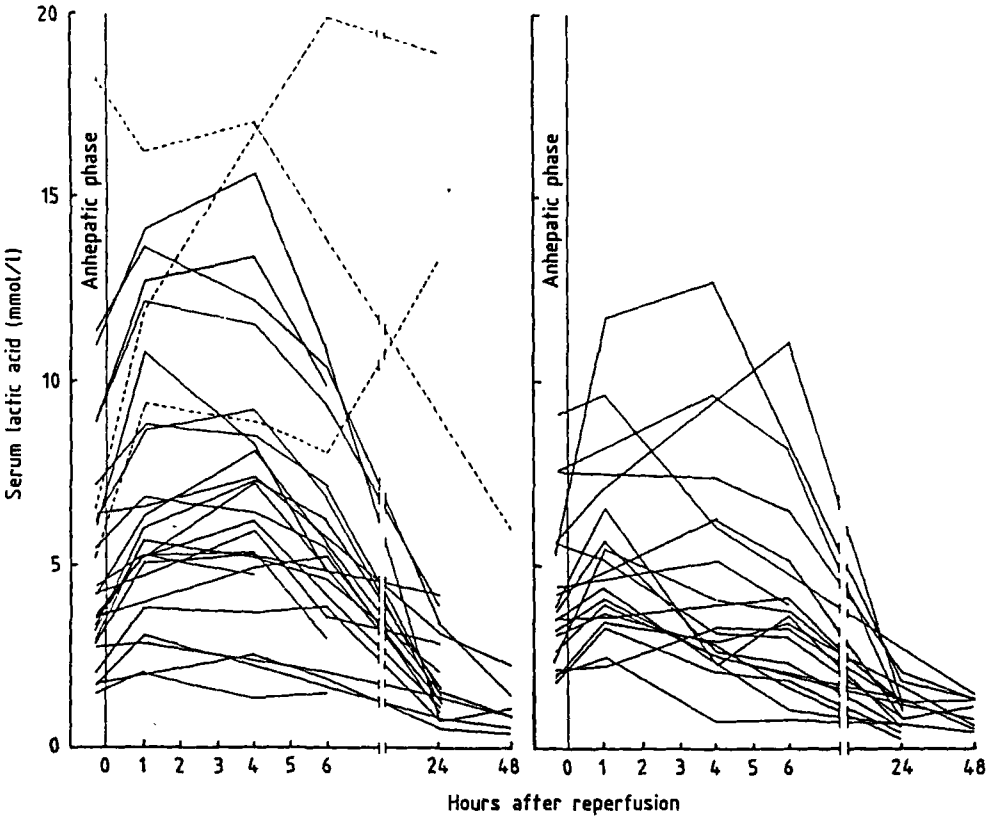

Fig. 4. Changes in plasma lactate concentration after recirculation in EC ($n = 27$) and UW ($n = 20$) (UWS + UWL) groups. Values of initial nonfunctioning cases are shown as *dotted lines*

REX Kit (Sanwa Chemical, Nagoya, Japan) and KETO-340 (a semi-automatic spectrophotometer designed for measurement of ketone bodies; Ihara Electric, Kasugai, Japan) [8, 22, 24]. Plasma lactate levels were also measured enzymatically [4].

Routine liver-related parameters, including serum aspartate aminotransferase (AST) level, total bilirubin concentration, and partial thromboplastin time (PTT) were measured daily until the 5th postoperative day by optimized standard methods.

Statistical analysis

Results are expressed as mean ± standard error. Statistical significance was determined by unpaired *t*-test. *P* values less than 0.01 were considered to be significant.

Results

Clinical outcome

At present, 31 of the 43 patients are alive, including 15 in the EC, 9 in the UWS, and 7 in the UWL groups.

Three of the 27 cases in the EC group were clinically diagnosed as having an initial nonfunctioning graft (INF) and required urgent retransplantation. All other patients recovered from the operation and survived longer than 1 week.

There were no instances of INF in the UW group, and all the grafts survived longer than 1 week. One patient who received a graft with the longest preservation time of 24 h 20 min developed bile duct necrosis due to impaired arterial perfusion on the 7th postoperative day and underwent retransplantation 42 days later.

Laboratory data

Figures 1, 2, and 3 show the changes in KBR after recirculation of the graft in the three groups. As shown in Fig. 1, in the EC group, KBR recovered over the critical level of 0.7 within 6 h after recirculation in 17 cases (63%). In 7 cases (26%), KBR increased over 0.7 within 24 h. KBR failed to recover in the other 3 patients (11%), and the diagnosis of INF was made in these cases.

As shown in Fig. 2, in the UWS group, KBR recovered within 6 h in 6 cases (60%) and within 24 h in an additional 4 cases (40%). Figure 3 shows the changes in KBR in the UWL group. KBR reached 0.7 within 6 h in 7 cases (70%) and within 24 h in the other 3 cases (30%). Recovery patterns of patients were almost identical among the three groups.

Figure 4 shows the changes in plasma lactate level of the EC and UW groups. Values of INF cases are indicated by dotted lines. In all cases, lactic acid accumulated in the anhepatic phase, and its concentration further increased immediately after recirculation of the graft. Normalization in lactate concentration was obtained on the 2nd postoperative day. Plasma lactate level remained extremely high throughout the postoperative course in INF cases. There was no significant difference in patients who survived among the three groups.

Table 2 shows the changes in AST, total serum bilirubin concentration, and partial thromboplastin time in the three groups; INF cases were excluded here. AST level on the 1st postoperative day was significantly higher in the UWL group than in the UWS group. There were no significant differences in the changes of total bilirubin concentration or PTT among the three groups.

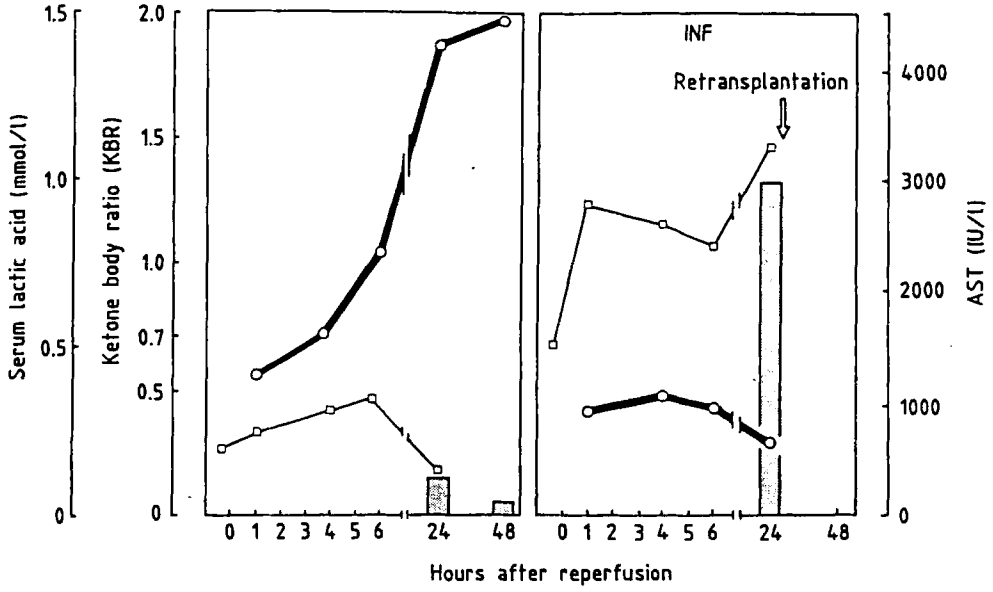

Fig. 5. Changes in ketone body ratio (KBR, o—o), serum lactic acid (LA, □—□) concentration, and aspartate aminotransferase (AST, ■) in two selected patients. *INF*, Initial nonfunctioning graft

Figure 5 shows two cases, one with a functioning graft and the other with a nonfunctioning graft. The patient represented on the left was a 48-year-old woman who underwent liver transplantation for hepatocellular carcinoma. The graft was preserved with UW solution and the cold ischemia time was 18 h 13 min. KBR increased promptly after the operation. Lactic acid concentration returned to a normal level and the AST was 330 and 109 IU/l on the 1st and 2nd postoperative days, respectively. On the 1st postoperative day the patient was extubated, and on the 2nd postoperative day she was able to call to her husband, who was sitting at her bedside.

The patient represented on the right was a 46-year-old man with hepatocellular carcinoma who received a graft preserved for 6 h 41 min with EC solution. After the operation KBR failed to increase; lactic acid and AST rose markedly on the 1st postoperative day, and the liver allograft produced only a small amount of bile. The clinical diagnosis of initial nonfunction was made and the patient underwent retransplantation the following day.

Table 2. Changes in aspartate aminotransferase (AST), total bilirubin, and partial thromboplastin time (PTT) in EC, UWS, and UWL groups. Numbers of patients are given in parentheses. Normal values: AST < 18 IU/l, bilirubin < 17 μmol/l, PTT 33–40 s. * $P < 0.01$

Aspartate aminotransferase (IU/l)					
Postoperative day	1	2	3	4	5
EC (24)	454 ± 77	252 ± 67	145 ± 40	67 ± 10	124 ± 68
UWS (10)	254 ± 39*	209 ± 75	166 ± 68	110 ± 42	106 ± 46
UWL (10)	802 ± 107*	713 ± 181	412 ± 104	139 ± 29	67 ± 12
Bilirubin (μmol/l)					
Postoperative day	1	2	3	4	5
EC (24)	78.9 ± 16.9	70.4 ± 13.6	81.4 ± 16.5	108.6 ± 16.2	121.3 ± 17.9
UWS (10)	102.5 ± 30.4	104.8 ± 15.8	105.3 ± 20.8	107.0 ± 22.0	120.8 ± 26.9
UWL (10)	105.4 ± 19.0	150.8 ± 52.5	153.3 ± 39.4	210.4 ± 55.9	282.6 ± 75.1
Partial prothrombin time (s)					
Postoperative day	1	2	3	4	5
EC (24)	47.7 ± 1.5	51.0 ± 1.9	45.6 ± 2.1	45.3 ± 2.2	45.8 ± 3.2
UWS (10)	49.5 ± 3.4	45.6 ± 2.6	47.9 ± 4.8	38.6 ± 2.0	40.4 ± 2.6
UWL (10)	49.9 ± 2.9	57.4 ± 5.1	43.3 ± 2.7	45.1 ± 1.8	45.1 ± 2.2

Discussion

The advantages of UW solution have been discussed by Belzer and Southard [1], who indicate that its effectiveness lies especially in raffinose and lactobionate, two impermeants used in place of glucose to prevent cell swelling during preservation. They also suggest one disadvantage of glucose, which is the main impermeant in EC solution, in relation to intracellular lactic acidosis during preservation. Glucose stimulates the undesired production of lactate and hydrogen ions, a process which continues to occur during preservation since the liver has the potential for anaerobic glycolysis during cold storage. On the other hand, cell swelling and intracellular acidosis may suppress mitochondrial function, one of the key factors in the metabolic integrity of the graft. The potential of UW solution should thus be evaluated not only in terms of clinical results but also by the functional recovery of mitochondrial function of allografts after restoration of the blood supply.

In the present study, immediate retransplantation for INF was necessary in three cases in the EC group (11%). By contrast, initial graft function was excellent in all cases in the UW group. Moreover, rapid recovery of KBR, which reflects hepatic mitochondrial REDOX state, was obtained in patients receiving grafts procured and preserved with UW solution even after long periods of preservation. As we have reported, the recovery of KBR may depend on the functional reserve of the graft, as well as on the metabolic load placed on the graft by the recipient. Hence, the early recovery of mitochondrial function, indicated by the early recovery of KBR, guarantees an uneventful postoperative course [20]. On the other hand, a delay in or lack of KBR recovery implies functional

failure of the transplanted organ [20]. The remarkable difference beween the two cases reported in this study clearly shows the importance of early postoperative recovery of KBR after liver transplantation. KBR also correlated well with the hepatic energy charge, a parameter for evaluating the high energy state of the liver, as reported by Ozawa et al. [14, 21].

However, the recovery of KBR in the UWS group was not significantly different from that in the EC group. Moreover, elevation in plasma lactate concentration immediately after reperfusion of the graft was not suppressed in the UW group when compared with the EC group, as shown in Fig. 2. Since elevation in lactate level after reperfusion may be caused by the release of lactate from the graft, it may be assumed that the intracellular accumulation of lactate during preservation was not suppressed effectively by UW solution. The subsequent decrease in lactate concentration was also the same with both UW and EC solutions, and normalization in the lactate level was obtained only after the recovery of KBR. Although some experimental results support the possibility that UW solution prevents intracellular acidosis of hepatocytes [11, 23], the suppression of intracellular acidosis by preservation solutions has been reported to be problematic [3]. Further experimental investigations, such as the direct measurement of tissue lactate concentration and pH after different periods of cold ischemia, are necessary to ascertain the effects of UW solution on lactate metabolism.

AST levels in the UWL group were also significantly higher on the 1st postoperative day than those in the UWS group. This result is compatible with that of Kalayoglu et al. [7]. Undoubtedly, it indicates ischemic damage to the graft caused by the prolonged preservation period. This is suggested by the fact that the patient receiving the graft with the longest preservation time developed bile duct necrosis. Further prolongation of preservation time cannot be recommended, although this complication cannot be explained by the extended preservation period alone.

In conclusion, preservation of human liver grafts for more than 10 h can be successfully performed with UW solution. These results are based on changes in KBR and on the lower incidence of INF. On the other hand, more clinical and experimental data are needed to clarify the metabolic changes occurring during preservation before the safety of liver preservation with UW solution for periods over 20 h can be confirmed.

Acknowledgement. This work was supported in part by a grant from the Alexander von Humbolt Foundation. N. Osaki and Y. Taki are recipients of fellowships from the foundation.

References

1. Belzer FO, Southard JH (1988) Principles of solid-organ preservation by cold storage. Transplantation 45: 673–676
2. Calne RY, Williams R, Rolles K (1986) Liver transplantation in adults. World J Surg 10: 422–431
3. Fuller BJ (1988) Control of pH during hypothermic liver storage – role of the storage solution. Transplantation 45: 239–241
4. Gutmann I, Wahlefeld AW (1974) L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. In: Bergmeyer HU (ed) Methods of enzymology. Academic Press, New York, pp 1464–1468
5. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO (1988) Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 46: 517–522
6. Jamieson NV, Sundberg R, Lindell S, Southard JH, Belzer FO (1988) A comparison of cold storage solutions for hepatic preservation using the isolated perfused rabbit liver. Cryobiology 25: 300–310
7. Kalayoglu M, Sollinger HW, Stratta RJ, D'Alessandro AM, Hoffmann RM, Pirsch JD (1988) Extended preservation of the liver for clinical transplantation. Lancet I: 617–619
8. Mellanby J, Williamson DH (1974) Acetoacetate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 1446–1451
9. Morimoto T, Ukikusa M, Taki Y, Koizumi K, Yokoo N, Tanaka A, Noguchi M, Yamamoto S, Nitta N, Kamiyama Y, Yamaoka Y, Ozawa K (1988) Changes in energy metabolism of allografts after liver transplantation. Eur Surg Res 20: 120–127
10. Neuhaus P, Broelsch C, Ringe B, Lauchart W, Pichlmayr R (1984) Experience with combined donor nephrectomy and hepatectomy. Transplant Proc 16: 235–237
11. Newsholme EA, Start C (1974) Regulation in metabolism. Wiley, London, pp 247–252
12. Nitta N, Yamamoto S, Yamaoka Y, Ozawa K (1988) Arterial blood ketone body ratio as an indicator of the no-return point in hepatic flow occlusion without venous shunt in dogs. Life Sci 42: 1973–1979
13. Ozawa K (1983) Biological significance of mitochondrial redox potential in shock and multiple organ failure – redox theory. In: Lefer AM, Schumer W (eds) Molecular aspects of shock and trauma. Liss, New York, pp 39–66
14. Ozawa K, Aoyama H, Yasuda K, Shimahara Y, Nakatani T, Tanaka J, Yamamoto M, Kamiyama Y, Tobe T (1983) Metabolic abnormalities associated with postoperative organ failure. A redox theory. Arch Surg 118: 1245–1251
15. Pichlmayr R (1987) Indication for liver transplantation. Dtsch Med Wochenschr 112: 20–22
16. Pichlmayr R, Bockhorn WH, Neuhaus P (1983) Lebertransplantation. In: Breitner B (ed) Chirurgische Operationslehre 6. Urban & Schwarzenberg, München Wien Baltimore, pp 1–19
17. Pichlmayr R, Ringe B, Lauchart W, Wonigeit K (1987) Liver transplantation. Transplant Proc 19: 103–112
18. Starzl TE, Iwatsuki S, Shaw BW Jr, Gordon RD (1985) Orthotopic liver transplantation in 1984. Transplant Proc 17: 250–258
19. Taki Y, Ukikusa M, Morimoto T, Yokoo N, Koizumi K, Noguchi M, Tanaka A, Yamamoto S, Nitta N, Kamiyama Y, Shimahara Y, Yamaoka Y, Ozawa K (1987) Short-term changes in blood ketone body ratio in the phase immediately after transplantation. Transplantation 43: 350–353
20. Taki Y, Gubernatis G, Yamaoka Y, Oellerich M, Yamamoto Y, Ringe B, Okamoto R, Bunzendahl H, Beneking M, Burdelski M, Bornscheuer A, Pichlmayr R (1990) Significance of arterial blood ketone body ratio measurement in human liver transplantation. Transplantation 49: 535–539
21. Tanaka J, Ozawa K, Tobe T (1979) Significance of blood ketone body ratio as an indicator of hepatic cellular energy status in jaundiced rabbits. Gastroenterology 76: 691–700
22. Uno S, Itoh M, Kurono Y, Yamaoka Y, Kamiyama Y, Ozawa K (1987) A simple and sensitive assay for blood ketone bodies using highly purified 3-hydroxybutyrate dehydrogenase. Clin Chim Acta 168: 253–255
23. Vessel ES (1963) Significance of the heterogeneity of lactic dehydrogenase activity in human tissues. Ann NY Acad Sci 94: 912–918
24. Williamson DH, Mellanby J (1974) D-(–)-3-hydroxybutyrate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 1836–1839
25. Yamamoto S, Nitta N, Wada Y (1988) Hepatic warm ischemia in pigs using a centripetal force pump. Surg Res Comm 3: 5–14