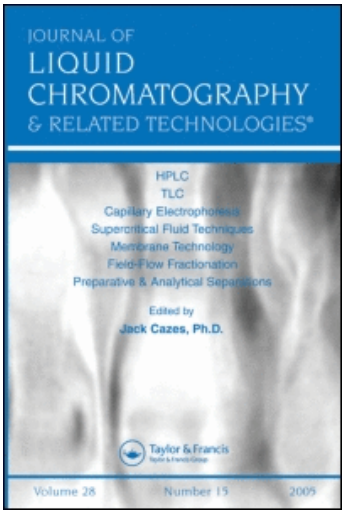

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LIPID PEROXIDATION STUDIED BY HPLC IN ISOLATED RAT LIVER: COMPARATIVE EFFICIENCY OF UW AND HTK PRESERVATION SOLUTIONS

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ABSTRACT

Lipid peroxidation on preserved isolated rat liver.

Lipoperoxidation (LPO) generated by activated oxygen species could be a cause of damage observed in isolated organs during normoxic reperfusion. Free and bound MDA, vitamin E and vitamin A analyses were performed by HPLC on extracts from livers preserved in two hepatic clinical preservation solutions; ATP level and intracellular pH were followed by ^{31}P NMR. The data indicate that bound MDA values increased during ischemia and decreased upon cold and warm reperfusion. This rather unexpected MDA accumulation during

ischemia supports the hypothesis that lipoperoxidation might be initiated by ADP-Fe^{+++}-O_2^- complex accumulating during this period. Furthermore, in lactobionate-solution preserved livers, we found during reperfusion a significant decrease in vitamin E content concomitantly with a decrease in bound MDA. This observation suggests a protective effect of vitamin E in lipoperoxide formation in livers stored in UW. This phenonenom is not observed in HTK-preserved livers.

INTRODUCTION

Much evidence now supports oxygen radicals as being a causative factor in a number of pathologies involving cell damage. Lipoperoxidation (LPO) initiated by oxygen-derived free radicals, plays an important role in hepatic injury associated with ischemia. A mechanism for the generation of free radicals during the reperfusion of an ischemic organ has been proposed by Mc Cord (1) and Schoenberg et al (2) : the basic source of the oxygen-derived free radicals is xanthine oxidase (XOD). During ischemia, xanthine dehydrogenase is converted to XOD and the substrates for XOD are generated by the breakdown products of adenine nucleotides. On reperfusion, oxygen is supplied to XOD, which converts hypoxanthine to uric acid and generates the superoxide anion and hydrogen peroxide, activated oxygen species which could then initiate LPO.

Liver damage may therefore cumulate effects of ischemia and reperfusion, which may occur during sequential

steps in organ procurement: cold ischemic organ preservation in storage solutions and/or reperfusion after transplantation (3).

Unfortunately, some evidence supporting the free radical hypothesis of ischemic reperfusion cell damage has often been indirect, by analysing the effect of free radical scavenger antioxidants or inhibitors of oxidant-producing enzymes (4-10). However, Henry et al (11) using an enhanced chemiluminescent technique for continuous monitoring of radical generation, have recently indicated that there is a decrease in free radical generation during ischemia and a pronounced increase during the early reperfusion period.

On the other hand, Tien et al (12) and Sugioka et al (13) were unable to find evidence for involvement of OH-radicals in lipid peroxidation on two *in vitro* model systems. They proposed that initiation of lipid peroxidation may be mediated by the "ADP-perferryl ion" which abstracts hydrogen directly from polyunsaturated fatty acids without the OH° intermediate action (14). An initiator of LPO was produced by the reaction of ADP-Fe^{++} with O_2 with subsequent formation of a ferric-type oxygen complex such as ADP-Fe^{+++}-O_2^-(15).

Using the procedure of cold ischemia and oxygenated reperfusion as a model of oxidative stress, we studied LPO and vitamin levels by high-performance liquid chromatography (HPLC), associated with ^{31}P nuclear magnetic resonance (NMR),

to analyze the ischemic breakdown of ATP, its reperfusion recovery, and the intracellular pH (pH_{in}).

The aim of this study was to evaluate the malondialdehyde (MDA) / thiobarbituric acid (TBA) response towards damage produced by LPO in the perfusion-ischemia-reperfusion sequence. This evaluation was performed with two cold storage solutions used in hepatic clinical transplantation: University of Wisconsin solution (UW) and Bretschneider's solution (HTK), containing mainly lactobionate or histidine (16) respectively, and free radical scavengers such as allopurinol (9, 10) or mannitol (14). The results are discussed with regard to the ADP-perferryl-ion hypothesis and to the complex relationship between MDA analysis performed by HPLC and LPO damage occuring in a biological complex system. The reciprocal action of vitamins A and E on LPO damage, analyzed by HPLC, is also discussed.

MATERIAL AND METHODS

Liver Perfusion.

Wistar rats (2-3 weeks old ; 60-150 g) were used as liver donors. They were fed *ad libitum* with complete food for

rats and mice (reference AO3, 91360 Epinay sur Orge, France) containing vitamin A: 12000 UI/kg; vitamin D_3: 3000 UI/kg; vitamin E: 30 mg/kg. The experiments were always started at the beginning of the afternoon. For all the rats tested, there was a good correlation between liver weight and body weight ($r= 0.778$; $n = 55$; $P<0.001$). Liver weight was considered as age-dependent in this body weight range. After intraperitoneal injection of pentobarbital (50 mg/kg), the liver was perfused at a flow rate of 3ml/min/g of liver wet weight and transferred to the NMR cell as previously described (17). The liver was perfused in a non-recirculating mode and the perfusate was gassed with 95 % O_2 and 5 % CO_2. The temperature was regulated by means of two thermostatic baths.

The protocol of the perfusion-ischemia-reperfusion sequence which reproduced the clinical procedure was as follows: (1) metabolic equilibration period: 30 min perfusion at 37°C then 15 min perfusion at 4°C with Krebs buffer followed by 15 min perfusion at 4°C with the preservation solution (HTK or UW); (2) 24 hour cold ischemia in the preservation solution; (3) 15 min reperfusion at 4°C in Krebs buffer, then (4) 30 min subsequent perfusion at 37°C in Krebs buffer. The composition of the three solutions used is given in Table 1.

The NMR data were obtained throughout the sequence. At the end of the different phases of the experimental protocol,

TABLE 1

Composition of Perfusion Buffer (Krebs) and Preservation Solutions (HTK and UW).

Substance	Amount (mmol/l)		
	Krebs	HTK[a]	UW[b]
Na^+	143	15	30
K^+	5	10	
Ca^{++}	2		
Mg^{++}	1.2	4	5
Cl^-	127	32	
HCO_3^-	25		
$H_2PO_4^-/HPO_4^{--}$	1.2		25
SO_4^{--}	1.2		5
Lactobionate			100
Raffinose			30
Mannitol		30	
L-Histidine		200	
pH (at 20°C)	7.35	7.15	7.40

[a] HTK was supplemented with 2 mmol/l L-Tryptophan and 1 mmol/l α-Ketoglutarate.

[b] UW-Lactobionate was supplemented with 5 mmol/l adenosine, 3 mmol/l glutathione, 8 mg/l dexamethasone, 1 mmol/l allopurinol and 50 g/l hydroxyethylstarch.

livers were freeze-clamped between precooled aluminium tongs in liquid nitrogen and the tissue was used for malondialdehyde and vitamin determinations, after -80°C conservation.

In order to measure the percentage of residual O_2 during ischemia, livers from one other group of rats were perfused at 37°C and then at 4°C in Krebs buffer gassed with 95% O_2 and 5% CO_2. Then they were submitted to cold ischemia in Krebs buffer with the surrounding atmosphere saturated by nitrogen, while in normal perfusion-ischemia-reperfusion sequence, the livers were submitted to cold ischemia in the preservation buffer with the normal air atmosphere (18). The oxygen percentage was then measured in Krebs buffer.

NMR Spectroscopy.

NMR experiments were performed in a Bruker AM400 spectrometer operating at 9.4 Tesla. The spectra were recorded with a ^{31}P probe accorded to 161.9 MHz, as previously described (18).

The ^{31}P NMR spectra allowed the quantification of intracellular phosphorylated metabolites. ATP content was estimated from the area of β-ATP resonance. The level of ATP calculated during the beginning of the perfusion (phase 1) was taken as the basal content (100 %) for estimating the variation

in metabolite levels throughout the entire protocol. The absolute levels were determined by comparing the β-ATP resonance area with the area of a known quantity of ^{31}P atoms of methylene diphosphonic acid (MDPA) contained in a reference capillary.

Intracellular pH was estimated from the chemical shift of intracellular P_i resonance at each temperature (37°C and 4°C).

This NMR non-invasive method allowed direct and continuous monitoring of the energy status on the same liver, so it was possible to work on a reduced number of samples.

Free and Bound MDA Determination by HPLC.

Approximately 150 mg of frozen liver tissue were homogenized in 1.0 ml of ice-cold 7 % $HClO_4$. The precipitated proteins were removed by centrifugation at 1000g for 10 min. To 50 µl of 0.5 % thiobarbituric acid (TBA) were added 300 µl of supernatant sample and the color reaction was activated by heating for 30 min at 100°C for free MDA analysis. The protein pellets were homogenized in 1.0 ml of 0.5 N NaOH and incubated in a 60°C water bath for 30 min. Protein concentrations of hydrolysed homogenates were determined by the method of Lowry et al (19) on 50 µl aliquots. For bound MDA analysis, the hydrolysed samples were then acidified to

pH 1 with 400 µl of 35 % $HClO_4$. After centrifugation at 1000g for 10 min, the clear supernatants were treated with TBA as for free MDA determination. HPLC analyses were then performed as previously described with tetraethoxypropane as a standard (20, 21). The values were expressed as pmoles of MDA per mg of liver proteins.

In our experimental conditions ($HClO_4$, pH 0.75±0.07; mean ± SD), aldehydic compounds (trans 2-hexenal, trans 2-octenal, trans 2-4 hexadienal, trans 2-4 nonadienal...) react with TBA giving yellow 450 nm-, orange 495 nm-, and red 532 nm- absorbing chromophores (22,23). However at pH<1, the yield of the red 532 nm- absorbing pigment produced by these aldehydes at 100°C during 1 hour was only 0.03 to 0.3% of that of MDA, and was considered as negligible (24), while the yield was 5-10 % at pH 3.5 (23).

Moreover, we verified that lactate and pyruvate, three-carbon oxidized products of anaerobic glycolysis produced during ischemia, did not interfere with the MDA/TBA chromogen spectrum on account of their oxygenated group.

In order to determine the *in vitro* instability of MDA in the presence of H_2O_2 (potential initiator of LPO during oxygenated reperfusion), liposomes of purified phospholipids (PL) of pig liver were prepared as follows: 10 mg PL in chloroform/methanol (2/1; v/v) were evaporated to dryness under nitrogen and dispersed in 10 ml 0.15 M KCl/ phosphate buffer pH 7.4. This suspension was then submitted to

sonication at 0°C under a stream of nitrogen. Liposomes were incubated at 37°C for 5 hours with H_2O_2 (0-50 mM) in the presence of 1 mM Fe^{++}/10 mM ADP. We used 0.5 ml of the incubated suspension to assay fatty acids after transformation into isopropylic esters and analysis by gas chromatography (26), and the same quantity was used to assay MDA after TBA reaction as described above for free MDA. These aliquots were taken at different incubation times. LPO can be initiated by H_2O_2/ADP-Fe^{++}, as shown by the decrease in polyunsaturated fatty acids (figure 1). A time-dependent decline in the amount of MDA/TBA adduct resulted from the reaction between MDA and H_2O_2 during incubation but not during heating with TBA (25); this shows the instability of MDA in the presence of a millimolar concentration of H_2O_2.

Vitamin Determination by HPLC.

Approximately 300 mg of frozen liver tissue were homogenized in 1.0 ml of water purged with nitrogen. Vitamins were extracted with a mixture of 2 volumes of ethanol containing retinyl acetate and tocopherol acetate as internal standard, and 4 volumes of hexane for 1 volume of homogenate. After centrifugation at 1000g the hexane layer was evaporated in a nitrogen atmosphere and then dissolved

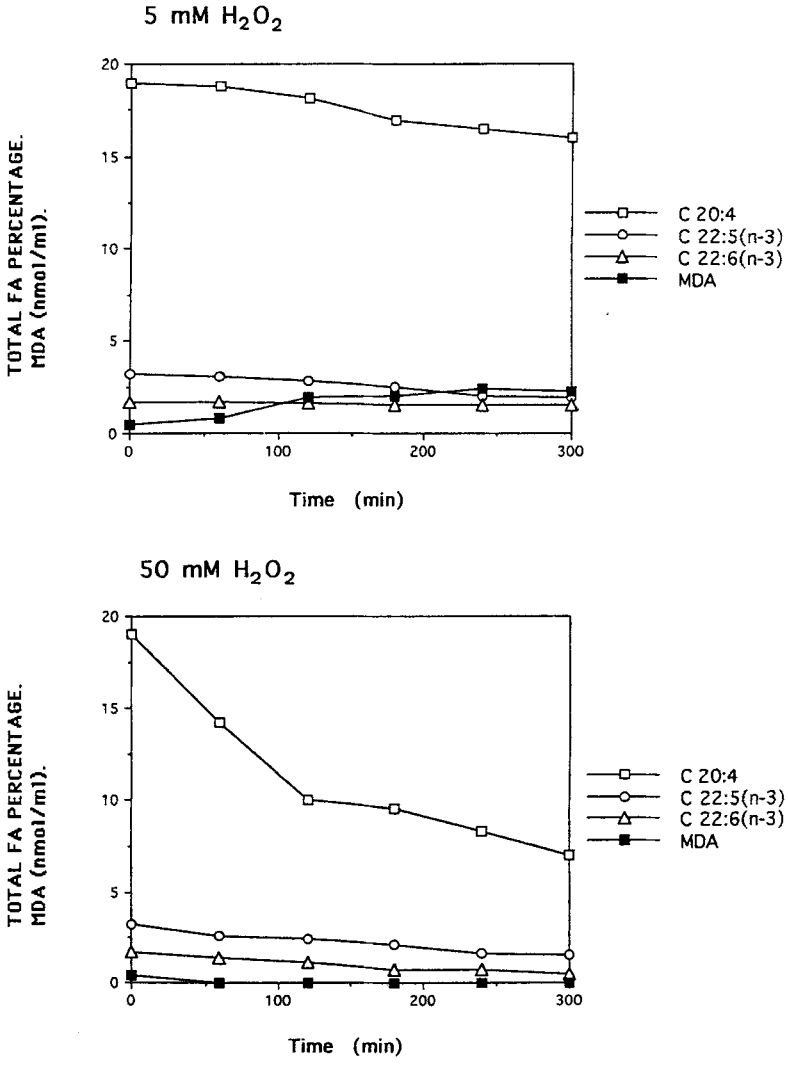

Figure 1: Liposome oxidation by hydrogen peroxide (5 and 50 mM): polyunsaturated fatty acids (FA) and MDA value produced by fatty acid oxidation expressed as nmol/ml of the incubation mixture.

in 1 volume of methanol and subjected to HPLC, as described previously (26).

The values are expressed as pmoles of vitamin E per mg of liver proteins or as the molar ratio of retinol to retinyl palmitate.

Statistical Analysis.

Data are presented as means ± SD or S.E.M. (details below). For comparison between unpaired data, the U test of Mann-Whitney was chosen because of the sample size. $P<0.05$ was considered significant. Linear regression analysis was used to assess the relationship between rat body-and liver-weights and between MDA values and age-dependent liver weights. Statistical analysis was done with the Stat View software.

RESULTS

Evolution of Energy Parameters and Intracellular pH during Perfusion, Hypothermic Ischemia and Subsequent Reperfusion

The ATP level (7.23±0.21 μmol/g of liver dry weight) during the initial 37°C perfusion period was taken as baseline content (100 %).

At the end of the 24-hour ischemia, ATP was negligible in livers stored in HTK and in UW preservation media. Cold reperfusion with oxygenated Krebs buffer induced an immediate increase in ATP and warm reperfusion restored to 65±11 % or 70±5 % the initial ATP content in livers stored in HTK or UW media, respectively (Table 2).

The initial pH_{in} (7.25±0.05) was taken as a control value. At the end of ischemic preservation, the pH_{in} decreased to 7.12±0.05 in HTK preserved livers. In the UW solution, the pH_{in} was difficult do determine with accuracy, owing to the high phosphate concentration. Rapidly after the reperfusion started, the pH_{in} reached a value which was not significantly different from the initial value for livers preserved in HTK and UW (Table 2).

Changes in Lipid Peroxidation during Hypothermic Ischemia and Reperfusion.

Lipid peroxidation was evaluated as free MDA or MDA bound to NH_2 groups after derivatization with thiobarbituric acid and separation of the red chromogen by HPLC.

In the initial period, there was a good linear correlation between free and bound MDA and liver weight (r=0.762 and

TABLE 2

NMR Determination of ATP Content and Intracellular pH of Isolated Rat Liver During the Perfusion-Ischemia-Reperfusion Sequence.

	Initial 37°C Perfusion in Krebs Medium	24-Hour Cold Ischemia in Preservation Media		Reperfusion in Krebs Medium 4°C	37°C
ATP	100% (n=13) (7.23±0.20 μmol/g liver dry weight)	HTK :	0% (n=10)	66±5% (n=5)	60±11%(n=5)
		UW :	0% (n=8)	77±11% (n=5)	70±5% (n=5)
pH_{in}	7.25±0.05 (n=13)	HTK :	7.12±0.05 (n=11)	7.26±0.05 (n=5)	7.18±0.06 (n=5)
		UW :	not determined	7.17±0.07 (n=5)	7.29±0.06 (n=5)

Results are expressed as mean±S.E.M.

r=0.759 respectively; n =8; P<0.025). So for the experimentation, only livers weighing in the range of 3 to 6.5 g were retained for homogeneous sampling and to eliminate the incidence of an age-dependent increase in MDA (27). During ischemia in both preservation media there was no significant correlation between free or bound MDA, and liver weight. Increasing the temperature from 4°C to 37°C led to an improvement in the correlation; then there was a significant correlation between bound MDA and liver weight only in livers stored in UW medium (Table 3).

Mean values of free and bound MDA for each period are shown in figure 2. There was no significant difference between free MDA values during the course of experimentation in either preservation media. Bound MDA values seemed to increase weakly during ischemia in both preservation media, and had a propensity to decrease during reperfusion especially in livers stored in UW medium. In fact, MDA showed changes in relation to duration of ischemia.

Therefore, when the bound MDA values were measured in the first hours of cold ischemia in both preservation media, there was an increase in the MDA value, reaching a maximum at about 1 or 2 hours. This initial increase agreed with a fast initial decrease in the ATP amount (figure 3) due to its degradation into ADP. The bound MDA value then decreased with ATP content and an equilibrium value was reached at the

TABLE 3

Linear Correlation between Free or Bound MDA and Liver Weight.

	Initial 37°C Perfusion in Krebs Medium	24-Hour Cold Ischemia in Preservation Media		Reperfusion in Krebs Medium 4°C	37°C
Free MDA	r=0.762; P<0.025 (n=8)	HTK :	r=0.326; NS (n=10)	r=0.222; NS (n=5)	r=0.725; NS (n=5)
		UW :	r=0.454; NS (n=8)	r=0.110; NS (n=5)	r=0.511; NS (n=5)
Bound MDA	r=0.759; P<0.025 (n=8)	HTK :	r=0.473; NS (n=10)	r=0.026; NS (n=5)	r=0.677; NS (n=5)
		UW :	r=0.087; NS (n=8)	r=0.804; 0.1<P<0.05 (n=5)	r=0.827; P<0.05 (n=5)

NS= not significant.

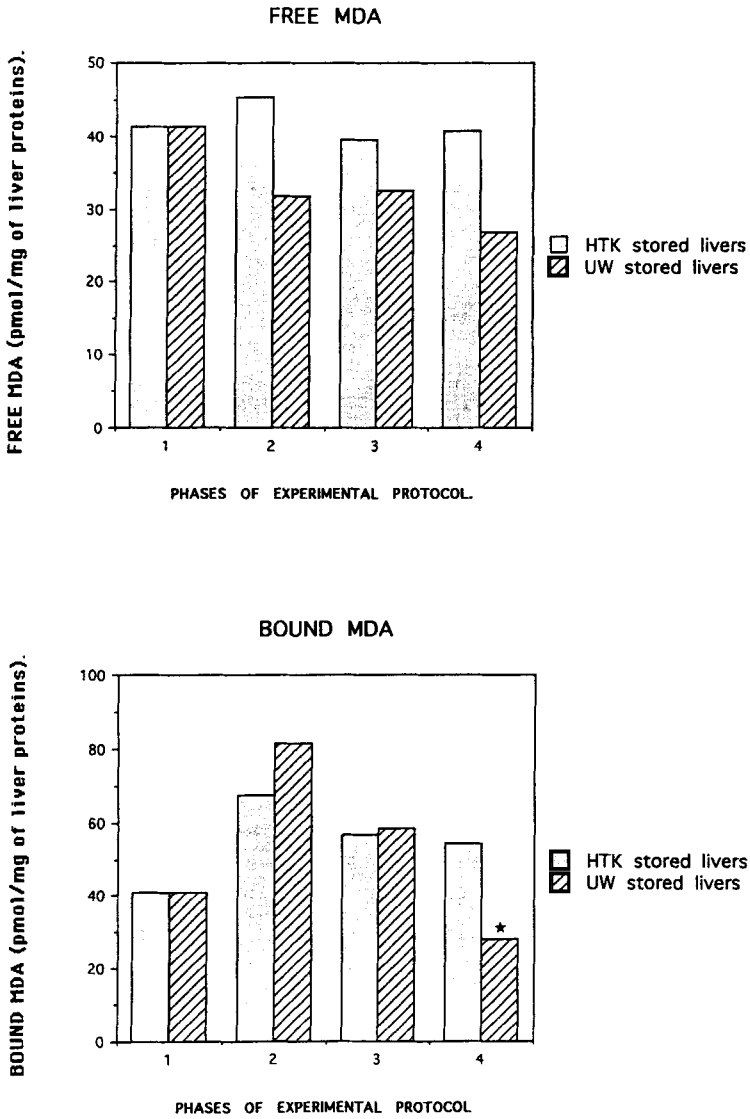

Figure 2: Free and bound MDA in HTK or UW stored livers. The phases of experimental protocol are as follows: -1- metabolic equilibrium period in Krebs buffer; -2- 24 hours cold ischemia in preservation solution (HTK or UW); -3- cold reperfusion in Krebs buffer; -4- warm reperfusion in Krebs buffer.

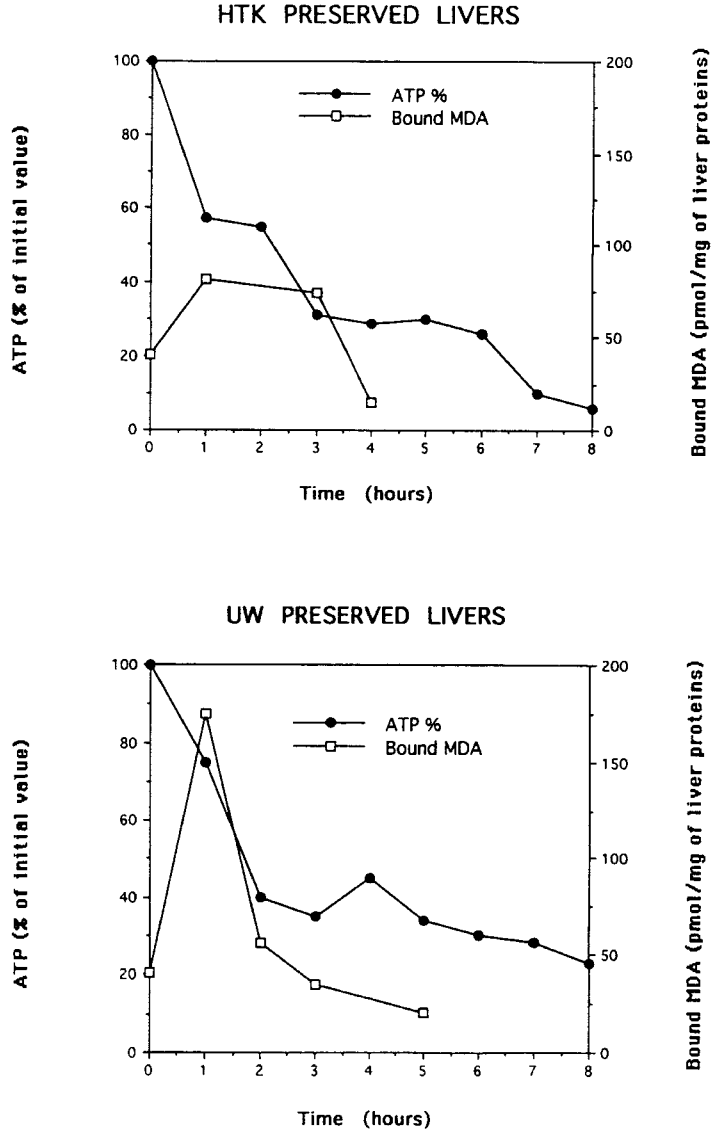

Figure 3: ATP content in HTK or UW stored livers and free MDA release in preservation solution during the first hours of cold ischemia.

end of ischemia. During the first two hours of cold ischemia, there was residual oxygen in the preservation medium (figure 4). The O_2 content at the beginning of ischemia was very high owing to the greater O_2 solubility at 4°C compared to 37°C. The O_2 content rapidly decreased with time, but was still 12% after 1 hour and 3% after 2 hours.

Free MDA was also measured in the surrounding liver medium. The different values obtained are shown in figure 5. The baseline value during perfusion at 37°C was 0.046±0.011 and decreased to 0.015±0.005 nmol/ml perfusate (n = 4, respectively) during cold perfusion. Free MDA in surrounding medium was increased for both stored livers during the first hours of ischemia. Free MDA measured value was negligible at the end of the ischemic period. The degradation of MDA in surrounding medium during prolonged ischemia could explain this low value. For livers stored in UW, reperfusion at 4°C in Krebs solution produced an immediate and large free MDA release in the perfusate, and then a rapid decline. For livers stored in HTK, reperfusion at 4°C produced a lesser release but then an enhancement during reperfusion at 37°C.

Changes in Vitamin Status during Ischemia and Reperfusion.

The vitamin E level in rat liver during the initial period (280.7±7.21 pmol/mg liver proteins; mean±S.E.M.) was

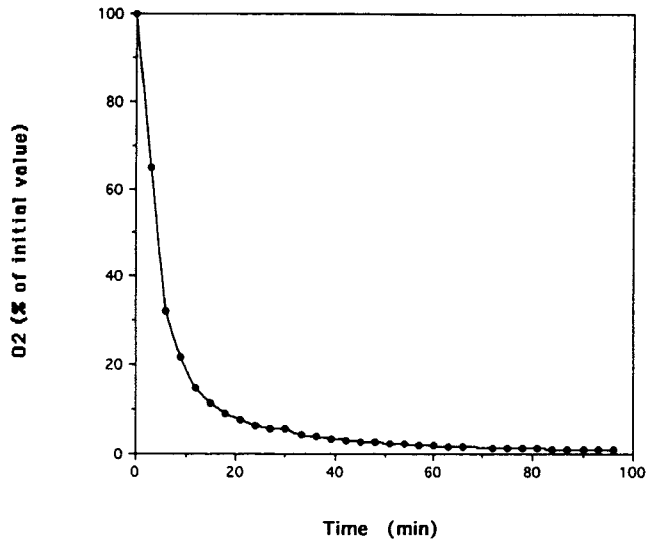

Figure 4: Residual O_2 concentration in the surrounding medium during the first hours of cold ischemia.

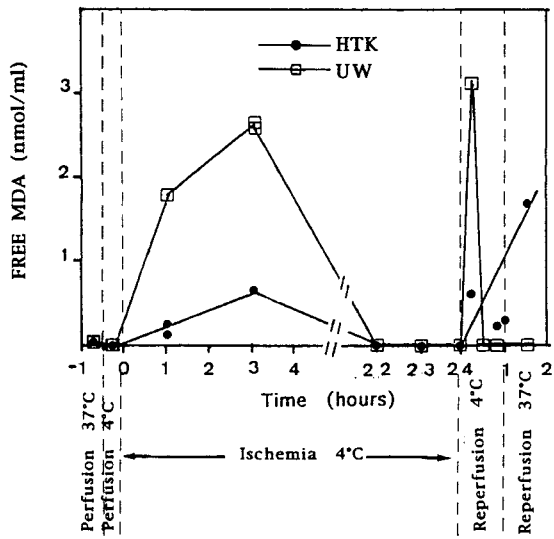

Figure 5: Release of free MDA in the surrounding medium during the perfusion, cold ischemia and reperfusion sequence.

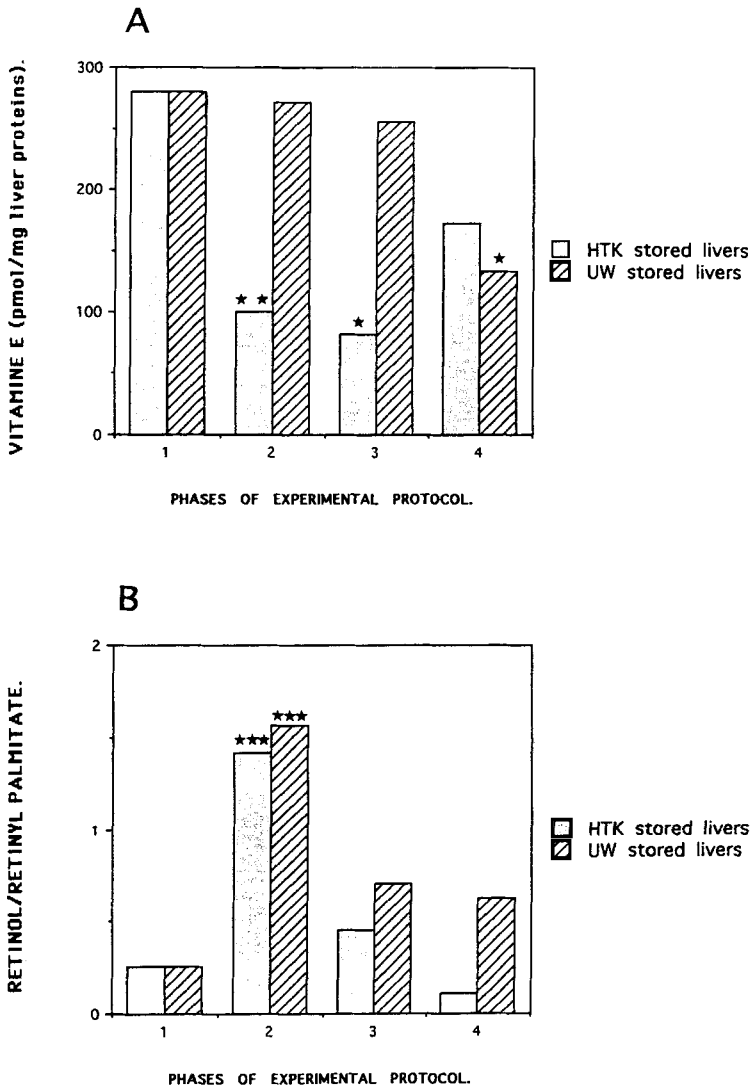

Figure 6: Vitamin status.
A- vitamin E content.
B- vitamin A status expressed as the molar ratio of retinol to retinyl palmitate.
The phases of experimental protocol are as in Figure 2.
* $P<0.05$; ** $P<0.01$; *** $P<0.005$.

considered as the baseline. Ischemia in HTK and subsequent reperfusion in Krebs medium induced a significant decrease ($P<0.010$) in vitamin E content (figure 6A). On the other hand, for livers preserved in UW, the vitamin E content decreased significantly ($P<0.05$) only with 37°C reperfusion in Krebs medium.

Excess vitamin A is stored in liver as a free form or as esters with long-chain fatty acids. For the control rats, the predominant forms were retinyl palmitate (77.4 %) and retinyl stearate (only 4.6 %). So we expressed the vitamin A status as the molar ratio of free retinol to retinyl palmitate, and its initial value was 0.26±0.09 (mean±S.E.M.; n=8). This value increased significantly ($P<0.005$) during ischemia in both preservation media and then, during the reperfusion in Krebs medium, decreased to a value which was not significantly different from the initial value (figure 6B).

The molar ratio increment was due to a release of free retinol from the ester forms. The baseline value (42.18±18.48 pmol/mg liver proteins; mean±S.E.M.; n=7) was then multiplied by 8.35 and 7.12 during ischemia for livers preserved in HTK and UW, respectively. The decrease in this ratio during reperfusion was due to changes in both free retinol and retinyl palmitate levels.

DISCUSSION

It is now well established that during ischemia there is a continuous degradation of ATP (2, 18, 28), giving ADP (during the first hours of ischemia) and then adenosine which diffuses extracellularly and is then degraded into inosine and hypoxanthine. The latter then accumulates in ischemic tissues. This degradation of energy-rich phosphates is accompanied by a decrease in the pH_{in} whatever the preservation solution, owing to an increase in inorganic phosphate and the accumulation of lactate produced by anaerobic glycolysis. After ischemia, the pH_{in} of HTK stored livers reached 7.12±0.05 because of the high buffering capacity of this histidine-containing solution. In UW preserved livers, the pH_{in} was not determined with accuracy owing to the high phosphate content. Cold reperfusion with Krebs medium very quickly restores the ATP level and the pH_{in} in both preservation media (18). During subsequent warm reperfusion, these values reached lower values (Table 2).

During ischemia, apart from the purine metabolism, xanthine dehydrogenase is converted to xanthine oxidase (29) which can catalyse the conversion of hypoxanthine to uric acid after reoxygenation, thus reducing oxygen to superoxide and hydrogen peroxide (30). These reactions consequently lead to a

generation of activated oxygen species which could overwhelm the protective intracellular mechanisms, such as vitamins E and A, and produce LPO. However, Tien et al (12) and Sugioka et al (13) have studied two *in vitro* systems in which they were unable to find evidence for OH° involvement in LPO. There is a real controversy in the literature as to whether OH° are absolutely necessary intermediaries in the initiation of LPO. Much evidence supporting the free radical hypothesis of ischemic reperfusion cell damage has often been indirect by analysing the effect of free radicals (4-9). The analysis of MDA, the end product of LPO, also gives inconsistent results; for example, an increase in TBA-reactive substances (TBA-RS) during ischemia and then enhancement during reperfusion (8,10, 31); or an increase in TBA-RS after only 2 hours of reperfusion (32); or an increase in TBA-RS during ischemia and then a decrease during reperfusion (10, 33). In our experiment, among TBA-RS we have analyzed the MDA/TBA value by a specific HPLC method (20) derived from Wong (21). The pH value <1 used in our method largely reduced the yield of 532 nm-absorbing chromogen produced by other oxidized products of LPO (see Material and Methods). Our results, which are in agreement with those of other authors (3, 10, 33), show a slight mean increase in bound MDA during ischemia in both preservation media with a subsequent decrease during reperfusion compared with the baseline value (figure 2). The

increase in bound MDA during ischemia could be due to the fact that even under global ischemic conditions, some oxygen is available to initiate LPO In our experiment, the initial perfusate was gassed with 95% O_2 and 5% CO_2 producing a low-grade oxygen pressure until 2-3 hours of ischemia (figure 4). Using spin trapping, Bolli et al (31) and Sakamoto et al (34) have reported a certain amount of oxygen free radical production during ischemia. Our results show that HTK and UW did not inhibit LPO (figure 2) in agreement with other authors (2, 5, 10, 15): whereas, on the one hand, HTK contains 30 mM mannitol which could trap OH° (14), on the other hand, UW contains 1 mM allopurinol which could inhibit xanthine oxidase (9, 10). MDA is more likely to be produced by LPO initiated by the reaction of ADP-Fe^{++} with O_2 (15, 35) as shown in figure 3. In line with this consideration, free iron ions may accumulate during ischemia by release from ferritin under anaerobic conditions (14). During ischemia, ATP breakdown leads to ADP production, but the presence of ADP is difficult to quantitate owing to the partial NMR invisibility of ADP. Whatever the case, in ischemic conditions, the connection of ADP, free iron ions and residual oxygen in preservation solutions (figure 4) could explain the LPO observed during ischemia. The study by Masini et al (8) supports this hypothesis: they observed that a perfusion with $FeCl_3$/ADP enhanced the incidence of ventricular arrhythmias, histamine release and MDA

production in isolated guinea pig heart. α-tocopherol is also depleted during ischemia, especially in livers stored in HTK (figure 6A), by scavenging the reactive lipid peroxy radicals (5, 15, 35-37) that propagate the autooxidation chain reaction of lipids. Moreover, we have shown that some of the MDA formed can be eliminated in the surrounding medium (figure 5). So the MDA value observed may be the result of several effects: the damage of polyunsaturated fatty acids initiated by the ADP-Fe^{+++}-O_2^- complex, the partial inhibitory effect of vitamin E (35) and the possible release in the surrounding preservation buffer.

After reperfusion, there was a decrease in bound MDA values (figure 2) accompanied by a large release of free MDA in the perfusate (figure 5) whatever the preservation medium. These results are in agreement with those of Ferguson et al (10) and Pickford et al (33). However, the latter authors have suggested that the fall in TBA-RS during reperfusion might be due to the swelling and weight increase caused by a protein-rich tissue oedema. Since the LPO markers were standardized by expressing values in terms of protein concentration of tissue homogenate, high protein levels might result in spuriously lower final values. In our experiment, HTK produced a turgescence at 4°C but lactobionate in UW is known to counteract cold cellular swelling. So Pickford's hypothesis can not be taken into account. The decrease in bound MDA

could merely reflect its enhanced release in the perfusate. On the other hand, oxygen solubility during reperfusion is higher in cold than in warm Krebs medium. So the oxygen supply is largely increased during hypothermic reperfusion and an oxygen excess might lead to H_2O_2 (38). The latter can be a precursor of free radical generation *via* the Fenton reaction, and may enhance LPO and MDA levels. However, the instability of MDA suggests that MDA could be directly decomposed by strong oxidants (figure 1). MDA could also be metabolically degraded *in vivo* by mitochondrial aldehyde deshydrogenase (39). So even if the TBA test assayed by HPLC is pratically specific toward MDA under our experimental conditions, the indirect and complex nature of the relationship between LPO and the TBA-test makes it difficult to correlate the MDA/TBA response to the quantification of peroxidative injury, in this particularly complex biological system (40).

Under normal nutritional conditions, the majority of total body retinol (90-95 %) is stored in the liver predominantly as retinyl palmitate. Liver accumulates dietary retinoids like retinyl esters, so the molar ratio of free retinol to retinyl palmitate varies with age (41) and diet (42). Therefore, our results on the total retinol status of control rats expressed as the molar ratio of free retinol to retinyl palmitate are in agreement with those of other authors (43, 44). Liver also plays a dynamic role in retinoid metabolism, generating retinol

from the stored retinyl palmitate through an ester hydrolase activity (45) as well as resynthesizing the esters through an acyl-CoA: retinol acyl transferase (46). In cold ischemic conditions, our results (figure 6B) indicate that retinyl esters are hydrolysed, particularly retinyl palmitate. After reoxygenation, free retinol is reesterified. At the present time, we do not know whether this increased hydrolytic activity is only oxygen-dependent, or whether it is initiated by a modified metabolism subsequent to LPO. An hypothesis could be that the α-tocopherol depletion produced by its reaction against the radical chain reaction initiated by the ADP-Fe^{+++}-O_2^- complex may influence retinyl ester hydrolysis (47, 48, 49).

Previous studies (18, 28) have demonstrated the superiority of lactobionate- (UW) and histidine- (HTK) containing media over the previously used Eurocollins' and Krebs-Henseleit's solutions on ATP and pHi_n recovery during post-ischemic reperfusion on rat liver.

Free and bound MDA analysis does not reveal significant differences between HTK and UW media. On the other hand, it is particularly difficult to link MDA level and peroxidative lipid injury in complex living systems (40), even if MDA is determined by a specific HPLC technique (20). The varied results in the literature takes into account the procedure's

diversity, and principally the complex relationship between LPO and MDA production.

However, UW makes it possible to preserve the vitamin E content during cold ischemia better than HTK. On the other hand, bound MDA decreases in livers stored in UW during cold and warm reperfusion to a greater extent than in livers stored in HTK. Finally, this study seems to support the superiority of UW over HTK, with regard to LPO and the protective effect of vitamin E.

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